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**THE DEVELOPMENT OF ROOTS AND ROOT SYSTEMS
IN WHITE SPRUCE [*PICEA GLAUCA* (MOENCH) VOSS] SEEDLINGS
AND THE INFLUENCE OF CULTURAL TREATMENTS ON ROOT
MORPHOLOGY, ANATOMY, AND THE CAPACITY TO CONDUCT WATER**

by

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ABSTRACT

Root development in *Picea glauca* seedlings was studied anatomically during the first year after germination. The cyclic pattern of elongation of individual roots was established about three months after germination. With progressing development, root hairs gradually diminished and colonization of roots by mycorrhizal fungi increased. The development of primary tissues in long roots, relative to the distance from the root tip, appeared to be related to their rate of root elongation. In these roots, the development of Casparian bands in the endodermis often occurred several millimeters away from the root tip. In elongating short roots, endodermal cells attained their primary state only 2-4 cells away from the proximal part of the apical meristem. In non-elongating roots, the secondary-state endodermis was connected to the metacutis just above the apical meristem. The development of Casparian bands was always prior to the maturation of the first xylem elements. The endodermis did not develop past the secondary state. Through the presence of passage cells, it remained functional until its disruption by secondary growth. Low frequency of plasmodesmata in the endodermis indicated that the plasma membrane - cell wall - plasma membrane type of transport was the main means of molecule exchange between the cortex and the stele in white spruce roots. Undifferentiated tissues of the root near the apical meristem were almost impermeable to fluorescent dye tracers Sulforhodamine G and fluorescein diacetate. The metacutis and the endodermis at the primary and secondary state were impermeable to the apoplastic tracer Sulforhodamine G.

Roots and root systems were structurally and physiologically affected by cultural treatments such as pruning and fertilizer application. Roots of seedlings grown at low nitrogen (N) supply were thin and their tracheids were narrow. Excess N did not significantly increase root diameter and tracheid dimensions, compared to the optimum supply. Dimensions of bordered pits were not significantly affected by the N level. The secondary development in roots advanced basipetally but exceptions were found indicating that cambial growth of roots could vary along the root regardless of the position relative to the root tip. Seedlings with different root systems modified by nursery culture exhibited different patterns of root growth after planting. Root elongation and root surface area increases immediately after planting were greater in container-grown than in mechanically box-pruned seedlings but this was unrelated to the longer-term performance of these seedlings. The initially low hydraulic conductance of root systems in box-pruned seedlings

increased significantly 6-8 weeks after planting while it remained unchanged or declined in container-grown seedlings. Root pressure, comparable to that reported for angiosperm seedlings, was found in white spruce seedlings during the first few weeks after planting. This is contrary to the general notion that conifers do not develop notable root pressure. The initiation and elongation of roots in unfertilized organic compartments was poor compared to root growth in unfertilized mineral compartments, especially in mechanically pruned seedlings whose roots proliferated in the latter compartments. The growth of roots in the organic substrates was enhanced by the addition of slow-release fertilizer to that substrate. The growth response of roots to slow-release fertilizer added to the mineral substrate was restricted to that compartment but root growth in both soil compartments was affected by the addition of slow-release fertilizer to the organic substrate. Root development in different types of planting stock was differently affected by the soil substrate type and the addition of the slow release fertilizer.

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¹Univariate analysis of variance

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²Multivariate analysis of variance

³Multivariate analysis of variance

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CHAPTER 1 INTRODUCTION

Growth, morphology, phenology, and physiology of the above-ground portion(s) of conifer seedlings have been extensively studied. Our knowledge in these areas, even if still incomplete, greatly exceeds our understanding of the processes taking place below ground. Root morphology of conifer seedlings is poorly described as is physiology of roots, water and solute uptake, respiration, and suberization, in relation to seasonal changes in root morphology. Johnson-Flanagan and Owens (1985a; 1985b; 1986) described the morphology, anatomy, and some aspects of physiology of the roots of interior spruce seedlings grown in styroblock containers. However, these studies had other objectives than the relationship between the morphology, anatomy, and water conducting properties of the roots.

Studies on roots of British Columbia conifers have focused on the influence of site preparation treatments on gross morphology (McMinn, 1982; Hallsby, 1995), juvenile instability (Burdett 1979; Burdett *et al.* 1986) and post-juvenile stability (Krasowski *et al.* 1996a). These studies considered root form of planted trees but not the relationship between structure and function other than the stability and, at best, inferred the relationship between root form and water/nutrient uptake.

Roots function in plant anchorage, uptake of water, selective uptake of solutes, and as site of production of hormones and substances important for the plant's metabolism (Sutton, 1991). The degree of fulfilment of these functions depends on the physiology and morphology of the root system. However, plant roots are not the only inhabitants of the soil environment. They must co-exist with organisms that make the soil their home. It will benefit the plant if this coexistence is harmonious.

The goal of these studies is to learn about the relationship between root structure and function. The development of long and short roots is studied in relation to their capacity for conducting water. Practical aspects of root growth, important to forestry, are addressed by studying the effects of different root pruning methods on the morphology and hydraulic properties of root systems and these are related to the performance of seedlings under experimental conditions and in the natural environment of forest sites. Effects of inorganic nitrogen on root anatomy, particularly on the anatomical characteristics of root tracheids, are examined to determine whether mineral nutrition could affect the ability of roots to conduct water. Effects of different

soil substrates on the growth of roots and the influence of slow-release fertilizer applied to different soils are studied to determine their effects on root and shoot growth and on mycorrhizal fungi associated with these roots. It is hoped that these studies will provide basic information on root development in white spruce (*Picea glauca* Moench [Voss]), a commercially important tree species in western Canada, and will supply practising foresters with information relevant to their work in forest regeneration.

CHAPTER 2: LITERATURE REVIEW

THE STRUCTURE AND FUNCTION OF ROOTS AND ROOT SYSTEMS

The acquisition of soil-based resources and the anchorage of a plant are the primary functions of root systems. Storage, production of growth regulators, propagation, and dispersal are considered secondary functions (Fitter, 1991). A root system represents the structural/functional integrity. It consists of individual roots that may vary widely in their structure and function (Fitter, 1991). The highly variable below-ground environments, forming an array of microsites within short distances from each other, require dynamic and responsive root systems whose plasticity and structural complexity enable them to live in association with a variety of soil organisms.

These associations play an important role in determining and modifying the growth and functioning of root systems in the soil. They are not accounted for in artificially created conditions such as hydroponic cultures (Atkinson and Last, 1994).

Morphology of roots and root systems

The limited range of distinct morphological features in individual roots limits their usefulness in taxonomic identification. The main morphological features of individual roots are their diameter, texture, color, and, sometimes, specialization. The variation in root diameter is great within and between species (Fitter, 1991). Roots of small diameters are formed under low-nutrient conditions (Christie and Moorby, 1975; Fitter, 1985). The senescence and turnover of small diameter roots far exceeds that of larger diameter roots (Persson, 1982; Vogt and Bloomfield, 1991). The variation in texture relates to the presence, number, and length of root hairs, the persistence of the cortex and external tissues, and the properties of bark in woody roots. Although there is a considerable variation in root color (mainly various shades of brown), the most common distinction is that between brown and white (unpigmented) roots (Fitter, 1991). Mycorrhizal associations usually affect morphological characteristics of roots (Sutton, 1969; Wilcox, 1991; Hooker *et al.* 1992a, b; Hooker and Atkinson, 1992).

In contrast to individual roots, the morphology of root systems varies widely. Root systems are described by the number and length of external (terminating in a meristem) and internal (joining)

links, the angle of branching and relative diameter of links (the rate of increase in diameter per linking magnitude) (Fitter, 1991). This author stated that, to date, there are no satisfactory classifications of the morphological types of root systems that encompass both the range of morphological diversity found among plant species and its functional significance. However, relating between morphology and function of root systems is a difficult task partially due to the fact that different functions are performed simultaneously. The seasonality of root growth and root turnover are not without effect on the morphology of root systems. Recent advances in techniques for observation and measurement of roots *in situ* and the development of digital image analyzing systems allow for a better understanding of the dynamics of root growth (Atkinson and Last, 1994)

Several studies described the morphology of conifer roots in seedlings (Wilcox, 1954, 1962a; 1964; 1968a; Hermann, 1977; Johnson-Flanagan and Owens, 1985a) and in mature trees (Eis, 1974; Kuiper and Coutts, 1992). Johnson-Flanagan (1984) listed four morphological types of roots occurring in conifers. A primary root (tap root or main root), long lateral roots, short lateral roots, and, in some species, adventitious roots. Wilcox (1964) presented a different classification of long roots (apart from the main root) which included long, almost unbranched pioneer roots, mother roots bearing many branches, and, also branched but thin subordinate mother roots. Both, Johnson-Flanagan (1984) and Wilcox (1964) based their classifications on earlier publications by various investigators. Thus, conifer root systems consist of various kinds of roots. Wilcox (1964) critically analyzed the common belief in functional significance of heterorhizy. He stated that, although morphological modifications undeniably relate to functional specialization, it should not be implied that different roots develop for separate functions which they can perform better than the others.

The morphology and growth patterns of container-grown seedlings of *P. glauca* were described by Johnson-Flanagan and Owens (1985a). These root systems were found to be greatly influenced by the limitations imposed by the container cavity that directed the growth of long laterals downward resulting in air pruning at the bottom opening of the container cavity. Additionally to a taproot, three classes of long laterals were distinguished: 1) white, elongating roots without root hairs, 2) absorbing roots with root hairs present beyond the zone of elongation, and 3) brown roots. The functional implications of these morphological features, were only assumed. The morphology of spruce seedling root systems was subject to changes resulting from cyclic growth periods of individual roots and, more decisively, the annual growth cycle. Brown roots were characteristic of winter time and periods of no elongation. White, elongating roots free of hairs occurred typically at the resumption of root elongation in the spring. Smaller elongating

roots and roots bearing root hairs predominated at other times. Wilcox (1954) found root systems of *Abies procera* Rehd. grown with little space limitation and without root pruning consist mainly of first order laterals bearing short roots. Second and further order long laterals did not become prominent until the second growing season.

The seasonal differences in root system morphology were also noticed by Wilcox (1964; 1968) in *Pinus resinosa* Ait. The unbranched pioneer-type roots were abundant during periods of growth surges in spring and late summer. Higher growth rates resulted in increased distances from the meristem to the first branch root. The lack of elongation of initiated laterals on elongating pioneer roots and on poorly branched portions of mother roots was believed to be the result of suppression of elongation of laterals by the apex of the parent root. Short roots were reported to be ephemeral structures, often colonized by fungi. Although short cycles of elongation separated by dormant periods could be observed within a single growing season (Wilcox, 1964), the majority of short roots remained small and persisted only one or two seasons or less.

Mycorrhizal associations

Baylis (1975) suggested that plants with many large diameter roots depend on mycorrhizae more than those with small diameter roots. Conifer roots fit the large-diameter type of plant category and mycorrhizal associations are common. In Pinaceae many roots are thick and grow in the soil at low densities (Fitter, 1991). The classification of mycorrhizae is based on the location of the fungal mycelia relative to the root structure but different types of mycorrhizal association (ecto, ectendo, or endomycorrhiza) apparently have no functional differences (Wilcox 1991). Read (1983; 1984) suggested that each type of mycorrhizae was associated with habitat type. He considered ectomycorrhizae to be characteristic of boreal habitats, the ectendomycorrhizae typical for cold, wet soils, and vesicular-arbuscular type common in warmer, dry soils. About 95% of Pinaceae are ectomycorrhizal (Newman and Reddell, 1987). Associations between the host plant and mycorrhizal fungi change with host's age. The same species of a fungus may form different kinds of mycorrhizae while associated with different hosts. E-strain fungi colonize conifer seedlings in the nursery (Mikola 1965) and usually form ectendomycorrhizae in association with *Pinus* and *Larix* and ectomycorrhizae with roots of *Picea*, *Abies*, *Tsuga*, and *Pseudotsuga* (Laiho, 1965; Wilcox, 1991). Some exceptions have been reported (Laiho, 1965; Thomas and Jackson, 1979; Wilcox *et al.* 1983).

Mycorrhizae modify root morphology and this modification depends on the fungus and host species (Wilcox, 1991). Root branching may be affected producing dichotomous short roots (Chilvers and Gust, 1982a;b), however, long roots can also be colonized (Wilcox 1968; Harley and Smith, 1983; Sutton and Tinus 1983; Scales and Peterson 1991a; b). Colonization of different root types may change with age of the mycorrhizae. Hepper (1985) showed that mycorrhizal infections initiated on the main root spread to branched roots while further colonization of the main root was later avoided. The susceptibility of roots to fungal infection decreases with age and progressing development as a result of cortex collapse and increasing suberization of tissues (Wilcox, 1991).

The external appearance of mycorrhizae may vary even for the same fungus when in association with different hosts (Scales and Peterson, 1991b). The mantle may or may not be present (Wilcox, 1991). Sometimes, pseudomycorrhizae or unclassified mycorrhizae are reported but their role and function remain obscure. They may have parasitic character (Wilcox and Gunmore-Neumann 1974; Wilcox and Wang 1987a;b).

The anatomy of various mycorrhizae have been described in a number of conifers including *Picea*. In *P. abies* L. Karst, *P. glauca*, *P. engelmannii* Parry Engelm., *P. pungens* Engelm., and *P. sitchensis* (Bong) Carrière, Laiho (1965) observed only extracellular hyphae and no mantle. However, Thomas and Jackson (1979) reported the presence of intracellular hyphae in the outermost cortical cells of Sitka spruce (*P. sitchensis*) and Scales and Peterson (1991b) cited G. Hunt (personal communication) who had observed a well-developed mantle on roots of container-grown *P. engelmannii*. Scales and Peterson (1991b) gave detailed morphological and anatomical description of ectomycorrhizae formed by *Wilcoxiana mikolae* var *mikolae* (Yang & Wilcox) Yang & Korf with roots of *Picea mariana* (Mill) BSP. They showed the presence of a mantle but there were no intracellular hyphae. The same species of fungus formed similar appearing mycorrhizae with *Pinus banksiana* Lamb. but this association was ectendomycorrhizal (Scales and Peterson 1991a). Massicotte *et al.* (1989) found differences in the anatomy of mycorrhizae formed by *Laccaria bicolor* (Maire) Orton with roots of *P. resinosa* compared to those formed with *Betula alleghaniensis* Britt. Kottke and Oberwinkler (1990) reported differences in the development of mycorrhizae in *Picea abies* and *Larix decidua* Mill. relative to the development of the endodermis. In the former, a Hartig net developed only after the development of the secondary endodermis. In *Larix*, the endodermal development into a secondary state was slow and the Hartig net was developed at the primary endodermal state.

Most of the above cited studies of mycorrhizae in conifers reported progressive degeneration and collapse of cortical cells in infected roots. However, normally there was no hyphal penetration through the endodermis into the stele. Haug *et al.* (1988) reported intracellular hyphae in the stele of *P. abies* but this was a parasitic rather than a symbiotic situation.

ROOT ANATOMY

Tyree and Karamanos (1981), noted that root anatomy is one of the main factors affecting water flow into and through roots; thus it cannot be ignored in studies of the dynamics of water permeability. Xylem development in relation to conductive capacity of roots has been extensively studied in maize (St. Aubin *et al.* 1986; Wenzel *et al.* 1989; McCully and Canny, 1988; Wang *et al.* 1991). There are also numerous studies of fluorescent tracer movements demonstrating apoplastic pathways in roots of various plants (Peterson *et al.* 1981; Enstone and Peterson, 1992a; 1992b; Moon *et al.* 1986) and, infrequently, symplastic pathways (Moon *et al.* 1986; Erwee and Goodwin, 1983; 1985).

Anatomical studies of conifer roots have focused on the root tip and meristems emphasizing the origin of tissues and their differentiation more than characteristics related to the conductive capacity of these roots. Bogar and Smith (1965) studied root tip anatomy, organization of the meristems in roots of different order and mycorrhizal association in Douglas fir. Wilcox (1954; 1962a), Leshem (1970), and Johnson-Flanagan and Owens (1985a) reported important differences between the anatomy of actively growing and dormant conifer roots. The most significant difference was the presence of a metacutis around the apical meristem of dormant roots. Leshem (1970) studied the metacutis in *Pinus halapensis* Mill. concluding that this suberized and lignified structure did not function in protection of the meristem against various stresses. Johnson-Flanagan (1984) reported that the metacutis was impermeable to an apoplastic dye and, since it joined the secondary endodermis through a bridge-like structure, the dye did not penetrate into the stele. Thus, both the metacutis and endodermis acted as apoplastic barriers. However, in heat-killed roots the dye moved through the symplast. Johnson-Flanagan (1984) suggested that this result indicated the presence of a symplastic barrier in heat-untreated roots.

Root anatomy and pathways of water movement

The changing availability of soil water and the growth of new roots continuously change the geometry of the water extracting system (Oertli 1991). The complexity of root anatomy constitutes a major difficulty in defining a single or even a typical pathway of water movement from the soil to roots and through the root (Moreshet and Huck 1991). Root tissues create the pathway for water movement in roots.

The external root tissues

The epidermis is the outermost layer of primary roots in many species (Esau 1965). Conifers do not possess a true epidermis arising from epidermal initials. The outermost cell layer is referred to as rhizodermis emphasizing its function rather than relative position (Bogar and Smith 1965; Wilcox 1954; 1962a 1968; Johnson-Flanagan, 1984). In grasses, seminal roots lose all their tissues outside of the endodermis during flowering and the endodermis becomes the most external tissue in direct contact with the surrounding environment (McCully 1987). Hypodermis is produced by some species immediately underneath the epidermis. Hypodermal cells have thick, often lignified cell walls (Drew 1979). Perumalla and Peterson (1986) reported the development of Casparian bands in hypodermal cells of corn (*Zea mays* L.) and onion (*Allium cepa* L.), which impeded apoplastic water flow into the cortex. In roots undergoing secondary growth, the action of the cork cambium and the subsequent development of the periderm create the external layer of the root (Esau 1977). Kramer (1946) reported that older roots that had undergone secondary growth were very active in water uptake in woody perennials.

Root hairs and mycorrhizae

The functional importance of root hairs and mycorrhizae in water absorption is not fully understood. Root hairs, although short lived, may create a total absorbing surface greater than that of axial and lateral root surfaces combined (Russell 1977). However, Cailloux (1974) found water absorption of root hairs of *Avena* not proportional to their surface. Jones *et al.* (1983) compared hydraulic conductivity of root hair cells, epidermal cells of hairless root portions, and cortical cells. They found no significant differences in conductivity of these different cell types and concluded that root hair membranes lack specialization for water uptake. This, however, was disputed by Hofer (1991) on the basis of turgor pressure measurements which showed lower

turgor pressure in root hair cells than in hairless epidermal or cortical cells. The enlargement of root surface area by root hair and mycorrhizal hyphae was expected to reduce the resistance to water flow in the soil portion bounding the root surface (Oertli 1991). He doubted, though, that there would be any real benefit to the plant from such an increase in root surface area unless a significant water potential gradient would be created in the rhizosphere and if the resistance to water movement through hyphae or root hairs would be lower than that of the soil.

Some authors consider the penetration of the soil by hyphae as beneficial because it effectively increases the conductivity of that soil for water flow (Hardie and Leyton, 1981; Allen, 1982; Graham and Syversten, 1984). But, Safir *et al.* (1972) reported that the difference in the hydraulic resistance between mycorrhizal and non-mycorrhizal roots could be eliminated by improving nutrition of non-mycorrhizal plants; thus the beneficial effects of the mycorrhizae might be related to improved plant nutrition rather than to structural properties. Similarly, the observed effects of greater resistance of mycorrhizal plants to water stress (although only to a certain level) (Hetrick *et al.* 1984) could result from the overall better condition of plants well supplied with nutrients by its mycorrhizae. Wilcox and Ganmore-Neumann (1974) cautioned against assigning too great a significance to mycorrhizae and preoccupation with research on short branch roots colonized by fungi. They stated that such preoccupation could lead to an *a priori* assumption of the direct relationship between the numbers of mycorrhizae and nutritional status of a seedling and that such an assumption would diminish the appreciation of absorbing abilities of long roots and their role as sites of mycorrhizal infection and lateral root initiation. To support this statement, findings by Bowen and Rovira (1976) were cited showing the same efficiency of phosphate uptake by some portions of uninfected long roots as that of infected short roots of *Pinus radiata* D. Don..

Cortex and endodermis

In primary roots, the cortex usually occupies a large portion of the root cross section extending between the external tissue(s) and the endodermis. Cortical cells possess relatively thin cell walls and intermesh with abundant intercellular spaces (Esau 1977). During secondary growth, intracellular spaces within the cortex enlarge and may eventually disrupt it (Curl and Truelove, 1986). The so called phi layer develops in cortical cells of some species of gymnosperms (Scott and Whitworth, 1928; Wilcox, 1962a) and angiosperms (Esau, 1943; Haas *et al.* 1976) but it does not appear to create an apoplastic barrier to solute movement and probably functions in mechanical support (Peterson *et al.* 1981).

The structure and development of the endodermis was described in detail by Clarkson and Robards (1975), who also demonstrated ultrastructurally its function as a barrier to the apoplastic flow of solutes. There are four states of endodermal development. The pro-endodermis is located close to the root apex and does not have a Casparian band but its cells have a distinct shape and ultrastructure. The Casparian band appears at state one, first in the radial walls. The plasma membrane adheres tightly to the cell wall, even during plasmolysis. The secondary state develops shortly, with a more extensive suberization of the cell wall which, eventually, becomes complete. Cellulose thickenings develop adjacent to the suberized cell wall at the third state- this may begin before the completion of the secondary state (Van Fleet 1961; Clarkson *et al.* 1971; Clarkson and Robards, 1975; Ferguson and Clarkson 1975). The fourth state, if it develops, leads to the deposition of phenolic compounds in the cell wall and cell death (Van Fleet, 1961). Jorns (1987) demonstrated Casparian bands in the primary endodermis of long roots of *Picea abies* as smooth regions of the cell wall with a tightly adhering plasma membrane. Subsequently, all cell walls of endodermal cells, but not of the passage cells, were covered on the inside by a lamellar layer of suberin. This was also shown ultrastructurally in roots of *Pinus sylvestris* L. (Warmbrodt and Eschrich 1985). Kottke and Oberwinkler (1990) compared the development of the endodermis in short, mycorrhizal roots of *P. abies* with that of *Larix decidua*. The latter species possessed the endodermis mainly at the primary state while the former had a secondary-state endodermis beginning to occur near the regions of xylem differentiation. Regularly spaced passage cells were present in both species. The authors reported electron-dense incrustations on the outer tangential walls of the passage cells but could not determine their chemical composition. Wilcox (1962a) also reported the presence of non-suberized cell walls in passage cells of dormant roots in *Libocedrus decurrens* Torr. In *Picea glauca* long roots, Johnson-Flanagan and Owens (1985a) reported the presence of passage cells in the secondary endodermis but these cells were sparse and sometimes their walls were suberized. The endodermis in all investigated conifer species did not progress beyond the secondary state. Kottke and Oberwinkler (1990) reported a difference in the advancement of the endodermal differentiation between dormant and actively growing roots. They agreed with the earlier opinion of Wilcox (1954; 1962a) that the differentiation of the endodermis relative to the distance from the root meristem depended on the rate of root growth and was advanced near the meristem in dormant roots where the secondary endodermis extended to the meticutized layer enclosing the meristematic portion of the root apex. This was also shown in roots of *Picea glauca* by Johnson-Flanagan and Owens (1985a).

The stele

Lateral roots originate from parenchyma cells located immediately beneath the endodermis - the pericycle (Esau, 1977). This positioning of lateral root initials may interrupt the continuity of the endodermal restriction to the apoplastic transport as growing laterals must penetrate the endodermis.

McCully and Canny (1988) stated that the most important anatomical measurement for assessing potential hydraulic conductance of roots is the diameter of the largest open tracheary element(s) in a given root segment. This property changes with the distance from the root tip as a result of the progressing xylem maturation. Varney *et al.* (1991) studied corn roots and partially confirmed the statement of McCully and Canny (1988). Unfortunately, Varney *et al.* (1991) did not determine whether the measured xylem elements were dead or alive. Wang *et al.* (1991) measured relative water content of root segments of soil-grown *Zea* and related their observations to the maturity of the xylem. Because they concentrated on root segments from near-apical regions, they failed to find a strong relationship between these properties and concluded that immature root apices were relatively isolated from water content fluctuation. McCully and Canny (1988) using tracer dyes, showed live late metaxylem of corn roots being non-conductive and, only narrow, open early xylem elements were conductive.

STUDIES OF WATER UPTAKE BY CONIFERS

The few studies of water uptake by roots of conifers focused on the differences between suberized and unsuberized roots (Addoms, 1946; Kramer, 1946; Chung and Kramer, 1975). These studies showed that older, suberized roots and root parts play an important role in the plant's water economy and mineral nutrition. Hydraulic conductivity of conifer roots has been found invariably and substantially lower than that of woody angiosperms and herbaceous annuals, (Kramer, 1946; Sands *et al.* 1982). The latter publication reported bean (*Phaseolus*) roots being about eight times more effective in conducting water than pine roots. This was attributed to the small diameter of conifer tracheids. It is not certain though, if this is the only reason for the observed low efficiency of conifer roots in water conductance or whether there are other reasons. The anatomical work of Sands *et al.* (1982), in contrast to their careful water relation work, was rather crude. If the size of tracheids is truly the main factor limiting the hydraulic conductivity, perhaps treatments that could affect tracheid size would also improve water conducting properties of conifer roots.

The dynamics of water movement in roots

There have been numerous reviews on water movement in roots focusing on the dynamics of that movement and hydraulic properties of water (or solutions) and the media it travels through (i.e. Weatherley, 1963; Brouwer, 1965; Newman 1974, 1976; Russell, 1977; Taylor and Klepper, 1978; Klepper and Taylor, 1979; Fiscus, 1983; Boyer, 1985; Passioura, 1981, 1982, 1988; Morshet and Huck, 1991; Oertli, 1991). All of them acknowledge that our understanding of the process of water movement from the soil into the root and the movement across the root into the stele is, at best, incomplete.

Water potential and fundamental types of transport.

The water potential is a thermodynamic property that is used to describe equilibria. Equality of water potential between two points is a necessary condition for equilibrium but it is not sufficient to preclude water flow as other components of a system must also be at equilibrium (Oertli, 1991). The water potential of the soil or a plant responds to changes in many variables such as temperature, external pressure, solutes, water content, matrix properties, and, depending on circumstances, other variables (gravity, for example). The difference in water potential between two points may create the driving force for water movement. There are three basic components of water potential: 1) external pressure, 2) solutes, and 3) matrix, although the existence of the third component is apparently controversial (Passioura 1980).

There are three fundamental types of transport: 1) diffusion, 2) convection, and 3) propulsion (Mitchell 1961). Diffusion is the movement of some components relative to others due to thermal motion of individual particles. Convection is the simultaneous movement of all components in the direction of decreasing concentration gradients. Propulsion pertains to the movement of a solid body and not to water movement. Sap transport in the xylem is by convection, whereas water movement in the cortex of a non-transpiring plant is by diffusion (Oertli, 1991).

Water transport in the rhizosphere

The movement of water in soil, which offers resistance to flow, requires a driving force to overcome that resistance. This driving force is created by water potential gradients. Soil

resistance to water flow varies, even for the same soil, depending on its moisture content and (closely related) its aeration. Dry soil has greater resistance to flow than saturated soil due to the filling of pores with air (large pores are filled with air first) which decreases the area effectively conducting water per soil cross section (Oertli, 1991).

The existence of a strong water potential gradient near the root surface is being disputed. It could be expected that removal of water from roots by transpiration and the increased resistance to flow in the drying soil would decrease water content near the root surface. This, in turn, should decrease water potential near roots creating suction pressure that would draw water toward roots. Oertli (1991) cites a number of publications that support this expectation and about an equal number of those that contradict it. Newman (1969), based on modeling work, concluded that soil resistance to flow becomes significant only near the wilting point.

Root shrinkage due to soil drying has been reported in the literature (Huck *et al.* 1970; Herkelrath *et al.*, 1977; Faiz and Weatherley, 1982; Taylor and Willatt, 1983). Transpirational removal of water from roots may cause them to shrink away from the drying soil eventually resulting in an air gap between the root surface and the soil. Such an air gap would greatly increase the resistance to water flow at the root surface creating an interfacial resistance (Tinker, 1976). This would also, perhaps transiently, reduce root surface active in water absorption predisposing the plant to even greater water stress. Passioura (1985) argued that there is a low risk of air gap creation at a root surface. He considered the main resistance to water movement to be in the endodermis rather than at the root surface; therefore, the water potential of the cortex should be near that of the soil. This would not be a condition promotive to the discontinuity of water flow. He also reasoned that mucigels and hyphae that cause soil particles to adhere to the root surface would further decrease the possibility of air gaps. Corn roots, for instance, are covered in certain segments with extensive mucigel-associated soil sheets (McCully 1987). However, this extent of soil adherence to mucilage-covered roots is not commonly reported in many other plants. In the composite transport model of the roots (Steudle *et al.* 1993; Steudle and Peterson 1998) the endodermis is not considered to be the main barrier to water movement through roots. Rather, it is presumed that apoplastic, symplastic, and transcellular pathways together play an important role in the passage of water across root tissues and they all contribute to the overall resistance to water flow. If this view is correct, the occurrence of air gaps at the root surface in drying soil could not be viewed as an unlikely or unusual situation.

It is possible that water transport between roots and the soil is not unidirectional. Again, the evidence in the literature is conflicting. Dirksen and Raats (1985) did not find any significant

water loss from roots of alfalfa (*Medicago sativa* L.). They concluded that the tension in the xylem and the resistance to radial flow in a root are sufficient to prevent significant water loss from roots to the soil. Baker and van Bavel (1986) found no difference between radial resistance of roots to water influx and efflux, but reported significant release of water from roots of couch grass (*Dactylon sanguinalis* [L.] Vill.). Richards and Caldwell (1987) also reported release of water from roots of *Artemisia tridentata* Nutt. Perhaps excessive drying of some parts of the root system can be reduced by water release from other, better hydrated roots or root parts. Protection of soil organisms living in close association with a given plant is another logical explanation of the release of water from roots to the soil.

Measuring water flow through roots

The resistance to water flow is usually measured by observing water potential differences between two points in the system while simultaneously measuring the volume of water flow. As noted by Moreshet and Huck (1991), measuring total resistance of a root system is usually straight forward but partitioning it to the individual components of that system is a problem.

Pressure probes and microprobes can be used for calculation of hydraulic conductivity of organs, groups of cells or single cells (Husken *et al.* 1978; Steudle and Jeshke, 1983; Jones *et al.* 1983). The method is based on controlled pressure application followed by the measurement of the time required for relaxation of that pressure in the examined specimen. When used to calculate flow through several cells, it is difficult to distinguish between the pathways of water movement (symplastic, apoplastic, or transcellular) so the calculated resistance to flow through these cells is bulked (Moreshet and Huck 1991). Hallgren *et al.* (1994) presented in detail how a pressure probe can be used in studies of water flow through conifer roots.

The most commonly used methods for water flow measurement are various modifications of a potometric method (reviewed in detail by Kozinka (1974)). Root systems or root segments are sealed in a vessel containing water or nutrient solution and the extraction of water from that vessel is measured either by volume or by weight. Water uptake by the whole root system can be determined rather easily by sealing the whole root system of a transpiring plant kept under steady state conditions and measuring water loss over a period of time. This method was used by Sanderson (1983) to determine the whole root system water uptake in barley (*Hordeum vulgare* L.). Air temperature and humidity must be strictly controlled to ensure accuracy of such measurements. Variation of the method involves collection of the exudate from the cut stem

stump protruding through an orifice in the vessel. The sap flow is induced by either pressurizing the roots or applying suction to the stump, the former method is used most often. Interesting variations of the potometric method were developed by Graham *et al.* (1974) and modified by Sanderson (1983). They measured water uptake by different root segments of axial roots and by individual lateral roots using micropotometry.

Potometry received much criticism in the literature. Passioura (1988) argued that decapitating, placing in nutrient solution, and pressurizing roots may create artifacts and yield results irrelevant to the behavior of intact plants growing in normal conditions. Pressurizing roots may push water into the intercellular spaces of the cortex, infiltrate them and transform them into conduits in the longitudinal transport of water (Salim and Pitman, 1984). This problem may be less severe if examined plants remain in the soil with large air-filled pores rather than in the nutrient solution (Passioura and Munns, 1984). Other problems potentially leading to artifact creation are: 1) root breakage while transferring plants between containers or by too vigorous aeration (Miller, 1985; Moon *et al.* 1986), 2) carbohydrate starvation of decapitated roots (Bowling *et al.* 1985), 3) effects on cellular physiology of gases such as oxygen (Termaat *et al.* 1985) and nitrogen (Miller, 1972) under partial pressure significantly higher than in the normal air, 3) circadian rhythms of root hydraulic resistance (Passioura and Munns, 1984), 4) anoxia (Kramer, 1983; Hanson *et al.* 1985).

Experimental measurements are often used in the development of theoretical models of water flow in plants. Some of these are very complex and based on numerous assumptions. An example of a popular model developed by Philip (1957) and Gardner (1960) used to calculate the flow is the cylindrical flow model discussed by Passioura (1988). It assumes the cylindrical geometry of a system in which water flows radially along pressure gradients from the soil into the root. It also assumes an even distribution of roots in the soil, ignores root overlapping (it assumes that each root has an access to the soil cylinder of a radius equal to half of the distance between roots), and does not account for variation in root absorbency along root length. Nevertheless, the model is in wide use (Passioura 1988).

Radial resistance to flow

Water entering roots must first cross the outermost root tissue(s) which, as said earlier, may be different in different plants, roots, and segments of the same root. Roots that possess epidermis may also have a layer of cuticle covering the epidermal surface, including root hair surfaces (Curl

and Truelove 1986). This would likely impede the movement of water, but the extent of such impediment is not known (Moreshet and Huck, 1991). Hypodermis, where present, is believed to serve as an initial "filter" of solutions entering the root (Peterson *et al.* 1981; Perumalla and Peterson 1986). The pathway through the cortex to the endodermal barrier appears to be relatively free of major obstacles to the solute movement (Moreshet and Huck, 1991). It is not certain how much intercellular spaces contribute to this movement. The intercellular spaces are filled with gases and they are usually interconnected longitudinally rather than radially. However, if filled with water, intercellular spaces may contribute to the flow pathway (Passioura 1988). There are three pathways available for water movement in roots: 1) through cell walls (apoplastic), 2) through the cytoplasm, via plasmodesmatal connections (symplastic), and 3) transcellular (symplastic/apoplastic). In transcellular transport, water would have to cross the cellular membranes several times, including tonoplasts and the plasma membrane, and move in cell walls. This kind of movement is also termed "vacuole to vacuole pathway" (Moreshet and Huck 1991).

Water movement can occur through the three pathways at the same time, but there is no agreement as to the contribution of each of these pathways to the total water flow through roots (Moreshet and Huck 1991). Weatherley (1982) suggested that the apoplast may have the least resistance to flow and calculated that cell wall microfibril capillaries may have up to three times higher conductivity than the symplast. He considered that narrow plasmodesmata must have high resistance to flow. Newman (1976) considered symplastic conductance to be far greater than that of the cell walls. He proposed that water travels in roots mostly in the symplast and only occasionally crosses the cell walls. If so, the endodermis would not be the major barrier creating the water potential gradient between the radial and axial transport as traditionally believed (Passioura 1988). On the other hand, possible creation of such gradient could perhaps be caused by the plasmodesmatal constriction in the endodermis (Gunning and Robards 1976). Passioura (1988), although not sympathetic to Newman's (1976) view, brought up two kinds of evidence he considered supportive to that view. The first one was anatomical - impermeable bands similar to the Casparian bands have been reported in species that possess the hypodermis (Peterson *et al.* 1981; Perumalla and Peterson 1986). The second argument brought up by Passioura (1988) is that pressure probe measurements of water passage through individual cells indicated approximately the same resistance to flow as that of the entire root system (Jones *et al.* 1983; Steudle and Jeschke, 1983). Passioura (1988), however, qualified this observation by stating that large resistance to flow through the endodermis combined with little resistance in cortical cell walls could produce the same effect. In the composite transport model of the root (Steudle *et al.* 1993; Steudle and Peterson, 1998), the contribution of different pathways is considered to be

variable and dependent on the nature of the force that drives the transport of water through roots. An apoplastic pathway is thought to be the main route for water transport under hydrostatic pressure as the driving force. The symplastic route is presumed to be the main pathway for osmotic pressure-driven transport. This model also assumes that the blockage of the apoplastic route by Casparian bands is not complete and could be bypassed. Although evidence in support of this model has been presented (Steudle *et al.* 1993; Peterson *et al.* 1993), this evidence could be differently interpreted and cannot be considered an unequivocal proof of the proposed model.

The potential endodermal resistance to water flow would depend on the state of the development of the endodermis. Sanderson (1983) speculated that the endodermis at the secondary state of development could possibly exercise less control upon the apoplastic transport than at state one. This would be due to the plasma membrane breaking away from the Casparian band during the development of a suberin lamellae at the secondary state. The lamella (its permeability is unknown) could allow the water to bypass the Casparian band. The continuing development of the endodermis and the deposition of waxes at the tertiary state would block the plasma membrane. Graham *et al.* (1974) measured water uptake of barley and marrow (*Cucurbita pepo* L.) root segments at various distances from the root apex and examined the developmental state of the endodermis in these segments. A similar study on barley roots was published by Sanderson (1983). In both studies water uptake was measured with micropotometers. In the two species examined in these studies the sudden decrease in water uptake was coincident with the development of the tertiary state of the endodermis. This occurred at about 70 mm and 90 mm from the root tip in barley and marrow roots, respectively. In conclusion, radial resistance to water flow is not uniform and varies with root age and development.

Axial resistance to flow

The relationship between the size and condition (living or dead) of xylem elements and water conductance was discussed earlier. Higinbotham *et al.* (1973) reported that xylem cells of maize roots remained alive at some distance from the root tip. St. Aubin *et al.* (1986) reported that metaxylem cells in soil sheathed maize roots were alive at large distances from the root tip. They attributed this to the potential functioning of these root segments in providing water to the adhering soil.

Crosswalls offer high resistance to axial flow at a distance of several centimeters from the root tip (Sanderson *et al.* 1988; St. Aubin *et al.* 1986; Clarkson, 1991). It could be expected that large,

open metaxylem elements would offer little resistance to water flow (Passioura 1988). Another possible impediment to the axial flow may be cavitation. Although cavitation has been reported to occur mainly in the stem xylem (Crombie *et al.* 1985; Tyree and Dixon, 1983, it could also occur in roots (Byrne *et al.* 1977).

Water uptake by root systems

One of the ultimate goals of research on permeability of roots to radial and axial water flow is the determination of a "hydraulic architecture" of root systems, in other words, determining which roots and which parts of these roots contribute the most to water uptake of the entire root system. This may be of critical importance to the development of seedling types for reforestation, and for the treatment of horticultural and agricultural crops, as root system development may be influenced by the nursery culture, pruning, and various cultivation treatments. The popular belief that only very young roots and terminal portions of older roots are significantly contributing to plant's water absorption has been long abolished. Addoms (1946) observed absorption of weak aqueous dye solutions by older, suberized roots of yellow poplar (*Liriodendron tulipifera* L.), sweet gum (*Liquidambar styraciflua* L.), and shortleaf pine (*Pinus echinata* L.). She observed microscopically that older roots absorbed large quantities of the dyes and noted differences among the species. It was noticed that the greatest concentrations of the dyes were localized around breaks around the base of branch roots, in lenticels, and in wounds. Apparently, the dyes did not enter axial roots at the base of branch roots in shortleaf pine. A reference to Preston's (1943) observations made on lodgepole pine (*Pinus contorta* Dougl.) was provided by Addoms (1946) to emphasize the difference between pines and the other examined species in this respect. Another interesting observation of Addoms (1946) was that older, suberized roots did not appear to absorb unless the younger, branch roots were excised and the wounds sealed. This suggested that the absorption by older, suberized roots would occur only if the necessity arose, perhaps due to increased xylem tension caused by the inability to feed the transpirational stream.

Kramer (1946) used small potometers attached to roots of plants remaining in the soil to observe water absorption in shortleaf pine, yellow poplar, and dogwood (*Cornus*). He found water absorption by all examined roots that varied from 3.2 to 17.0 mm in diameter even though many had thick layers of cork. He also noted significant differences in absorption by roots of different species. Kramer (1946) concluded that water absorption through non-elongating, suberized roots must be of vital importance to overwintering trees. Chung and Kramer (1975), supported this conclusion by potometrically quantifying absorption of water and radioactive phosphorus by loblolly pine (*Pinus taeda* L.) roots.

A new and interesting method of determining the major sites of water uptake by whole root systems was presented by Varney and Canny (1993). They quantified the build-up of a water soluble apoplastic dye in different roots or root portions. The dye dissolved in water was applied as a mist to exposed root systems contained in an aeroponic chamber. The separation of the dye from water at the apoplastic barriers caused accumulation of the dye in a given root segment. The dye accumulation was assumed to be proportional to water absorption by this portion of the root and its concentration was determined spectrometrically.

Tritium has been used for studies of water uptake by roots (Bishop and Dambrine 1995; Walker and Richardson, 1991). The isotope was applied to roots at different depths and xylem sap or water vapour captured from transpiring leaves were analyzed by scintillation counting. Since deuterium and tritium are water isotopes, they are ideal tracers for water movement studies. Unfortunately, there are no techniques allowing their use in microscopy.

The apoplastic and symplastic permeability of roots

The permeability of tissues to water must be distinguished from their permeability to solutes. Many investigators make direct inferences to water permeability while in fact they trace the movement of solutes, either as radioactive ions or chemically complex dyes. Laüchli (1976) questioned the use of K-fluorescein by Strugger (1939) as the dye may cross cellular membranes or not, depending on the pH. He criticized the use of heavy metal salts as tracers for water movement due to the large size of the particles. However, he also presented the study by Crowdy and Tanton (1970) as an example showing that apoplastic water transport in the epidermis of wheat (*Triticum*) leaves was confined to the cell walls, even though they used EDTA-Pb salt as a tracer.

The apoplast

The apoplast consists of a system of pores in cell walls, and cell wall surfaces outside the plasma membrane. Intercellular spaces, much larger in diameter than pores in cell walls are normally occupied by gases and may not form channels for water and solute movement, particularly since they are oriented longitudinally rather than radially (Clarkson, 1991). Different substances may be deposited onto or into cell walls affecting their permeability to different solutions (Laüchli, 1976). Cellulose microfibrils vary in diameter from 3-30 nm and are separated by spaces forming a three-dimensional lattice (Robards, 1970). The variation in microfibril arrangement ranging from regular to random, reported in the latter study, created a transport pathway of frequently changing directions and resistance to flow. Supposedly, small particles would be carried by the water moving in these channels (Clarkson, 1991). Carpita *et al.* (1979) found that the apoplast may impose a pore-size limitation to the transport of large molecules. Clarkson (1991) noted, that secretion of very large macromolecules (molecular weight of 100 000 and up) by roots deficient in phosphorus did not conform to the pore-size limit and that other, unknown apoplastic channels might exist.

Electrostatic effects of the components of apoplastic pathway on transport of solutes are of great importance. Cell walls are predominantly negatively charged due to the presence of disassociated carboxyl groups of galacturonic acid chains, pectins, chemicelluloses, cell-wall proteins, and phospholipids. Additionally, the charge of polar residues of the plasma-membrane bound proteins affects the space between the plasma-membrane and the facing cell wall surface (Laüchli, 1976; Clarkson 1991). The electrostatic effects limit the effective pore size for transport of anions. They are pushed away from the negatively charged pore surfaces and are excluded from entering small diameter pores (Clarkson, 1991). Cations are bound by the negatively charged sites (Laüchli, 1976). The charge of the plasma membrane surface is mixed and changes with pH, but the negative charges of headgroups of phospholipids bind several molecules of water each, creating a rigid coat obstructing the movement within the space between the plasma membrane and cell wall surface (Clarkson, 1991).

Strugger (1939) pioneered the use of dye tracers in studies of transport in plant tissues. Many studies have followed since. The experiments of Addoms (1946) employed polychromatic dyes. Similarly, Huisinga and Knijf (1974) studied the movement of safranin O and basic fucsin in roots of broad bean (*Vicia faba* L). They reported that these dyes penetrated the endodermis. However, Peterson *et al.* (1981) disputed that report suspecting that dye concentrations used by Huisinga and Knijf (1974) might have been toxic to the studied plants. Toxicity of tracer dyes

eliminates many polychromatic stains from use as tracers (O'Brien and Carr 1970). Fluorescent dyes are commonly used as tracers of the movement of aqueous solutions in plants due to their high detectability at low concentrations which reduces potential toxic effects of the dye (O'Brien and McCully, 1981).

Enstone and Peterson (1992b) tested apoplastic permeability of root apoplast in broad bean and onion. They demonstrated that the number and diameter of open xylem elements increased with the distance from the root tip. Moon *et al.* (1986) localized apoplastic barriers in mangrove roots by the lack of dye (Cellufluor) accumulation beyond the periderm in mature roots and the exodermis and endodermis of progressively younger regions of fine roots. They also demonstrated penetration of the dye through these barriers if they intentionally damaged the root causing discontinuity of these tissues. They showed that there was no apoplastic leakage through the endodermis at the sites of root junction in the studied species. The leakage at the sites where lateral roots penetrated the endodermis, apparently breaking the continuity of the Casparian band was reported in a number of species (Dumbroff and Peirson, 1971; Peterson *et al.* 1981). Another possible uncontrolled apoplastic route through the region where the Casparian band has not yet matured, somewhat above the root apex (Perry and Greenway, 1973), was shown to be not a major apoplastic route in mangrove (Moon *et al.* 1986).

Ashford *et al.* (1988) used Cellufluor to study apoplastic permeability of the mycorrhizal sheath in *Pisonia* and related their observations from the tracer study to the ultrastructural study of this mycorrhizal association. They found that in the regions near the root cap, where the sheath was still undifferentiated the tracer penetrated into the root epidermis. However, the differentiated fungal sheath contained electron dense extracellular material in the interhyphal spaces making the sheath impermeable to the tracer. This extracellular material apparently consisted of phenolic compounds.

Hanson *et al.* (1985) examined what proportion of the water flow through root systems of red pine (*P. resinosa*) seedlings passed to the xylem without ever crossing a semipermeable membrane. Moon *et al.* (1986) performed a similar study on gray mangrove (*Avicennia marina* [Forsk] Vierch) roots. The former study used hydroponically cultured plants. Moon *et al.* (1986) used plants remaining in soil-filled pots. In both cases a highly mobile fluorescent dye was applied at non-toxic concentration to the culture medium or as an aqueous solution to the soil. Roots were pressurized and exudate was collected at time intervals. The presence of the dye in the exudate was measured spectrophotometrically and expressed as percentage of its concentration in the applied solution. Both studies showed low presence of the dye in the xylem

exudate when the roots were undamaged. These results indicated the blockage of the entry of the apoplastic dye into the xylem of intact roots. Moon *et al.* (1986) also examined roots anatomically finding no evidence of leakage of the apoplastic dye at root junctions of gray mangrove.

The symplast

The development of the symplast concept has been attributed to Münch (1930) but its firm establishment in biology resulted from the studies of Arisz (1960; 1969). According to this concept, the symplast is the sum of cytoplasmic compartments contained within the plasma membranes of all living cells in a plant. Plasmodesmata provide the continuity of the symplast and the great significance of their functioning has been recognized (Gunning and Robards, 1976). There are several interpretations of the structure of plasmodesmata and their relationship with the endoplasmic reticulum. Clarkson (1991) presented the current view on the structure and function of plasmodesmata remarking that there was still considerable uncertainty about their function as variable valves controlling the effective surface area of exchange channels between cells. The studies on plasmodesmata have not been made on roots but it is believed that their functioning in roots may be the same as elsewhere in a plant (Clarkson 1991).

Plasmodesmata occupy less than 2% of the membrane area but they are more conductive of electrolytes than membrane surfaces (Overall and Gunning, 1982). Spanswick (1972) showed that the passage of electric current through plasmodesmata constituted up to 10% of the total electric current transfer between cells, much more than could be explained by their percentage in the membrane surface area. Terry and Robards (1987) used a small-molecule synthetic peptide marked with a fluorescent tracer to show that the effective exclusion limit of the plasmodesmata was about 3 nm. However, as noticed by Clarkson (1991), conclusions from studying the movement of labeled macromolecules in the symplast could be suspect, as similar molecular weight of different molecule-marker aggregates might not warrant the same mobility in the symplast. Further, the evidence from viral studies showed that viruses much larger than the size of the plasmodesmatal opening could pass from cell to cell (Weintraub *et al.* 1974; Gibbs, 1976). Olesen (1980) proposed that the size of the channel between the desmotubule and the membrane lining the plasmodesmatal pore could vary in size resulting in sphincter-like properties of plasmodesmata. Some studies provided thorough evaluation of plasmodesmatal frequencies in regions expected to carry a particularly heavy load of transport activities (Gunning and Hughes,

1976; Thomson and Liu, 1967). While these studies partially confirmed the suspected relationship between plasmodesmata numbers and the extent of symplastic transport, this relationship was not found to be straight forward. Robards *et al.* (1973) found 2.5 times as many plasmodesmata exit on the pericycle side of the radial endodermal wall of barley roots than on the cortical side. Clarkson (1991) speculated that the flux leaving the endodermal plasmodesmata could increase due to the junction of symplastic and apoplastic flows.

A truly symplastic dye, as a truly apoplastic dye, cannot cross the plasma membrane so its movement is confined only to the compartment it is applied to. The use of microinjected symplastic dyes in plant tissues is relatively new and the method suffers from numerous problems. The major one is the molecular exclusion limit which varies from organ to organ, from tissue to tissue, and perhaps even within the same tissue (Erwee and Goodwin, 1985). These authors discussed the plant's symplast as a non-uniform means of communication consisting of numerous domains which could differ in their molecular exclusion limit. Such limits have been defined for only a few plants or their organs (Goodwin, 1981, 1983, Erwee and Goodwin, 1983; 1985; Tucker, 1982).

FACTORS THAT INFLUENCE ROOT GROWTH AND ROOT SYSTEM DEVELOPMENT

Root systems of forest trees develop in response to the interaction of complex genetic, physiological, and environmental factors. The limiting influences of individual factors, that can be meaningfully assessed, are modified by these complicated interrelationships obscuring the understanding of the overall regulation of root growth and development (Sutton 1991). Findings from research on roots and root systems of agricultural plants cannot be extended directly to roots of forest trees due to fundamental differences between forestry and agriculture (Tamm, 1950). In contrast to many agricultural crops, root systems of trees, apart from the ephemeral roots (Fogel, 1983), are of long-lasting perennial character.

Barlow (1983) categorized interacting factors influencing root growth and development as: 1) environmental, including physical and chemical characteristics of the external environment, 2) internal physiological correlations dependent on the physiological state of the plant, its maturity, genetic makeup, and the position of the root on a plant, and 3) internal physiological determinants closely related to the anatomical (cell wall constitution and extensibility, suberization) and water-relation dependent (permeability, turgor) characters. Sutton (1991) subdivided the factors

influencing root growth differently. He separated them into soil factors and soil-plant interface factors resulting from the interaction between the plant and soil. The brief review presented below follows Sutton's (1991) outline in a condensed format.

Soil factors

Physical soil characteristics influence root development by presenting physical obstacles to root growth and by influencing factors such soil temperature, aeration, and moisture (Sutton, 1991).

Physical properties of the soil

These include characteristics such as soil bulk density, compaction, texture, structure, porosity, and soil strength. These characteristics are closely interrelated and, in a general way, determined by soil particle sizes (the texture), their aggregation (the structure), and, consequently, the space occupied by voids and pores. Bulk density is the measure of particle density per unit of soil volume, thus it is indicative of the space occupied by the pores (Williams, 1971). These characteristics, in turn, determine soil strength (Letey, 1985) defined as its ability to resist an applied force (Taylor, 1974). The physical/mechanical soil characteristics are the determinants of natural soil compaction (understood as a product of natural processes) and its responses to external compacting forces (such as those imposed by the use of heavy equipment) that alter pore sizes and distribution increasing mechanical impedance (Sutton, 1991).

Apart from modifying soil environment, the mechanical properties of the soil can directly and dramatically influence root system morphology. Detailed accounts of such effects can be found in reviews by Sutton (1991), Rogers and Head (1969); Russell (1961), and Taylor *et al.* (1972), in more general terms and in particular reference to soil compaction Bennie (1991), to mention only some. Without getting into details, the general trend for direct modifications of root growth by soil mechanical properties is a decrease in length of individual roots and increased branching of root systems with increasing soil resistance to penetration by the roots. In radiata pine, for example, fewer and shorter first order laterals were produced at high soil bulk density while the number of second order laterals increased. The reverse occurred at low bulk density (Sands and Bowen, 1978; Nambiar, 1980). The depth of rooting can be restricted and the direction of root growth affected by increasing root extension and proliferation into soil portions offering lesser obstruction to root growth. Soil compaction can reduce mycorrhizal infection of roots, as was reported for corn (Entry *et al.* 1996).

Soil temperature

Gregory (1983) found shoot meristem temperature of pearl millet more closely related to root development than soil temperature at a 5 cm depth. However, others state clearly that soil temperature has a profound effect on root growth processes (cell division and cell expansion) reflecting on root initiation, branching, root length and diameter, as well as on the growth direction, root senescence and suberization, and root turnover (Kaspar and Bland, 1992).

In *Pinus radiata* (Bowen 1970; Nambiar *et al.* 1979) and *P. resinosa* (Wilcox and Ganmore-Neumann, 1974; Andersen *et al.* 1986) increased root temperatures stimulated root elongation and reduced root system branching. Exactly the reverse was reported for *P. echinata* (Sword and Brissette, 1993). While the effects of root zone temperature on root growth and development may be species dependent, it should be noted that the applied temperature treatments (plus other experimental conditions) as well as the methods of estimating the responses differed in these studies. Root length is viewed as a more sensitive indicator of soil temperature effects on root growth than root dry weight (Bowen, 1991). This author did not dispute the predominating view that increases in soil temperature result in increased production of primary root axes. However, he stated that soil temperature effects on the production of lateral roots cannot be easily generalized as the available reports are often conflicting and that the reported increases in lateral root production may reflect generally greater root growth at higher temperatures. Bowen (1991) stated that, by conventional wisdom, root diameter was inversely related to soil temperature.

Soil temperature is a factor regulating the proliferation of new roots after planting. Stone and Shubert (1959) reported the most dramatic increase in proliferation and length of roots of *Pinus ponderosa* Dougl. when soil temperature increased from 10 to 15°C. In *P. radiata* this temperature ranged between 11-14°C, (Nambiar, 1980). Seedling age played an important role in modifying the development of first order laterals in *P. radiata* - while the response at the age of 3 weeks was dramatic (Bowen, 1970), it was negligible in 8 month old seedlings (Nambiar *et al.* 1979).

Soil temperature extremes may be limiting to root growth in different geographic locales in different ways. Sutton (1991) cited Bilan (1968) who had shown high soil temperature in Texas resulted in virtual absence of laterals in the uppermost 5 cm of the soil. Low soil temperature was found to limit root growth and rooting depth in many boreal forest soils (Sutton, 1969).

Balisky and Burton (1995) suggested low soil temperature as the main cause of poor rooting at high elevation sites in British Columbia. Nambiar *et al.* (1979) found the extent of root suberization inversely related to soil temperature. Bowen (1991) suggested that this may have strong implication for water and nutrient uptake as well as for the susceptibility to diseases. However, this could be due to reduced rates of root elongation at low temperature rather than enhanced suberization. Reviews of the literature on the subject of soil temperature effects on root growth, whether of general (Bowen, 1991; Kaspar and Bland, 1992) or forestry-specific (Sutton, 1991) character, indicate a strong interaction between soil temperature and many other soil factors such as moisture, aeration, structure, mineral composition, nutritional status, and soil microorganisms.

Theodorou and Bowen (1971) found mycorrhizal fungi grow best between 18-29° C under controlled conditions but these temperatures did not correspond to those typically found in soils under ambient conditions. Wilcox and Ganmore-Newmann (1974) stated that the trend for decreased branching and increased root elongation under higher root zone temperatures was the same for infected and uninfected roots. Bowen (1991) remarked that we tend to forget that mycorrhizae involve two organisms which may not have the same response to the limiting factor. This would enable the plant to compensate for the losses in length or function of its roots resulting from deleterious factors if these factors do not affect the associated fungus.

Soil moisture

It is generally assumed that root systems play a decisive role in plant adaptation to moisture deficit (Vartanian, 1994). This author cited numerous studies reporting stimulated lateral branching of root systems in response to moisture deficit. Growth of roots can be less restricted than shoot growth or it can be stimulated by soil drying (Sharp, 1990; Sharp and Davies, 1979; Jupp and Newman, 1987). Sword and Brissette (1993) reported increased lateral branching of shortleaf pine roots under drought conditions. Krasowski and Owens (1991) reported an increased rate of root dry weight accumulation in container-grown seedlings of *Thuja plicata* J. Donn. grown under moderate water stress, compared to well watered seedlings, but also an overall decrease in root and shoot dry weight under prolonged drought and under drought combined with short day treatments. Nambiar *et al.* (1979) discussed an interrelationship between moisture stress and soil temperature effects on growth of new roots (termed root regeneration). They stated that low soil temperature lowered leaf water potential, possibly through restricted water uptake. This reduced photosynthesis and the amount of photosynthate available to roots resulting in poor root regeneration. Similar results were obtained on Douglas fir (*Pseudotsuga*

menziesii Mirb. Franco) seedlings (Lopushinsky and Kaufmann, 1984). Another type of moisture influence on roots and through the roots on the entire plant is that caused by excess moisture. Permanently saturated soils are not accessible for roots of most tree species (Sutton, 1991) but even transient flooding and water logging can result in severe damage to root systems or to the whole plant. This is due to reduction in oxygen availability to roots slowing their respiration and reducing or stopping growth (Jackson and Drew, 1984) by affecting the process of cell division rather than cell enlargement (Lopez-Saez *et al.* 1969).

Soil water status influences the plant by changing its growth rates and/or developmental patterns. It also influences the physical and mechanical soil properties such as nutrition, aeration, mechanical impedance, and temperature (Eavis and Payne 1969). Soil hydraulic conductivity is greatly influenced by soil water content (Gardner and Ehlig, 1962). Soil water potential is more important to plant growth than soil water content (Letey, 1985). Thus, it can be said that soil moisture influences plant growth, including root growth, either directly by altering physiological processes or indirectly via interactions with other soil factors (Sutton, 1991).

Roots are capable of some physiological adjustment to low soil moisture by accumulating organic solutes. This ability to adjust osmotically differs in different species. Roots of jack pine (*P. banksiana*) were found to be more capable of osmotic adjustment than roots of white spruce (Koppelaar *et al.* 1991). These authors postulated that osmotic adjustment is the rapid-type of response to soil water deficit that prolongs turgor and sustains root growth and soil exploration for some time. Roots are also capable of non-hydraulic (that is, not caused by reduced water supply to shoots) root-to-shoot signaling of declining soil water availability (Ebel *et al.* 1994; Gowing *et al.* 1993; Tardieu, 1993; Tardieu and Davies 1993) resulting in closure of stomata and suspension of leaf expansion (Zhang and Davies, 1990). Such adjustments were reduced in corn with mycorrhizal roots (Augé *et al.* 1994).

Soil aeration

Many plants suffer or die under conditions of excess soil moisture. Soil oxygen is rapidly depleted by respiration of roots and soil microorganisms (Drew and Stolzy, 1991). Limitation to aerobic respiration affects metabolism and slows or stops growth of roots; thus, the most metabolically active parts near the root tip are the first to be found below critical oxygen pressure (Saglio *et al.* 1984). Not only a plant but also the soil responds to oxygen limitation. Accumulation of reduced compounds in the soil increases with the duration of flooding and with

increasing soil temperature reaching levels that can be toxic to plants (Drew and Lynch, 1980; Drew and Stolzy, 1991).

Some species are capable of metabolic, morphological, and anatomical adaptations to low soil oxygen levels and these may even prosper in frequently flooded areas. As remarked by Drew and Stolzy (1991), a distinction must be made between the tolerance to flooding and to anoxia because species with roots morphologically or anatomically specialized to withstand floods supply oxygen to flooded parts of the root system through these adaptations. The tolerance to anoxia is based on metabolic adaptations that enable roots to function anaerobically for some time (Jackson and Drew, 1984; Drew 1988). Structural changes caused by flooding are triggered by ethylene production and successive hormonal signals to other roots and to shoots (Jackson and Drew, 1984; Drew and Stolzy, 1991). Lenticels develop on roots and at the stem base improving air access to the roots (Hook 1984). Even more specialized are wetland species with knee-roots and pneumatophores facilitating gas exchange (Gill, 1970; Curran *et al.* 1986). The development of cortical air spaces - aerenchyma (Drew *et al.* 1979; Sutton and Tinus, 1983) allows for longitudinal air transport in the roots of swamp-inhabiting species. Continuous pathway of such air spaces was found in slash pine (*Pinus elliottii* Engelm.) growing on permanently saturated soils (Sutton, 1991).

Carbon dioxide concentration in the soil air can be many times higher than that of the atmospheric air (Hillel, 1980). The effects of this gas on the roots are not well understood (Sutton, 1991). Very high levels of CO₂ in the soil can severely inhibit root growth but Pearson (1966) found such levels to occur rarely except after excessive addition of organic matter to the soil (Sutton, 1991).

Soil nutrients

Soil nutrients originate from the solid organic and inorganic material of the soil. Only a fraction of soil nutrients is dissolved in the soil solution at a given time but this is the only state at which nutrients can be acquired by roots (Jungk, 1991). Different elements vary in their chemical properties, mobility in the soil, and the requirement for uptake by plants (Stone 1983). Dynamics of nutrient movement in the soil and soil/plant root interface are not the subject of this review. It should be remarked that the availability of nutrients in the soil, their mobility, and uptake preferences under certain soil conditions have strong effects on plant growth and health. These

subjects have been reviewed many times (Olsen and Kemper, 1968; Nye and Tinker, 1977; Barber, 1984; Barber and Silberbush, 1984; Jungk, 1991; Marschner, 1991).

While plant nutritional status affects root growth and root morphology, the uptake of nutrients from the soil is itself dependent, among other factors, on the morphology and growth of roots. This dependency of nutrient acquisition on the size of the root system is especially significant if the mobility of soil nutrients is low (Jungk, 1991). Thin roots are more efficient in nutrient absorption than thick roots as the ratio of root surface area to the volume of exploitable soil decreases with root diameter increase (Barber, 1984). Increase in root length may also contribute to root surface area increase; thus root length is important to plant nutrient supply (Barber and Silberbush, 1984). Root hair proliferation can increase root soil penetration significantly and this is believed important to the acquisition of such immobile nutrients as phosphorus (Foehse *et al.* 1988). On the other hand, high concentration of P and nitrate caused a decrease in the number and length of root hairs (Hendriks *et al.* 1981). Mycorrhizae are known to supply plants with P, particularly under low levels of soil P (Tinker, 1984). High P content would be then expected to lower the need for abundant mycorrhizae. Indeed, Nambiar (1980) reported reduced mycorrhizal colonization of *P. radiata* roots with increasing soil fertility.

Nambiar (1980) reported an increase in shoot to root ratio of seedlings growing under high fertility conditions. The number and length of first-order laterals were unaffected by soil fertility but the length of higher order lateral roots increased. Nitrogen availability was found to be strongly related to the morphology of root systems. High N resulted in short, thick roots while low N induced production of long thin roots (Bosemark, 1954). However, an increase in root diameter observed by Coutts and Philipson (1976) in seedlings of Sitka spruce (*P. sitchensis*) with split root systems occurred only in roots from the N-rich compartment and not in those of the same plant remaining under low N supply.

The concentration of root growth, particularly that of fine roots, in nutrient-rich parts of the soil is another modification to root morphology in response to nutrient distribution (St. John *et al.* 1983). Balisky *et al.* (1995) criticized the type of planting stock produced and planting practices applied in British Columbia for neglecting the forest floor life. In the opinion of Balisky *et al.* (1995), reforestation practices in British Columbia favoured root establishment in the mineral soil and denied planted seedlings the access to organic layers. This could retard their growth and hinder survival. Indeed, organic soils are nutrient-rich sites of high nitrogen content resulting from organic matter turnover (McColl and Powers, 1984). Sutton (1969) reported the concentration of fine roots in the interface between the forest floor and mineral soil which was

cited by Balisky *et al.* (1995) to support their opinion. However, Sutton (1991) cited Pritchett and Fisher, (1987) who stated that the occupation of forest by roots was often temporary due to rapid drying of this soil layer on exposed sites. Therefore, the recommendations of Balisky *et al.* (1993) may be applicable to shaded forest environments such as those obtained under partial cutting rather than to planting on clearcuts or in cleared patches.

Soil organisms

Sutton (1991) remarked that soil organisms are usually ignored in laboratory experiments. He cited a number of publications indicative of the importance of soil organisms to the modification of root growth and function directly or indirectly - through their effects on the soil. Soil organisms can improve soil structure and porosity (Jenny, 1980). Among various microorganisms inhabiting the soil, earthworms are particularly beneficial to plants as they increase rooting depth, loosen the soil, secrete growth-promoting substances (Lee, 1985), and acidify the soil through release of respiratory carbon dioxide which promotes solubilization of mineral nutrients (Sutton, 1991).

While the microorganisms do not have to live close to the roots (Sutton, 1991), many microorganisms are permanently inhabiting root surfaces and the soil near the roots. Thus, the rhizosphere is a dynamic environment whose chemical and microbial characteristics differ significantly from those of the soil outside of the rhizosphere (Alexander, 1961; Bowen and Rovira, 1991). Inorganic composition of the soil in the rhizosphere is affected by selective uptake of ions by the roots and their efflux from the roots. Rhizosphere content of immobile ions, phosphate in particular, is dramatically affected by the root uptake (Lewis and Quirk, 1967). Microbial populations in the rhizosphere thrive especially in organic substances at the root/soil interface. These include root exudates (passively released from living cells), root secretions (actively released from roots), lysates, plant mucilages (mainly polysaccharides produced by roots and by bacteria), and mucigel (gelatinous combination of mucigel, bacteria, and their metabolic products) (Rovira *et al.* 1979).

Plants can be harmed by soil microorganisms through disease-causing rhizobacteria, the production and release of cyanide, nutrient unavailability, and biocontrol of beneficial microorganisms. Positive effects of associations between roots and soil microorganisms include nitrogen fixation, mycorrhizae, biocontrol of harmful organisms, plant-growth promotion by the release of growth stimulators, and improvements in the availability of nutrients (Bowen and

Rovira, 1991). Assimilation of N is viewed as the second (after photosynthesis) most important process for plant growth and development (Vance, 1991). For many plants, improved N availability results directly from symbiotic associations with N-fixing bacteria such as *Rhizobium* and *Frankia* producing nodules on the roots (Nutman, 1965). In forest tree and shrub species such association occur in Betulaceae (Vance 1991). Symbiotic associations with N-fixing bacteria contribute to the overall increase in the available soil N, benefiting other plants not capable of such associations (Hendrickson and Burgess, 1989). There are also numerous species of non-symbiotic N-fixing microorganisms that live in the soil. *Azotobacter* and *Beijerinckia* are the two long-known species of ecological importance but many others have been discovered (Kapulnik, 1991a).

An important group of microorganisms associated with roots are plant-growth-promoting rhizobacteria (PGPR), represented by genera such as *Arthrobacter*, *Bacillus*, *Enterobacter*, *Serratia*, and *Pseudomonas* (Kapulnik, 1991b). These organisms stimulate root hair production improving water and nutrient uptake (Okon, *et al.* 1988). Some species of these bacteria release disease-preventing antibiotics; of these *P. fluorescens* is probably best known (Howell and Stipanovic, 1979). Other beneficial effects of these bacteria include the production of iron-transport enhancing compounds (siderophores) (Neilands and Leong, 1986), and reduction of cyanide release by other microorganisms (Schippers *et al.* 1987).

Roots have to cope with the presence of other plant's roots in the soil. Exudations and leaching from neighbouring roots can affect a plant positively or negatively (Curl and Truelove, 1986). Competition for nutrients in the soil may severely limit root growth, and more needs to be known about resource sharing by roots of different species in different soil types, and at different times of the year (Sutton, 1991).

Plant factors

Root system morphology expresses a combination of influences exerted by soil properties and those of the plant itself. The latter include genetic factors and those related to the physiological state of the plant (Sutton, 1991). Theodorou and Bowen (1993) reported significant family to family variation in root morphology (expressed by the number and length of lateral roots of different order) and in the uptake of P and N in *P. radiata* grown in different soils. Theodorou *et al.* (1991a) found differences in sensitivity of different families of *P. radiata* to high soil strength and low aeration. For the same species, these authors concluded that particular genotypes

displayed different rooting characteristics while on different sites. The rooting characteristics included the short root density, percent of mycorrhizae, fine root length, and the number of active (white) roots (Theodorou *et al.* 1991b). The family x site interaction was further modified by seasonal influences. Generally, genetic effects on root morphology are not as strong as those imposed on the above-ground parts of the plant and not as influential as those caused by soil conditions (Sutton, 1991).

The importance of the relationship between shoot and root growth has long been recognized but the understanding of its nature is still incomplete. In temperate perennials, such as many conifers, the general seasonal pattern of root growth is similar and characterized by spring and fall flushes of growth interrupted by a period of low growth activity, even though individual roots can show cyclic growth surges at different times of the growing season (Wilcox, 1954; 1968; Johnson-Flanagan and Owens 1985a, b). It has been postulated that the prominent decline in root elongation results from preferential photosynthate partitioning to shoots during the period of rapid shoot elongation (Stone *et al.* 1962; Ritchie and Dunlop, 1980). Root respiration, an indicator of metabolic activity, was found to be related not only to environmental conditions under which white spruce seedlings were kept, but also to the stage of shoot development (Johnson-Flanagan and Owens, 1986). Fielder and Owens (1989) not only confirmed this in seedlings of Douglas fir, but found respiratory patterns to be different between coastal and interior varieties of Douglas fir indicating an endogenous control of this process in addition to environmental influences.

All plant species tend to maintain a certain shoot to root biomass ratio (Klepper, 1991). The approximate percent of total biomass allocated to roots is lowest in tropical evergreens (10%) and deciduous temperate trees (20%). It is the highest in desert and alpine plants (80-90%). Boreal evergreen species tend to allocate about 25% of biomass to roots (Schulze, 1983). This homeostasis is an expression of functional balance that is not easily disturbed by environmental conditions and cultural practices (Brouwer and DeWit, 1969). Roots and shoots are rarely at the same temperature (Klepper, 1991). Air temperature affects photosynthesis; thus, it is a factor important to root growth and development through its effects on the availability of photosynthate for the growth of roots (Torrey, 1986; Coutts, 1987; van den Driessche, 1987). However, it is light that is most critical for root growth and development, again through the availability of photosynthetically fixed carbon for the growth of roots. Increases in root elongation with increasing photosynthetic photon flux density (PPDF) have been demonstrated for many plants (Barney, 1951; Eliasson, 1968; Aguirrezabal *et al.* 1994). The fact that increased carbon availability stimulates root growth is further supported by experiments supplementing carbon

dioxide to various agricultural plants (Chaudhuri *et al.* 1990; Del Castillo *et al.* 1989; Rogers *et al.* 1992). Bethlenflavay and Pacovsky (1983) stated that less root growth under low light conditions is due to greater investment of carbon to leaf expansion. The importance of light to the growth of new roots in conifers has been documented (Abod *et al.*, 1979; van den Driessche, 1978; 1987). However, van den Driessche (1991) wondered whether the dependency of conifer root growth on light was related only to photosynthesis or whether it involved some other light-dependent growth promoter. The reason for this doubt was the poor relationship between measured photosynthesis and production of new roots, reported by some researchers (Zaerr and Lavender, 1974; Abod *et al.* 1979; van den Driessche 1978). Nevertheless, van den Driessche (1991) demonstrated poor production of new roots in Douglas fir under low CO₂, this effect being even greater after girdling. Thus, he concluded that light effects on the production of new roots appeared to be related only to photosynthesis.

Reports on the effects of light on mycorrhizae have been conflicting. Some studies found light stimulating to mycorrhizae (Hayman, 1974; Johnson, 1976) other did not (Furlan and Fortin, 1977; Thompson *et al.* 1990). These discrepancies may be explained by differences in responses of different species of fungi to different light regimes (Borges and Chaney 1993). Kinuzawa (1965) reported that the extent of mycorrhizal infections in *Pinus densiflora* Siebold & Zucc could be related to light intensity rather than to day length. Benefits of mycorrhizae to the host plant are usually reduced under low light (Bethlenflavay and Pacovsky, 1983).

The non-hydraulic signaling from roots to shoots, discussed previously, demonstrates that drought conditions, even when experienced only by a portion of the root system, can result in modification of stomatal behaviour and the rate of leaf expansion. Another example of root to shoot signalling is that resulting from mechanical stresses to roots (Davies and Zhang, 1991) and root confinement (Ternes *et al.* 1994). Both cited reports showed reduced growth of the above-ground portion of affected plants and suggested that abscisic acid (ABA) could be the chemical signal causing this growth inhibition. Elevated levels of ABA also resulted from flooding the roots and this increase correlated in time with changes in leaf stomatal conductance (Zhang and Davies, 1987)

Regulation of growth rate, pattern and distribution in plants is mediated via changes in the concentration of plant growth regulating substances (Sutton, 1991). All five major groups of plant growth regulators (PGR) are synthesized in roots, their site of synthesis is specific and different from the site on which they act (Itai and Birnbaum, 1991). PGR's never act separately *in vivo* (Pilet, 1991). The regulation of plant growth by PGR's is through their complex

interactions that involve changes in levels of one regulator impacting on levels of the others, simultaneous changes to a number of PGR's in response to environmental stimuli, and simultaneous changes in PGR's in response to developmental processes (Itai and Birnbaum, 1991). This complicates the understanding of the mechanisms of growth regulation by PGR's as exemplified by the changing concepts of regulation of gravireaction in roots. The theory of auxin control of this process survived unchallenged for nearly 50 years (Pilet, 1991). The view that gravireaction was regulated by the balance between stimulators (auxin) and inhibitors (ABA) did not survive that long. The current (and sometimes questioned) view not only considers the involvement of auxin and ABA but also the possible regulatory roles of other PGR's, the involvement of calcium in gravitropic signal transduction, and the regulation of auxin transport by ATPase-driven proton pumping (Pilet, 1991).

The role of PGR's in the regulation of root extension, radial growth, and initiation of lateral roots is obscure. According to Itai and Birnbaum (1991), the common assumptions about stimulation of lateral root development by cytokinins and inhibition of root elongation by auxin are currently under review. Charlton (1983) inhibited elongation of laterals by applying high levels of indoleacetic acid (IAA). However, from discussion presented by Charlton (1991) it is obvious that effects of exogenously applied hormones on lateral root initiation cannot be understood as representative of their endogenous mode of action. The balance of endogenous levels of auxins to cytokinins has been suggested as regulatory to lateral root initiation (Barlow, 1986; Morris, 1986) but it is not known how long this view will persist. The role of PGR's in regulating cambial activity of roots is even more obscure. For the lack of information, its role in roots is inferred from research on stem cambium.

CHAPTER 3. THE DEVELOPMENT OF WATER CONDUCTING CAPACITY IN THE LONG AND SHORT ROOTS OF *PICEA GLAUCA* [(MOENCH) VOSS].

ABSTRACT

Perceptible variation in the elongation of individual roots in *Picea glauca* (Moench [Voss]) seedlings occurred in late spring, during the third month after germination. The number of non-elongating roots increased during summer together with a decline in the occurrence of root hairs and an increasing development of mycorrhizae. After a surge of root elongation in the fall, root growth stopped in October. The development of primary tissues in long roots of *P. glauca* in relation to the distance from the root tip varied, apparently due to differences in rates of root elongation of different roots. In non-elongating roots, secondary-state endodermis extended to the metacutis surrounding the root apex. *Casparian bands* developed in long roots shortly before the maturation of the first protoxylem. The process of suberization of inner faces in endodermal cells began from their inner tangential walls but rapidly spread to all other cell walls. The tight adherence of the plasma membrane to Casparian bands was relaxed during the suberization process. A prominent decline in the amount of phenolics in endodermal cells and the abundance of cisternal endoplasmic reticulum suggested the utilization of phenolics in the development of suberin lamellae and the involvement of the endoplasmic reticulum in this process. Few cytoplasmic vesicles were observed indicating that the delivery of the material for the deposition onto cell walls involved a non-vesicular transport mechanism. Plasmodesmata were not observed in radial walls at any time throughout the differentiation and development of the endodermis. They occurred sporadically in tangential cell walls, inner rather than outer, and their remnants could be found in suberin layers, although the suberized cells appeared dead. Passage cells remained alive, possessing only Casparian bands and no lamellate suberin layers. In short roots, the ultrastructural characteristics of the endodermal development were as in long roots but Casparian bands occurred only 2-4 cells above the root apex of elongating roots followed shortly by the secondary-state development. In non-elongating short roots the secondary endodermis also joined the metacutis by a bridge-like structure. In both long and short roots the endodermis did not develop beyond the secondary state and persisted as a continuous sheath until it was ruptured during early secondary growth. Short roots did not undergo secondary growth and their endodermis was continuous with that of the parent root.

Most short and some long laterals of *P. glauca* formed mycorrhizal associations with fungi from *Ascomycetes* and *Basidiomycetes*. In long roots, the Hartig net occurred only in brown regions, where the secondary-state endodermis had developed. In short roots, the Hartig net was never present in the apical region surrounded by the metacutis. The metacutis was impermeable to an apoplastic tracer Sulforhodamine G and to the symplastic tracer fluorescein diacetate. Root tips of actively growing roots were almost impermeable to the apoplastic dye, especially the meristematic region. The movement of the apoplastic dye into the root was stopped at the primary and secondary-state endodermis. Some apoplastic dye entered into the developing stele of long roots but not of short roots through a region of elongation, acropetally from the primary-state endodermis. The symplastic dye did not migrate to the apical meristem region but was present in the subapical region. The penetration of the symplastic dye into the stele declined where the secondary-state endodermis was present. The symplastic dye poorly penetrated surface layers of more mature root regions. The symplastic dye was observed in passage cells of the secondary-state endodermis. The plasma membrane - cell wall - plasma membrane appeared to be the main route for the transport of solutes into the xylem of *P. glauca* roots.

INTRODUCTION

The diameter of the largest open tracheary element(s) has been considered the most important measurement characterizing hydraulic conductance of roots (McCully and Canny, 1988). The lack of open metaxylem elements up to 30 cm above the root apex of axial maize roots (St. Aubin *et al.* 1986) led McCully and Canny (1988) to question the common notion that most water absorption from the soil occurs in regions proximal to the root apex. Branch roots of maize became a subject of morphological (Varney *et al.* 1991) and anatomical Wenzel *et al.* (1989) studies as these roots were believed to contribute in major way to maize water transport. Roots of monocotyledons are morphologically and anatomically different from woody conifer roots. Nevertheless, the remark of Wenzel *et al.* (1989) that branch roots have been neglected in the investigations of root structure applies to conifer roots as well. The development of water conducting capacity, understood as the differentiation of primary xylem elements, the transition from the primary to secondary growth, and the development of the endodermis relative to that of the xylem has not been frequently studied in short laterals of conifers. Differentiation of precursory xylem and phloem and variation in the configuration of primary xylem of *Libocedrus decurrens* long laterals were described by Wilcox (1962a; 1962b). It was also Wilcox (1964) who described in detail the pattern of differentiation and maturation of primary xylem in long lateral roots of *Pinus resinosa*. A rarely cited study of Rypáček *et al.* (1976) briefly described the formation of the vascular cylinder in roots of *Abies alba* Mill.

Anatomical studies of conifer roots rarely ventured beyond root tips of long laterals forming major axes. Most anatomical studies on conifer roots have focused on apical meristems and the origin of primary tissues (Allen, 1947; Pillai, 1964; Bogar and Smith, 1965; Wilcox, 1954; 1962a; 1968b). Since root growth has been invariably synonymous with root elongation, structural changes associated with periods of root elongation or non - elongation have also been studied (Wilcox, 1954a; 1968; Leshem 1970; 1974; Johnson-Flanagan and Owens, 1985a). The development of long roots of container-grown white spruce was described by Johnson-Flanagan and Owens (1985a). That study was performed on potted seedlings during their second year of growth and reported the cyclic nature of root growth and the existence of distinct morphological types of long lateral roots. The preponderance of each of these types varied seasonally and inferences were made from the morphological characteristics about their elongation rates and functional role. The endodermis was briefly described for elongating and root hair-bearing roots and for brown, non-elongating roots but no Casparian bands were shown. The only electron

microscopic demonstration of the Casparian band in long lateral roots of a conifer is probably that by Jorns (1987).

Studies of conifer short roots have usually focused on mycorrhizal associations between these roots and different fungi (Kottke and Oberwinkler, 1988; Massicotte *et al.* 1989; Scales and Peterson, 1991a; b). Studies of the relationship between the differentiation of the endodermis and mycorrhizal development were made on short lateral roots of *Picea abies* and *Larix decidua* (Kottke and Oberwinkler, 1990) and on rootlets of *Pinus sylvestris* (Warmbrodt and Eschrich, 1985). These reports provided useful information about the anatomy of these roots; however, the studied plants were grown in sterile cultures rather than in the soil.

The present study was undertaken to describe the development of the endodermis in long and short branch roots of *Picea glauca* grown in unsterilized soil media. The state of the endodermis was related to the developmental state of the adjacent xylem in these roots at different times during the growing season. The apoplastic and symplastic permeability of the roots were tested with fluorescent tracers in elongating and brown, non-elongating roots. Although the study was not intended to describe details of ectomycorrhizae, nearly all short branch roots sampled for the study were mycorrhizal and a brief description of their anatomy was necessary.

MATERIALS AND METHODS

Plant material and sampling

Stratified seeds of a single white spruce seed source native to central British Columbia were sown in early March, 1996 into Spencer Lamaire⁴ containers ranging from small to very large in size, filled with a mix of peat moss and vermiculite. Several hundred seedlings were obtained. Seedlings were grown in a greenhouse equipped with an overhead watering system coupled with fertilizer injector providing adequate moisture and nutrition throughout the growing season.

Sampling for microscopic studies was made monthly from early May until November, choosing 4-5 random plants from containers of progressively increasing size. One sample was taken from plants stored frozen (-1°) from November until late February, then thawed, potted, and placed in a warm growth chamber for five days to reactivate root elongation. Root segments were sampled from the tap root, the first and the second order long laterals from various distances on the roots. One root of each type was sampled from each seedling. The entire root tip portion, up to 5 mm long, was excised from each sampled root. Then, segments of 2-5 mm long were taken from distances beginning at 6, 10, 15, 25, and 45 mm above the tip. Distal ends of sampled root segments were cut obliquely to mark segment orientation and to allow determination of distances from the tip during sectioning. Short branch roots were sampled intact with a small portion of the parent root at the junction or, if the roots were longer than approximately 2 -3 mm, only a short segment that included the tip was sampled.

Preparation of specimens for microscopy

Three times during the 1996 growing season (in May, July, and October) the same sampling procedure as outlined above was followed to collect specimens for paraffin embedding. These specimens were fixed in formaline-acetic acid-alcohol (FAA) (Sass, 1958), run through Johansen's (1940) tertiary-butyl alcohol dehydration procedure and embedded in Tissue Prep. Longitudinal and transverse sections were cut at 6 μ m with a rotary microtome and stained with safranin and hematoxylin.

⁴Spencer Lamaire Ltd., Edmonton, Alberta

For transmission electron microscopy, immediately after excising root segments with a sharp, thin razor blade, the segments were dropped into 4% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2) with 0.15 M sucrose. The immersed segments were further cut approximately in half longitudinally or a radial segment was sliced off on one side to improve the penetration of chemicals into the specimens. Glutaraldehyde fixation continued overnight at +4°. Fixation under mild vacuum was tried but it usually separated the cortex from the stele at the endodermis and the procedure was discontinued. Specimens were next washed for 2 hours in four changes of buffer and sucrose mix in which the fixative was replaced with distilled water. Postfixation in 1% osmium tetroxide in the same buffer and sucrose mix was carried out at room temperature for 1 h and for an additional 1 h in the refrigerator. After four rinses with the buffer over 12 h at 4° C the specimens were slowly dehydrated in an ethanol series. Specimens were gradually infiltrated with a low viscosity epoxy resin (Spurr, 1969) dissolved in propylene oxide over several days, then the solvent was replaced by pure resin that was cured at 60° overnight.

Semi - thin and ultra-thin sections were cut with glass and diamond knives, respectively, on a Reichert UM ultramicrotome. Semi-thin sections were stained with Richardson's stain (Richardson *et al.* 1960). Ultra-thin sections were stained for 1.5 h with 2% uranyl acetate, then for 12 min in 0.2% lead citrate (Reynolds, 1963) and viewed on a Hitachi electron microscope.

Histochemistry

Cryostat - sectioned, unfixed material, paraffin, and resin embedded specimens were used for histochemistry, as called for in particular procedures. Staining for lipids with Sudan black B was done according to Bronner (1975), the PAS Schiff's reaction for polysaccharides, and ferric-ferricyanide staining for phenolics were made as described by Clark (1981). A fluorescent berberine-aniline blue staining method of Brundrett *et al.* (1988) was used for suberin and lignin. Images of stained specimens were compared to unstained controls to assess the degree of natural fluorescence.

Apoplastic and symplastic tracers

Sulforhodamine G and fluorescein diacetate (FDA) (Sigma) were used as apoplastic and symplastic tracers, respectively. Toxicity tests were performed using ten seedlings per tested

concentration of Sulforhodamine G (0.02%; 0.05%, and 0.1% w/v were tested).

Sulforhodamine G was dissolved in tap water, pH was adjusted to 5.8. FDA stock solution was prepared by dissolving 0.1g of the fluorochrome in 50 ml of acetone. Then, the stock solution was added by drops into 2000 ml of water until saturation. Since only somewhat less than half of the prepared stock solution could be added, the concentration of the solution applied to the plants was less than 0.005%. Fluorochrome solutions were tested on seedlings remaining in the soil by watering with the dye solution to soil saturation once, than watering normally for 2 weeks, removing seedlings from the pots, and comparing foliage and root appearance to that of untreated controls. The same concentrations were also tested on seedlings brought into hydroponic culture. The plants were kept in dye solutions for 3 days, then transferred to a weak nutrient solution (soluble plant food) free of fluorochromes for another 4 days after which the plants were assessed. No toxic effects were found for 0.02% Sulforhodamine G but 0.01% was used for tracing. No toxic effects of saturated FDA solution were observed.

Individual plants were gently removed from containers and their root systems were dipped in water to remove some of the growth medium. The seedlings were then suspended in mildly aerated tap water and placed at 20° C under 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$ of incandescent and fluorescent light (16 h photoperiod) for at least 24 h, sometimes for 48 h. Then the plants were carefully transferred to another vessel and suspended in aerated dye solution. Aluminum foil wrap was used to protect dissolved fluorochromes from the light. Various incubation times were tried. Best results with Sulforhodamine G were achieved after at least 4, preferably 6 hours of dye application. FDA was applied for 1.5- 2.5 hours. Double staining was tried a few times by suspending the root system in Sulforhodamine for 4-5 hours, then adding drop by drop the amount of FDA stock solution known to be required for saturation of the working solution, and allowing additional 2 hours of incubation. The plants were tested during periods of active growth, when white roots were present, and during dormancy when there were no white roots.

Conventional epi-fluorescence microscopy and confocal scanning laser microscopy (CSLM) were used to examine the roots. Free hand sections of roots were mounted in immersion oil which prevented the spreading of the dyes for a few minutes. Whole mounts of white, semi-transparent root tips were viewed under the Zeiss LSM 410 confocal microscope at wavelengths of 488 and 568 nm (single and double staining). Dormant, brown, opaque roots had to be hand sectioned even for CSLM. Cover slips were supported by small pieces of PlastecineTM to prevent squashing the specimens. Single optical sections were saved as these were clearer than images created by transparent overlay projections.

Observations of root system development

Observations of root growth and root system development were made on seedlings sampled for this study and on other seedlings of the same seed source growing in containers, pots, and boxes of various sizes in 1996-98. These seedlings were sampled at various times during and after the growing season, as required for particular purposes.

RESULTS

First year growth and development

The radicle elongating from a germinating seed formed the main axial root often called the tap root. The young tap root quickly developed abundant root hairs. Few branch roots developed during the first month after germination. The newly formed first order laterals elongated and initially produced few branches. They were also covered with abundant root hairs throughout their length but especially at a short distance above the tip. During the first two months all the roots were white, thin, and appeared to be rapidly elongating. There was no evidence of developing ectomycorrhizae. About two months after germination the elongation of individual roots began to vary. Some roots continued to elongate while others slowed or appeared to stop their elongation. Gradually, the root system became clearly heterorhizic in appearance with long roots bearing long and short, stubby branch roots (Fig. 1). Short roots away from the root tip became yellowish to brown in appearance and many became mycorrhizal. Root hairs were sparse in regions where mycorrhizae developed.

As the development of a root system progressed, individual long roots attained the appearance characteristic of their elongation status. Elongating long lateral roots developed poorly branched segments extending for a considerable distance above the root tip (Fig. 1). These were the roots that Wilcox (1968) had called pioneer roots. It was common for these long pioneer-type root segments to be thicker and more tender than portions further up towards the root collar where the cortex collapsed. The pioneer-type segments bore numerous short roots but lacked elongated branches. The small branch roots were white only a short distance behind the apex of the elongating parent root. They rapidly turned yellowish-brown and gradually became mycorrhizal. It appeared that the rate of fungal infection during the elongation of long roots lagged behind the elongation rate of the parent root and the maturation rate of the associated small roots.

Above the unbranched, elongated segments of long lateral roots were the portions that bore numerous branch roots. Some lateral roots on the pioneer-type segments also elongated, particularly when their parent root slowed its elongation. When this happened, multibranched portions of the root, called mother roots by Wilcox (1968), developed instead of the pioneer-type root portions. Individual roots could resume elongation more than once during the growing

season and change their appearance from that of the mother root to the pioneer-type root, and so on. It appeared that there was little if any relationship between the elongation of a parent root and the elongation of roots it was bearing. There could be elongating and non-elongating individual roots on a parent root that could be itself elongating or not.

It appeared that the tap root does not conform to the above described pattern of elongation. The tap root bore not only those laterals that were positioned at wider (but less than 90°) angle to its axis but it often forked at a sharp angle usually forming a long lateral root. In the basal portion of the root system the tap root was prominent, since its diameter increased due to secondary growth. However, with increasing distance from the root collar and with each fork it formed, the prominence of the tap root diminished (Fig. 1). It usually formed no pioneer - type segments and major branches occurred closely behind the root tip indicating a slow rate of elongation (Fig. 1). Frequently, the tap root stopped elongating early in the growing season and did not resume elongation that year.

The proliferation and elongation of roots was most prominent during late spring and in early autumn. Although there were many non-elongating roots in mid-summer, there were also many white, elongating roots, and some root systems appeared to be actively elongating roots at that time while others had apparently slowed. Radial growth of major axial roots must have proceeded during the summer as these roots visibly thickened following the completion of shoot elongation. This was simultaneous with stem thickening near the root collar. At the end of the first growing season, root systems of one-year-old white spruce seedlings were heterorhizic with long and short lateral roots of various developmental orders. Most short roots were mycorrhizal and several morphotypes of ectomycorrhizae could be found on the same root system. Long roots were infrequently mycorrhizal; however, some long laterals with a prominent dark mantle (probably formed by *Mycelium radicis atrovirens* [MRA]) occasionally extended over several centimeters basipetally from just above the root tip. Mycorrhizae were not seen on older portions of long laterals. Fine, short branch roots near the root collar were often shriveled at the end of the first growing season and some had died. *Thelephora* and MRA frequently occurred on the short laterals near the root collar.

The anatomy

Elongating long laterals had large, prominent root caps (Fig. 2). The meristem of elongating white spruce roots was as described for other conifers: it had two distinct poles forming its

proximal and distal portions separated by a group of centrally positioned cells (Fig. 3). The stele and the inner cortex were formed from derivatives of the proximal meristem. The distal meristem contributed to the development of the column consisting of regularly arranged cells whose plane of division must have been strictly defined. The column developed into the central portion of the root cap (Figs 2-5). The obliquely dividing cells of the distal meristem produced the flanks of the root cap and the outer cortex. As a result of root elongation the upper flanks of the root cap extended above the meristem and there was no clear distinction between the last layer that could be qualified as the root cap and the first layer that would be definitely the cortical layer. Long roots that slowed their elongation had a reduced column and a small root cap (Fig. 4), compared to rapidly elongating roots. Phenolized cells that marked the innermost portion of the cortex and the pericycle were found closer to the apical meristem than in rapidly elongating roots (Figs. 4, 5). Elongated secretory cells were also only 6-7 cell layers above the proximal meristem of roots that ceased their elongation (Fig. 5). In brown long roots of dormant seedlings open primary xylem and secondary endodermis were present just a few cell layers above the proximal meristem. The variation in root elongation rates and status during the annual growth cycle of individual roots apparently impacted on the position of differentiating tissues of the root, relative to the distance from the root tip. There were lateral roots of the same developmental order sampled at the same time that differed in the distance between the root tip and the first open primary xylem element by more than 1 cm.

Lateral roots were initiated from the pericycle. The developing lateral primordium pushed through the cortex of the parent root stretching its endodermis (Fig. 6). A circular group of cells could be distinguished in the centre of the lateral root primordium and it appeared to form the future meristematic region of the new root (Fig. 6). An emerging lateral root displayed initial signs of apical organization (Fig. 7). There was a small circular group of cells dividing periclinally and obliquely toward the tip and forming its rounded dome. These cells could be treated as an equivalent of a distal meristem. Cells in the proximal region divided transversally forming more regularly arranged files extending inward into the new root and forming what would become its future stele (Fig. 7). The rounded flanks of the emerging root were derived from obliquely dividing cells originating at the distal end of the meristematic region and from cells at the flanks of the proximal meristem. Like in long roots, there was no distinct single layer of cells of discrete origin that would form the outermost layer of cells on the new root. Rather, two or three cell layers were produced that formed an external layer that became progressively phenolized and necrotic toward the base of the growing lateral root (Fig. 7).

The emerging lateral root ruptured the cortex and the endodermis of the parent root. The new lateral root had not yet developed its own stele and endodermis. Consequently, the apoplastic seal of the parent's endodermis was temporarily disrupted. This, however, was repaired as the developing root rapidly differentiated its own endodermis that connected to the endodermis of the parent root (Fig. 8). This occurred before the differentiation of vascular tissues in the new root took place. If the root were to remain small, it soon stopped elongating and vascular tissues developed quickly, reaching close to the root apex (Figure 9). The apical meristem remained small and spherical with no prominent quiescent region (Figs. 9, 11). The meristematic region was small and no conspicuous column or root cap were discernible in short laterals. Instead, the whole undifferentiated region of the root apex, including the meristem, was separated from the cortex by the structure known as the metacutis (Plaut, 1909; 1918; Wilcox, 1954; 1962; Leshem, 1970; 1974; Johnson-Flanagan and Owens, 1985a). It was formed by a lignosuberized layer (Leshem, 1974; Wilcox, 1962a; 1964a; 1968) enveloping the root apex and connecting to the endodermis (Figs. 9, 11-13).

Short roots of white spruce were usually mycorrhizal. A Hartig net developed in the cortex but not in and beyond the endodermis (Figs. 9-10). The endodermis of non-elongating short, fine roots was at the secondary state of development up to the bridge with the metacutis surrounding the root apex (Figs. 12-13). The apical region, cut off from the cortex by the metacutis-endodermis junction, also remained uninfected (Figs. 9, 11). The endodermis - metacutis continuum was well demonstrated by the berberine/aniline blue method of Brundrett *et al.* (1988) as shown in Figures 12-13. The fluorescence of the metacutis stained with berberine was more yellowish than the fluorescence of the endodermis. Cell walls of the metacutis stained poorly with Sudan Black which indicated low lipid content. Endodermal cells at the secondary state stained faintly with Sudan Black and only a thin coat around the inner face of the suberin layer stained more darkly. This was likely the staining of the thin remnants of the cytoplasm that contained lipid material. Thus, the secondary endodermis of white spruce contained suberin but little or no waxes.

The endodermis was at the secondary state throughout the length of non-elongating short roots. Many endodermal cells were squashed between the cortex and the stele and their opposite tangential walls were sometimes pressed against each other (Fig. 10). Many cells appear dead but the continuum of suberized walls was maintained throughout the root length. Passage cells were separated by various numbers of other endodermal cells. They lacked a suberized layer on their tangential walls (Fig. 14). In some root regions, passage cells were separated by only one or two fully suberized endodermal cells (Fig. 14). Elsewhere, passage cells were not seen among

several consecutive endodermal cells (Figure 13). The endodermis extended into the parent root joining its endodermis (Figs. 15; 16).

In short and in long roots, the endodermis was always at the primary state before the first mature primary xylem element appeared in the stele. The distance between the root tip and the position at which this occurred varied among roots, apparently in relation to their elongation status or elongation rate (although this was not quantified), especially in the long roots. The only time when all roots were at similar stages, regarding the development of the xylem and endodermis relative to the distance behind the root apex, was during seedling dormancy when all roots were brown and not elongating. At that time, they had the secondary endodermis at the junction with the metacutis and open primary xylem just above that junction as shown for long roots in Johnson-Flanagan and Owens (1985a) and for short roots further in this text.

The lack of a meaningful relationship between the distance from the root tip and the developmental advancement of the endodermis and the xylem can be seen on Figs. 17-21. A section through a taproot shown on Fig. 17 was taken 14 mm above the root tip in May, 1996. The first open primary xylem elements had just begun to appear on root sections. Electron micrographs taken from this specimen showed that the endodermis was at the transitional stage between the primary and the secondary states since the suberization of the inner tangential walls had begun. Some tap roots that ceased their elongation in May had open xylem elements and the stele enclosed by the secondary endodermis less than 1 mm above the meristem. Tap roots of spruce were either diarch or triarch. All sectioned lateral roots were diarch, regardless of the development order of their origin.

The second-order lateral collected in July and sectioned 15 mm above its tip (Fig. 18) had only 6-7 open primary xylem tracheids and its stele was enclosed by the endodermis at the secondary state, with its inner and outer tangential walls suberized, except for occasional passage cells. The tip of this root was yellowish - brown. Another second - order lateral collected in July and sectioned 15 mm from its yellowish - brown tip had already secondary xylem at this distance from the root tip (Fig. 19). The endodermis was at the secondary state and some of its cells, apparently passage cells, appeared to be still alive (Fig. 20) while others collapsed. White, elongating roots collected in July often had no open primary xylem several mm above the root tip but primary endodermis was always found before open xylem elements occurred.

The portion of a root section shown on Fig. 21 came from 5 mm above the tip of a second order lateral collected in November. The root was completely brown due to the presence of necrotic

cells at the root surface. The endodermis was located close to the root surface which at this stage consisted of more than a single layer of collapsed cells. Few open xylem elements were present at this distance from the root tip and the endodermis was at the secondary state. The differentiation of the primary xylem in roots that approached dormancy was slower than the development of the endodermis to its secondary state.

In elongating short roots xylem differentiation was rapid and xylem poles consisting of several cells could be found not far behind the root apex. Near the root apex, they were surrounded by the primary-state endodermis (Fig. 22). However, this did not persist. A few cell layers in the basipetal direction suberization of the inner tangential walls progressed rapidly while some more open xylem elements appeared (Fig. 23). When the root ceased its elongation, the endodermis just behind the root apex rapidly attained the secondary state. Its inner and outer tangential walls were coated with suberin although the endodermis enclosed only a few differentiated xylem elements (Fig. 24). All sampled short branch roots had only primary xylem, always diarch. This was evident from the centripetal differentiation of the primary xylem from two opposite poles. Living short roots could resume elongation and develop into long lateral roots. It did not appear that the development of ectomycorrhizae precluded further elongation of the short roots. Short laterals that overwintered as mycorrhizal roots were observed elongating in the spring, shedding the outer cortical cells as their breadth increased.

The development and fine structure of the endodermis

Ultrastructural features of endodermal development were similar in long and in short roots so they are described concurrently. Cells with dark phenolic contents occurred in the region where the endodermis began to differentiate in long (Fig. 4) and short (Fig. 9) roots. However, phenolics were present not only in cells of the future endodermis but also in neighboring cortical cells and in the future pericycle (Fig. 25). The proendodermis was identifiable only by its position in the innermost layer of cortical cells. Cells of the proper cortex were larger and more rounded than proendodermal cells that tended to be rectangular rather than circular (Figs. 25, 27). Most phenolics occurred in the form of regular droplets and amorphous deposits on inner faces of tonoplasts (Fig. 25). The fusion of smaller vacuoles containing phenolics appeared to contribute to the deposition of the electron-dense phenolic material on tonoplasts of larger vacuoles (Fig. 26). Plasmodesmata were sometimes observed in tangential walls (Fig. 25-26) but not in radial walls of the proendodermis.

The proendodermis and the developing pericycle were similar in appearance and they were distinguishable from each other by their position in the root. There was no evidence of the Casparian bands in proendodermal cells, thus radial and tangential walls did not visibly differ in their appearance. A distinct feature observed at this stage of the development was the presence of large clear vesicles near or adhering to radial and tangential walls of the proendodermis and to outer tangential walls of cortical cells (Figs 27-29). These vesicles usually occurred next to strands of rough endoplasmic reticulum (RER) (Fig. 28). When adhering to the cell wall, they were separated from the cytoplasm by the plasma membrane (Fig. 29). It was unknown whether these vesicles were endo- or exocytotic and what was the significance of their occurrence at that time. The stele adjacent to the proendodermis was undifferentiated.

Radial walls of the developing endodermis remained thin and bent (Fig. 30) under the pressure from the enlarging stele. Casparian bands in the primary-state endodermis appeared as smooth portions of radial cell walls to which the plasma membrane adhered tightly (Fig. 31-32). There was a discernible decline in the amount of phenolics in endodermal cells but tonoplasts were still covered with a phenolic coat (Fig. 32). Vacuoles occupied most of the cell volume and nuclei were rarely seen at this stage. The cytoplasm was reduced to a narrow band along the cell walls and poorly developed plastids without starch and mitochondria were present in endodermal cells (Figs 31-33). The cytoplasm had an active appearance due to the abundant endoplasmic reticulum and numerous small vesicles in its vicinity (Fig. 33). The plasma membrane had abundant granular material on its inner and outer faces (Fig. 33). Root segments representing this developmental stage were troublesome in preparation for microscopy. The penetration of chemicals into the tissue was obstructed apparently by the Casparian bands and cortical cells often separated from the endodermis. Stain precipitation was also a persistent problem.

The endodermis did not remain long in the primary state, especially in short roots. In the long roots it remained at its primary state for a few cell layers prior to the occurrence of the open primary xylem. Once open xylem elements began to appear on root sections, the endodermis transited to the secondary state. The process of laying down the suberin coat on the inner endodermal cell walls must have been rapid since endodermal cells that just began to produce the coat of suberin were found next to those where this process appeared to be complete (Fig. 34). The suberin layer was first deposited on radial walls, over the Casparian bands and on inner tangential walls. Outer tangential walls became suberized last (recall Figs. 23 -24). The plasma membrane stained darkly at this time which suggested that it was actively participating in the deposition of suberin onto the cell walls (Figs. 34 - 38). The suberin layer was deposited over the Casparian band on both inner sides of primary radial walls (Fig. 35) but only on the inner

endodermal sides of tangential walls (Figs. 36-38). The plasma membrane did not adhere as tightly to the Casparian bands as it did earlier. Areas where the plasma membrane was distanced from the Casparian band were evident, especially in plasmolyzed cells where the plasma membrane pulled away from the partly suberized radial wall (Fig. 35). Microtubules occasionally occurred near the suberizing cell walls (Fig. 36) but the abundance of the endoplasmic reticulum in endodermal cells (Figs. 34, 37-38) was more striking. The plasma membrane did not adhere tightly to the suberin layer being deposited on tangential walls (Figs. 36-38).

The suberin layer covering the inner faces of cell walls of the endodermis appeared lamellar under the electron microscope (Fig. 39). Spiked protrusions from the primary cell wall into the suberin layer were commonly observed (Figs. 39-40). In suberized cells the cytoplasm was reduced to an electron - dense layer coating the inner face of the suberin layer. The plasma membrane was indistinguishable from the rest of this dark, thin, amorphous material (Figs. 39-40). The fully suberized cells of the endodermis appear dead. The suberin layer was not deposited on inner cell walls of passage cells. They remained alive until the endodermis became completely crushed and disrupted during secondary growth. Until then, living passage cells with their plasma membranes adhering to radial cell walls over the Casparian band could be found next to the dead, suberized endodermal cells (Fig. 40). The stele, increasing in its breadth, stretched the secondary endodermis bending its radial walls (Figs. 41, 43). Pericycle cells (Fig. 41) and cortical cells (Fig. 43) next to the collapsed endodermis remained alive. Gradually, the endodermal walls were destroyed by stretching (Fig. 42) and squashing (Fig. 44). The primary wall broke first but the suberin layers, apparently more flexible than the primary wall, persisted somewhat longer (Figs. 42, 44).

Plasmodesmata rarely occurred in tangential walls of endodermal cells, including the passage cells. They were not found in radial walls before or after the deposition of the Casparian band. However, plasmodesmata connecting the endodermis with the pericycle and with the cortex were occasionally seen, sometimes even traversing the suberin layer (Fig. 45). It is unlikely that these plasmodesmata were functional when the suberization was complete since the endodermal cells appeared dead. The presence of plasmodesmata in suberized endodermal cells was infrequent and more commonly the tangential cell walls consisted of a primary cell wall of rough, grainy appearance and a solid, smooth layer of lamellar suberin (Fig. 46).

Suberized cell walls of white spruce endodermis were intensely fluorescent when stained with berberine which causes the fluorescence of suberin, lignin, and callose (Brundrett *et al.* 1988).

Staining for lignin with HCl/phloroglucinol (Jensen 1962) failed to detect lignin in the endodermis of white spruce. However, this test caused rapid disintegration of tissues and it was not well suited for precise localization of small amounts of lignin. Nevertheless, it was rather certain that white spruce endodermis did not develop beyond the secondary state.

Mycorrhizae

Most short roots were mycorrhizal. Fungal hyphae were never detected in the region of the root apex, whether enclosed in the metacutis in non-elongating roots (Fig. 47), or in elongating roots prior to the development of the metacutis. The surface of the apical dome was covered by 3-4 layers of collapsed cells full of phenolics. These cells had characteristically smooth cell walls (Fig. 47) that resembled Casparian bands on radial walls of endodermal cells. Cells on the surface of the apical dome gave a yellowish-green fluorescence (Fig. 12) the same as the metacutis bridge connecting to the secondary endodermis. Basipetally from that bridge, fungal hyphae were abundant often forming a distinct mantle (Figs. 48-50) and penetrating between the cells (Fig. 48) to form the Hartig net in the cortex. Hyphae were commonly seen next to phenolized cells on the root surface (Figs. 48-49) and of the cortex (Fig. 51). Amorphous material commonly occurred between mantle hyphae. Surfaces of mycorrhizal roots stained faintly pink when subjected to the PAS reaction and faintly bluish-black with Sudan black B. This indicated that the amorphous material likely contained some polysaccharides and was probably of mucilaginous character. The Sudan staining suggested the presence of lipids. Cells on the root surface had natural fluorescence under UV light, greatly enhanced by the berberin staining, suggesting suberization of their cell walls. Indeed, many collapsed cells had characteristically smooth cell walls that appeared suberized (Fig. 49).

The morphotypes of ectomycorrhizae found on roots of young white spruce indicated that these mycorrhizae were formed with *Ascomycetes* and *Basidiomycetes*. Several different morphotypes could occur on the same root system and it was usually difficult to identify the fungus occurring on micrographs. The hyphae on Fig. 50 had a distinct dolipore, parenthesis type of their septal apparatus peculiar to *Basidiomycetes* (Webster, 1980; Kendrick, 1992). The Hartig net developed in the cortex but no hyphae were found in endodermal cells and in cells of the stele (Fig. 51).

Apoplastic and symplastic permeability of white spruce roots

Sulforhodamine G applied to young, elongating roots accumulated in the apoplast of the root cap and of the rhizodermis. Little stain entered into the apoplast of the inner portion of the root tip (Fig. 52). The symplastic tracer stained the majority of cortical cells in white tips of elongating roots but it did not stain the meristem and the rhizodermis, faintly stained the root cap and the undifferentiated stele (Fig. 53). Free-hand sections taken 6 mm above the tip of an elongating white lateral showed the existence of an apoplastic barrier surrounding the differentiating stele in which open xylem elements have not yet occurred (Fig. 54). No open xylem elements were present in the stele and the apoplastic tracer was present in cell walls of cortical cells and did not cross behind the endodermis. In regions closer to the root apex, where the proendodermal cells had not yet developed Casparian bands, there was no clear-cut border at which the apoplastic tracer was stopped, however, the undifferentiated stele was nearly free of the apoplastic stain (Fig. 55). In more mature regions the apoplastic tracer was stopped at the endodermis but faint fluorescence from the cell walls of the protoxylem indicated that some stain must have entered the stele in more distal regions of the root (Fig. 56). In more mature regions of the root, the symplastic tracer did not appear inside the cortical cells indicating that it did not cross into the root through the rhizodermis. It did, however, stain the contents of cells in the stele (Fig. 57) which suggests that the dye was also transported in the stele from regions closer to the root apex. Note that the symplastic tracer stained contents of passage cells (Fig. 57). In elongating short roots, the meristematic region was poorly permeable to either tracer. The apoplastic tracer did not penetrate into the stele of the short roots during their period of elongation but the symplastic tracer infiltrated into the stele above the meristematic region (Fig. 58).

In dormant long laterals, neither the apoplastic nor the symplastic dye penetrated into the meristematic area enclosed by the metacutis. Accumulation of the apoplastic tracer in the cortex above the metacutis/endodermis bridge is shown on the longitudinal section presented in Fig. 59. The stoppage of the apoplastic dye at the root surface covered by suberized cells of the metacutis is shown in Fig. 60. Bright fluorescence from the apoplastic dye that accumulated near the endodermis was observed at junctions between parent and lateral roots (Fig. 61). The apoplastic dye was present throughout the cortex of both roots but it apparently accumulated the most at the junction of their endodermis, if judged by the brightness of the fluorescence shown on Fig. 61. Some staining from the apoplastic tracer is also seen inside the stele of both joining roots (Fig. 61). It is uncertain if this staining originated from the dye transported from other regions of the root or whether the dye was smeared into that area while a hand section was made. Brown roots

were not transparent enough to permit tracing the dyes with the laser beam of a confocal microscope and had to be cut to allow the penetration of the beam into the root.

DISCUSSION

Seedlings of *Picea glauca* grown with little or no spatial restriction to root expansion developed well spread, heterorhizic root systems consisting of a tap root and numerous short and long lateral roots of various developmental orders. No adventitious roots occurred during the first year of growth. Heterorhizy is not a feature common to all conifers since it does not occur in the Cupresseaceae (Wilcox, 1962a). The white spruce seedling root system morphology resembled descriptions of root systems of other young coniferous seedlings such as *Abies procera* Rehd. (Wilcox 1954) and *P. resinosa* (Wilcox 1964). Root systems of white spruce were more extensively branched than those of one-year-old noble fir (Wilcox, 1954) which produced only first order long laterals during the first growing season. Although rates of growth were not measured in the current study, morphological and anatomical observations indicated that the growth of the tap root was rapid only for a few weeks after germination. With the progressing development of long laterals the tap root slowed or ceased its elongation and its prominence gradually diminished, like in noble fir (Wilcox, 1954).

Wilcox (1964) considered the periodicity of elongation and no-elongation cycles of individual roots to be a naturally occurring, inherent phenomenon that required to distinguish between patterns of activity of individual roots and of root systems. The individual pattern of elongation of single roots (Wilcox 1954; 1962b; 1964; 1968; Johnson-Flanagan and Owens 1985a; 1985b) did not become visible during the first growing season for at least two months after germination, until numerous laterals had developed. Earlier, the developing seedlings apparently needed all their roots to elongate simultaneously. The presence of abundant root hairs during early root system development suggests that the seedlings require the few roots they possessed to maximize their nutrient-absorbing capability. This could be important at that time since mycorrhizae had not yet developed. The later established independent growth of individual roots (Johnson-Flanagan and Owens 1985a; 1985b) could be interpreted as an expression of a highly controlled regulation of growth in a root system.

Seasonal variation in the abundance of elongating, hairless roots versus hair-bearing laterals found in white spruce seedlings during the second growing season (Johnson-Flanagan, 1984; Johnson-Flanagan and Owens 1985a) was not encountered in the current study that covered only the first year of growth. Root hairs, initially abundant, gradually diminished during summer while the majority of short roots developed mycorrhizae. The morphological classes

distinguished by Johnson-Flanagan and Owens (1985a) might imply a possible functional specialization, probably of a transient nature, requiring morphological modification of certain roots. The elongating laterals without root hairs could be the roots responsible for rapid expansion of the root system. Many such roots were found during the first year of growth in the currently reported study, especially following the surge of root growth in the fall, but the slower-elongating, hair-bearing long laterals, referred to as absorbing roots by Johnson-Flanagan and Owens (1985a), were not common in white spruce seedlings during their first year of growth. The elongation of new lateral roots on a newly formed portion of an elongating root appeared to be temporarily suppressed. The development of pioneer-type roots could result from such a suppression. Wilcox (1962b; 1964; 1968) suspected the existence of apical dominance over lateral root elongation. The release from apical dominance would result in a transition from the pioneer-type to multibranched roots. In white spruce seedlings, symptoms of apical dominance and its release were also observed. If indeed the pioneer roots were the expression of apical dominance of the axial root over the elongation of its newly formed laterals, these pioneer-type roots would be most suitable for studies of growth regulation in roots.

In the continental climate of the central interior of British Columbia winters are harsh and last from five to six months. Root elongation is completed by October and all roots remain brown until spring. It is unlikely that any radial growth of roots occurs during winter. However, are the roots really dormant? In the course of numerous studies on conifer seedlings, including white spruce, the author has observed that elongation of some individual roots could be evoked throughout winter by placing seedlings at growth promoting conditions, even when bud break does not occur. Thus, in the true sense of the definition by Doorenbos (1953), root systems of white spruce are never dormant. As noted by Wilcox (1968), resumption of root elongation does not involve all root apices at once. The condition of root dormancy must be understood as an inhibition of elongation only and can pertain merely to individual root apices. Thickening of axial roots during periods of no elongation occurred commonly, suggesting that secondary growth and root elongation were not interdependent.

The cessation of root elongation was accompanied by the development of a brown cup-like structure resulting from the formation of the metacutis, also referred to as dormancy layer(s) (Plaut, 1909; 1918; Wilcox, 1954; 1962a, 1964; 1968; Johnson-Flanagan and Owens, 1985a). The metacutis in long roots of white spruce developed in a similar manner as described for *A. procera* (Wilcox 1954) and *P. resinosa* (Wilcox 1968) and the structure was similar to those described for these two conifer species. In *P. resinosa* (Wilcox, 1968), the secondary endodermis extended acropetally for several cells into the metacutized root apex which was

deeper than in white spruce. The metacutis layer covering the apical dome was coated with remnants of up to three layers of collapsed cells but the metacutization usually occurred in only one layer of cells, except for the tip of the apical dome and the bridge to the endodermis where it could involve 2-3 cell layers. The common feature of the metacutis cells was the suberization of their cell walls, cell orientation, and the presence of dense phenolic substances filling their entire lumens. The orientation of the metacutis cells was typical for the collapsed cells present at the root surface and their tangentially elongated shape was due to the collapse of the radial cell walls. The elasticity of suberized radial walls of the metacutis must have been substantial as they did not break upon collapsing and the continuity of the metacutis was maintained all around the apical dome. In long roots, the metacutis marked the position of the approximate boundary between the cortex and the upper flanks of the root cap (Wilcox, 1962a). In short roots, it marked the boundary between the cortex and the undifferentiated region of the root apex.

There could be some variation in the morphology of the metacutis even within the same genus (Plaut 1909). In contrast to many other plants, including conifers such as *A. procera* (Wilcox, 1954), *L. decurrens* (Wilcox, 1962a), and *P. sylvestris* L (Warmbrodt and Eschrich, 1985) the metacutis of *P. glauca* lacked lignin. This was also reported by Johnson-Flanagan and Owens (1985a) as well as a high concentration of polysaccharides occurring in metacutized cell walls. The gradual browning of long roots that ceased their elongation was caused by the necrosis of the root cap cells located outside of the metacutis and by the necrotic layers formed by the cortex basipetal to the metacutis bridge. In short roots the metacutis covered the surface of the apical dome but the cup-like appearance was not attained for the lack of a root cap. Many short and long laterals aborted during the first growing season. Apart from the death of roots due to obvious external causes, there were no detectable morphological symptoms that would allow prediction of which roots were to die.

Apical organization of long lateral roots has been described in many conifers (Allen, 1947; Bogar and Smith, 1965; Wilcox 1954; 1962a; Johnson-Flanagan and Owens, 1985a). The last citation relates to the description of apical organization in long laterals of white spruce. In the currently reported study, short roots displayed signs of apical organization at the primordial stage, during the root's emergence through the cortex of the parent root. In contrast to the long laterals, the distal initials in short roots were not producing the column and, consequently no conspicuous root cap was formed. Thus, it appeared that the distal initials located at the acropetal tip of the spherical group of meristematic cells did not contribute substantially to the formation of a short lateral root. As in long roots, the origin of dermal cell layers could not be traced to a single distinct initial or a separate group of initials. The dermal cells of short roots differentiated

from the outermost cells of the cortex. Thus, the origin of the dermal layer in short roots was less complex than in long roots where remnants of the inner root cap cells could be deposited on the root surface forming additional layer(s) over the outermost cortical cells (Wilcox, 1962a). It was noted that surfaces of short roots were coated throughout their length by an amorphous substance weakly fluorescent when stained with berberine and staining weakly with Sudan dyes suggesting that it could be partly made of lipids. A weak positive response to the Feulgen reaction indicated a possible polysaccharide content, however, this staining could have been due to the presence of polysaccharide root secretions at the root surface. This outermost protective layer could guard the root against infection by parasitic organisms (C. Peterson, 1998, pers. comm., H. Massicotte, 1998, pers. comm.). It could protect the root from drying. However, this layer was not a barrier to the apoplastic tracer entry into the cortex. Unfortunately, the dermal layers of conifer roots have not been investigated in detail and their properties remain little known. Much more has been said about the ambiguity of the origin of these cells and potential problems with the appropriate terminology for the conifer dermal layers (Wilcox 1954; 1962a) than has been done to determine their functional properties. The phenolization of these cells, their potential role in the secretion and exudation to the root surface, and the properties of their cell walls could be of great importance not only to the root but also to soil organisms that associate with root surfaces. The dermal layers of conifers are definitely in need of a careful study.

Not surprisingly, the variability in the distances where the primary tissues matured was much greater in long elongating roots than in short roots. The dependence of the differentiation and maturation of primary root tissues on the rate of root growth was well documented by studies on *A. procera* (Wilcox 1954), *L. decurrens* (Wilcox, 1962a), and *P. resinosa* (Wilcox, 1968). It was apparent from these studies that the rate of root elongation could be much more variable than the rate of differentiation of primary tissues. Since short lateral roots elongated slowly, due to the apparent suppression from the elongating parent root, and elongated only a short distance, the differentiation and maturation of their primary tissues occurred not far behind the apical meristem.

The development of vascularization in long roots of white spruce resembled closely that of noble fir described by Wilcox (1954). Apart from the main root, that was sometimes triarch, all sampled roots of white spruce, long or short, had diarch xylem configuration. In contrast to noble fir that had a centrally located resin duct, white spruce roots usually had resin ducts near the outer limits of the xylem poles, adjacent to the early protoxylem. This was similar to the positioning of resin ducts in *P. resinosa* but no horse-shoe configuration of the protoxylem (Wilcox 1964) occurred during its early development in white spruce. It appeared that the

differentiating protoxylem of red pine left space for the development of a resin duct at each pole, whereas the resin ducts of white spruce apparently pushed themselves into the protoxylem displacing it outwards. The development of the primary xylem was centripetal but the metaxylem elements that were the last to mature produced the largest cells in the center of the stele, as shown in Chapter 4. In short roots, the arrangement of mature xylem elements was less regular than in long roots, the size differences between the late metaxylem and earlier differentiated elements of primary xylem was inconspicuous, and resin ducts were irregularly positioned or absent, as in *P. resinosa* (Wilcox 1964). No secondary growth was observed in short laterals.

As noted by Wilcox (1954) distinguishing between proendodermis and the pericycle during early differentiation of these tissues in elongating long roots was difficult due to numerous periclinal divisions in the inner layer of the cortex. In white spruce, the phenolization of the proendodermis was only partly helpful in its early localization. The characteristic phenolic droplets described by Van Fleet (1961) were not exclusive to the proendodermal cells of white spruce and occurred in the cortex and in the pericycle. Phenolics were; however, more abundant in the proendodermis and the pericycle than in the inner cortical cells and the whole cytoplasm of pericyclic and proendodermal cells could be darkly stained with phenolics coating cellular organelles. Proendodermis was easier to discern in short than in long roots mainly due to the ease of tracing the endodermal cells acropetally from regions where the distinct primary state was attained, which was not far away from the proendodermis. In short laterals, the pericycle was also phenolized during early differentiation and the clearness of the proendodermal appearance shown by Kottke and Oberwinkler (1990) in short roots of *P. abies*. and *L. decidua* was not encountered in *P. glauca*. The amount of phenolics in short mycorrhizal roots of *P. sylvestris* L (Warmbrodt and Eschricht, 1985) was also strikingly less than in roots of *P. glauca*. Since the studies of Kottke and Oberwinkler (1990) and of Warmbrodt and Eschrich (1985) were not made on soil-grown roots, one could wonder whether the differences in the degree of phenolization of tissues in the roots was characteristic for the species or a modification by cultural conditions.

In long roots of *A. procera*, Casparian bands were first found in the endodermis opposite protoxylem at the time when the first protoxylem matured. When two or three protoxylem cells matured, Casparian bands were deposited in all endodermal cells enclosing the stele (Wilcox, 1954). In long and in short laterals of white spruce, the deposition of Casparian bands began before the maturation of the first protoxylem elements. Casparian bands were always present in all endodermal cells encircling the stele when it contained the first mature protoxylem element. There was also no conspicuous difference in Casparian band deposition between cells located

near the xylem poles and other endodermal cells indicating a more uniform development of the endodermis in white spruce than in noble fir. Warmbrodt and Eschrich (1985) also reported a uniform development of endodermis around the developing stele of cultured mycorrhizal rootlets of *P. sylvestris*. In *L. decurrens*, the deposition of Casparian bands did not occur until 6-8 mature protoxylem elements were visible. Thus, the deposition of Casparian bands relative to the maturation of the protoxylem could differ among conifer species. In white spruce, the development of sieve areas in primary phloem did not occur until the suberization and the development of the secondary-stage endodermis commenced which was similar to the situation in *A. procera* (Wilcox, 1954). In *Libocedrus*, the phi-layer of cortical cells with heavy cell wall thickening had developed before the deposition of Casparian bands began (Wilcox, 1962a). Although cell walls of cortical cells were sometimes thickened, no heavy thickening similar to that described by Wilcox (1962) was present in either long or short roots of white spruce.

The ultrastructure of the developing endodermis of long and short roots of white spruce was similar. The presence of large clear vesicles encircled by the plasma membrane of proendodermal cells and pericyclic cells was not reported by previous investigators of conifer roots. The role of these structures remains unexplained. They could represent transport activity in these cells but from where to where could not be determined. It was also unknown if their presence was related to the transport of substances necessary for the deposition of suberin to develop Casparian bands. The Casparian bands in white spruce roots were as described by Jorns (1987) for long roots of *P. abies* L. and by Kottke and Oberwinkler (1990) for short roots of *P. abies* and *Larix decidua* L., as well as for detached, cultured mycorrhizal rootlets of *P. sylvestris* (Warmbrodt and Eschrich, 1985). The distance at which the Casparian bands were first found varied considerably in long roots, apparently due to the previously discussed relationship between the rate of growth and the rate of differentiation of primary tissues. In elongating roots, Casparian bands closest to the root tip were found between 6-7 mm from the root tip and the furthest about 15 mm above the root tip. In *P. abies*, Casparian bands were first found 25 mm from the root tip (Jorns, 1987). This could be interpreted as indicative of slower growth rates in roots of white spruce used in the current study than in Norway spruce studied by Jorns (1987). The primary-state endodermis could extend over a few centimeters of the root before the transition to the secondary state began. Root segments in these regions remained white. Once started, the suberization process was quickly completed. In dormant long and short lateral roots, the endodermis was always at the secondary state at the junction with the metacutis.

In white, short roots, Casparian bands could be first found 2-4 cells away from the proximal part of the meristem. Kottke and Oberwinkler (1990) reported that Casparian bands were found in the

second row of endodermal cells from the meristem in *P. abies* and in *L. decidua*. Warmbrodt and Eschrich (1985) reported first Casparian bands approximately 180 μm from the root apex. It should be remarked that short roots of white spruce could vary considerably in length and this could reflect on the positioning of differentiating tissues during root elongation. Nevertheless, the primary state of the endodermis occurred a very short distance from the meristem in all the above discussed species. Also similarly, the secondary state developed shortly. In white spruce, a suberin layer was deposited first on the inner tangential walls and on radial walls of endodermal cells, then on the inner tangential walls. This was consistent with the report of Johnson-Flanagan and Owens (1985a). In *P. abies*, suberin was deposited first on the outer tangential walls (Kottke and Oberwinkler, 1990). The deposition of the suberin layer must have been rapid since it could be seen just beginning in one cell and be already complete in the neighboring endodermal cell.

The ultrastructure of endodermal cells during the process of suberin deposition implied the possible involvement of the rough endoplasmic reticulum in this process, possibly by the production or processing of materials for deposition onto the cell wall by the plasma membrane. Although numerous vesicles were seen near the RER, there was no clear evidence of vesicular transport between the endodermis and the plasma membrane. The perceptible decline in the amount of phenolics in endodermal cells suggested their utilization in the process of suberin synthesis. The plasma membrane was slightly distanced from the Casparian band during the deposition of a lamellate suberin layer which could lead to the relaxation of the control of transport through the endodermis as suggested by Sanderson (1983). However, if such relaxation really occurred, it would last only for a short time as the suberization appeared to be proceeding rapidly.

Passage cells occurred frequently in both short and long roots. In longitudinal sections, they appeared smaller, with shorter tangential walls than the elongated cells that would later suberize. Their arrangement was not as regular as reported by Kottke and Oberwinkler (1990). However, it might be difficult to evaluate the regularity of the sequential occurrence of passage cells in the endodermis as it would require sectioning parallel to the same layer of endodermal cells throughout the section. This is easier said than done, especially while producing longitudinal sections. Thus, it could be that the passage cells in *P. glauca* were less regularly spaced than those of *P. abies* and *L. decidua*, or perhaps the drawings presented by the above cited authors were slightly idealized. The passage cells of white spruce were alive during the secondary state of the endodermis and did not develop suberization as did the other cells of the endodermis. The electron dense incrustations of the outer tangential walls of passage cells reported by Kottke and Oberwinkler (1990) were not seen in white spruce. However, the berberine-induced yellowish

fluorescence of the outer tangential walls of passage cells, that these authors thought to be related to the incrustations, was occasionally seen in white spruce. Since this was not a frequent occurrence, it could be that passage cells with incrustations were simply not encountered on the studied sections of white spruce roots. Kottke and Oberwinkler (1990) suggested a possible role of these incrustations in stabilizing the passage cells against the pressure from the growing root and recommended further investigation into the function of these structures.

Matured secondary endodermal cells died and only granular remnants of the protoplast could be discerned in their lumens. There was no conspicuous difference in the thickness of the lamellar suberin layers on different cell walls, in contrast to the work by Kottke and Oberwinkler (1990) reporting greater thickness of the inner tangential walls in the secondary endodermis in the two conifer species they investigated. The spike-like protrusions from the primary cell wall were not reported in other conifers. They were probably of the same nature as the material of the primary wall and could have resulted from sporadic continuation of the cellulose during the process of Casparian band formation since they were found only on radial walls. This could be possible as infrequent microtubules were found even during the deposition of the suberin lamellae. As in other conifers, the endodermis remained in the secondary state until it was destroyed by the pressure from the enlarging stele when secondary growth began. Short roots did not undergo secondary growth and the endodermis remained unruptured throughout their length and into the body of the parent root where it merged with its endodermis.

Kottke and Oberwinkler (1990) rightfully disputed the statement of Johnson-Flanagan and Owens (1985a) regarding the non-functionality of the secondary-state endodermis in long roots of white spruce. The non-living cells of the secondary endodermis could be termed passively functional since they blocked the apoplastic pathway into the stele but they could no longer control the selective transport of molecules between the stele and the cortex. With passage cells remaining alive, the secondary-state endodermis of white spruce should be viewed as a functional structure. Very few plasmodesmata were found in endodermal cells and in passage cells throughout the development of white spruce endodermis and they were never seen in radial walls. Thus, the transport through the endodermis of white spruce appeared to depend more on the transcellular (Steudle and Jeshke, 1983; Steudle, 1994; 1995) than on the symplastic pathway. Jorns (1987) did not find plasmodesmata in radial walls of long laterals of *P. abies*. Kottke and Oberwinkler (1990) observed plasmodesmata in tangential and radial walls of proendodermal cells of short roots in *P. abies* and *L. decidua* but reported the subsequent decline in their number up to complete disappearance of plasmodesmata in the secondary state endodermis. There was no explanation of the manner of disappearance of plasmodesmata during

the development of endodermal cells and if and how the emptied pits in cell walls were repaired. In white spruce, plasmodesmata persisted even through the suberization process and their remnants were found even when their functionality was dubious. The frequency of plasmodesmata found in the secondary state endodermis was about as low as at the earlier developmental stages.

A number of papers have described details of structural features of ectomycorrhizae formed by various fungi with different conifer species (Laiho, 1965; Alexander *et al.* 1987; Kottke and Oberwinkler, 1988; Massicotte *et al.* 1989; Scales and Peterson, 1991a; b). Structural differences were shown not only for mycorrhizae formed by the same fungus with angiosperm species and with conifer species (Kottke and Oberwinkler, 1988; Massicotte *et al.* 1989; Scales and Peterson, 1991b) but also with different conifer species (Scales and Peterson, 1991a; b). The development of mycorrhizae, in relation to the development of the endodermis in short roots was studied by Kottke and Oberwinkler (1988; 1990) in *P. abies* and *L. decidua*. These studies were closest in nature to the current study on *P. glauca* roots. Kottke and Oberwinkler (1990) stated that there were no differences in the development of the endodermis between roots from sterile culture, roots inoculated with different fungi in culture, and several types of mycorrhizae collected from the field. A slow rate of transition from the primary to the secondary state of the endodermis in *Larix* was responsible for the presence of the Hartig net in root regions with the primary-state endodermis and the spreading of the hyphae into regions near the apical meristem. In *P. abies*, the secondary state of the endodermis was attained faster, so the Hartig net developed only in regions with the secondary-state endodermis and the entry of the hyphae into the root apex was stopped at the metacutis/endodermis bridge. The development of the endodermis and the Hartig net in *P. glauca* corresponded with the situation in *P. abies*. The apical region of *P. mariana* was shown to be free of fungal hyphae (Scales and Peterson, 1991b). The absence of the Hartig net in the apical region could be a characteristic of the genus *Picea* and could be the result of the relationship between the development of the endodermis and metacutis and the development of the Hartig net. Kottke and Oberwinkler (1990) considered the condition in larch as better suited for the effective exchange of substances between the host and the fungus than occurred in spruce. However, the secondary condition of the endodermis did not seem to hamper the development of the Hartig net in short roots of white spruce where the well developed hyphae and its numerous organelles could be a symptom of the prosperity of the fungus. Mycorrhizae also developed on some long laterals of white spruce. A developed Hartig net was never found on white portions of elongating roots, it may be speculated that the spread of hyphae in long roots was similarly dependent on the development of the secondary-state endodermis in short roots.

Numerous studies have shown that the endodermis acts as a barrier to the apoplastic movement of different types of tracers into the stele of roots (Clarkson and Robards 1975; Robards and Robb, 1974; Nagahashi *et al.* 1974; Robards *et al.* 1979; Peterson and Edgington, 1975; Peterson *et al.* 1981a; Moon *et al.* 1986). The blockage of the apoplastic movements of Sulforhodamine G dye by the endodermis of white spruce roots was shown in this study. The dye entry into the stele was blocked by the endodermis at its primary and secondary states. In elongating long laterals, small amounts of the apoplastic dye, judging by the low intensity of the fluorescence, accumulated at the root surface and in the root cap region but the meristematic zone and the undifferentiated portion of the root immediately above the apical meristem remained unstained. Peterson *et al.* (1981) investigated the movement of two apoplastic tracers in roots of corn and concluded that neither moved into the vascular system through the root apex. They cited numerous studies in support of the notion that the cell walls of living root tips were impermeable to various apoplastic tracers (i.e. Lüttge and Weigl 1962; Weigl and Lüttge, 1962; Rasmussen, 1968; Tanton and Crowdy, 1972). The impermeability of the root apex of elongating corn roots to apoplastic tracers could be due to the presence of polysaccharide secretions (Karas and McCully, 1973) but it is also possible that thin, immature cell walls in the apical and subapical regions could be impermeable to the apoplastic tracers. Peterson *et al.* (1981) suggested that endodermal cells without differentiated Casparian bands need to reach some critical stage of maturity before they become permeable to apoplastic tracers. Possibly, the same could be true for other cells. In immature root regions of elongating long laterals of white spruce that apparently lacked developed Casparian bands, very little Sulforhodamine G could be found in the thin cell walls of the differentiating stele. However, cell walls of the differentiated primary xylem usually showed faint fluorescence typical for Sulforhodamine G indicating the presence of the tracer at these sites. This was not observed in unstained controls since lignin and the dye fluoresced at different excitation wavelengths. One advantage of the Sulforhodamine G dye was that almost no natural fluorescence of the plant tissues occurred in unstained controls at the excitation wavelength required by the dye.

Since the fluorescence of the xylem occurred even in regions where the endodermis had stopped the entry of the tracer into the stele, it appeared that the dye entered through regions located distally to regions containing matured xylem elements. Although little dye was found in the portions of undifferentiated stele where the Casparian bands had not developed, such regions could extend over various distances in roots, depending on their rate of elongation and differentiation of primary tissues. Another potential site of uncontrolled entry of an apoplastic tracer into the stele could be at the sites of emergence of laterals, when the endodermis of a parent root becomes discontinuous. Experiments with localized applications of an apoplastic

tracer performed by Peterson and Edgington (1975) suggested that, at least in some plants, this could occur, but they also implied that this is a situation of short duration. Several other studies concluded that apoplastic dye penetration into the stele did not occur at root junctions (Dumbroff and Peirson, 1971; Karas and McCully, 1973). Hanson *et al.* (1985) reported that a mobile apoplastic tracer applied with pressure to roots of *P. resinosa* was initially present in the exudate but later it was not. They attributed the initial presence of the dye in the exudate to a pressure-induced membrane leakage due to the osmotic shock that was followed by an osmotic adjustment. Similar observations were made by Moon *et al.* (1986) on *Avicennia marina* (Forsk.) Vierh). Finally, Sanderson (1983) suggested that there is a potential loss of tight control over the apoplastic transport by the endodermis during the initial stages of the secondary-state development. The ultrastructural observations of the current study suggest that this may be true. However, this could not be presently confirmed. Possibly, more than one site of leakage contributed to the observed presence of the apoplastic tracer in the xylem. Considering the low intensity of fluorescence of Sulforhodamine G from the xylem, the uncontrolled entry of the dye appeared to be minor.

There was no obvious entry of the apoplastic tracer into the apical and subapical regions of elongating short roots, probably due to a rapid transition between the region of immaturity and the site of Casparian band development. The apoplastic tracer did not penetrate the secondary endodermis of short laterals throughout their length. It was also stopped by the metacutis of short and long dormant roots and did not appear to penetrate the secondary-state endodermis above the root apex. The faint fluorescence in the stele seen on some micrographs could have been caused by smearing and displacement of the dye into areas that it previously did not occupy. Unfortunately, optical sectioning of brown roots could not be performed due to the opacity of the roots and hand-sectioning was necessary to prepare such roots.

The symplastic tracer FDA entered into root tips of elongating long laterals just above the apical meristem but little of its fluorescence was visible in the root cap and even less in the apical meristem. Some regions of the symplast can be inaccessible to larger molecules due to the size exclusion limit (Goodwin, 1981; Erwee and Goodwin, 1983; 1985) or, possibly, any molecules could be subject to metabolic control in the symplast. In elongating short roots, the entry of the symplastic dye into the root was mainly just above the root apex and the intensity of the fluorescence declined several hundred micrometers basipetally. This would be consistent with anatomical observations showing the development of the secondary-state endodermis just above the root apices of elongating short laterals. The decline in the intensity of the fluorescence in that region, compared to the more distal region, was possibly due to the dilution of the dye. The

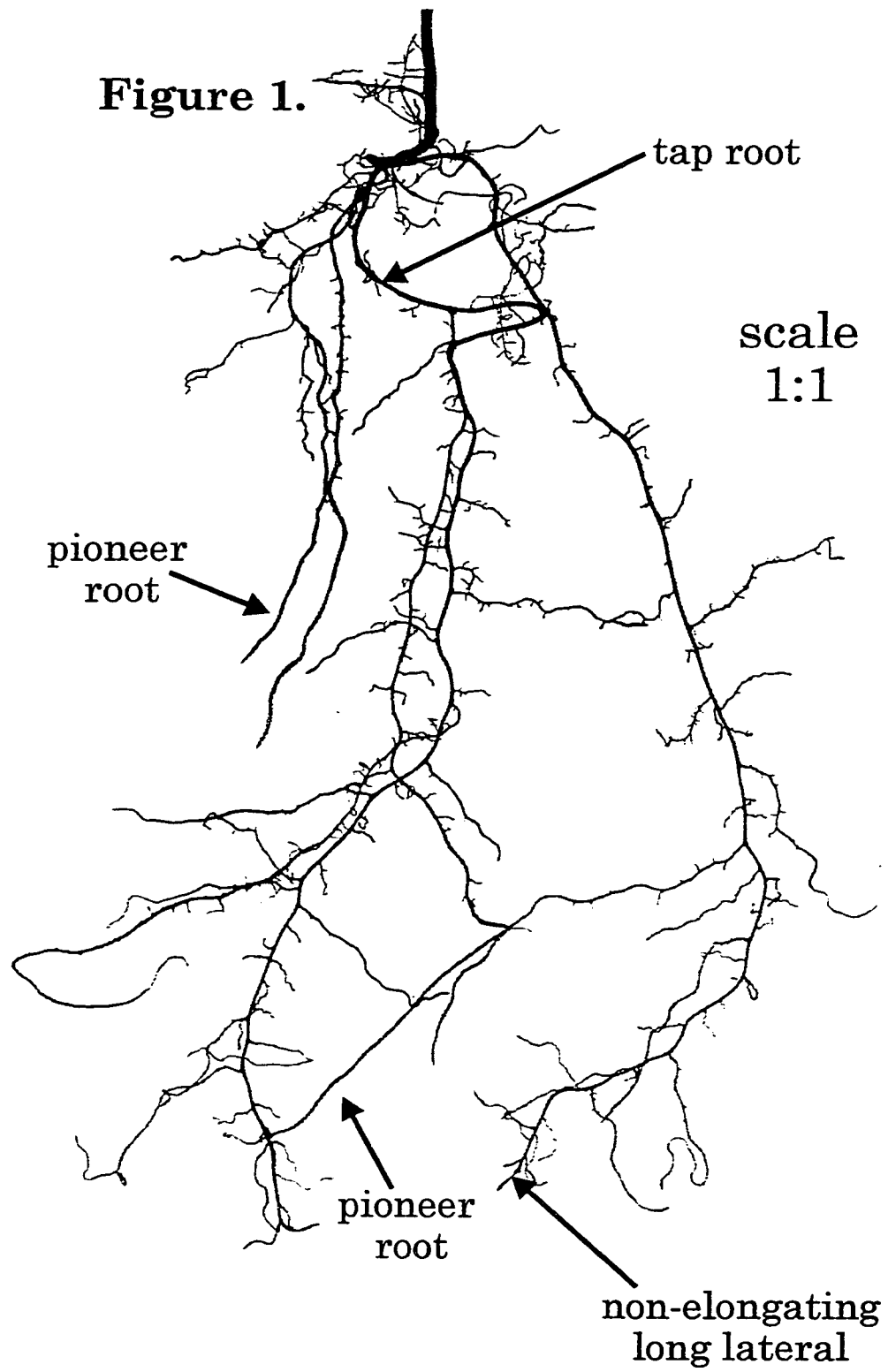
symplastic tracer generally failed to enter even the cortex of more mature regions of the root, being stopped at the root surface. Its presence in the stele in these regions showed that it was taken up distally. The presence of the symplastic dye in passage cells suggested that they were alive and that the transport through them was feasible. However, the conclusiveness of tracing the movement of dyes such as FDA through the symplast of roots may not be unchallenged. The dye was applied externally and had to cross the plasma membrane to enter into the symplast. If it could go in, it could also go out and move through the apoplast. FDA was, thus, capable of demonstrating a plasma membrane-apoplast-plasma membrane pathway which could not be distinguished from a strictly symplastic pathway. The former type of transport is likely the prevailing type in white spruce roots. This kind of transport would be reduced by the secondary-state endodermis due to the cell wall suberization in cells other than passage cells.

The composite transport model of the root (Steudle and Peterson, 1998) assumes that Casparian bands are not completely impermeable to water and that the apoplastic blockage can be bypassed. Experiments with tracer dyes do not allow to verify this assumption as the stoppage of apoplastic tracers by the endodermis demonstrates only a barrier to the movement of the dye and not to the movement of water. The experimental evidence presented by Steudle *et al.* (1993) and Peterson *et al.* (1993) as supportive of the composite transport model and of small contribution of the endodermis to the overall radial resistance to water movement in roots can be disputed. If the symplastic pathway is the main contributor to the water movement in roots, puncturing the endodermis may not cause any dramatic change to the flux of water through roots. Wounding roots and killing the living tissues present in the radial pathway of roots also does not necessarily demonstrate the contribution of these tissues to the overall resistance to the radial flow of water. The significant increase in hydraulic conductivity of roots in which the tissues outside of the endodermis were removed and the endodermis was injured could be interpreted as the result of the removal of the apoplastic barrier in the endodermis (its tangential walls became exposed and Casparian bands could be easily bypassed) and not necessarily as the result of removing a significant portion of the composite pathway.

The experiments with symplastic and apoplastic tracers indicated the existence of mechanisms that controlled movement of certain molecules in the apoplast and in the symplast. Numerous questions remain to be answered, especially regarding the control of the movement of molecules in the symplast. The involvement of fungal hyphae in the control of symplastic transport would be of special interest considering the extent of mycorrhizal development on spruce roots. Ashford *et al.* (1988) demonstrated the blockage of apoplastic transport into roots of *Pisonia* by a fungal sheath on their surfaces. It remains unknown in what way mantles can modify the

apoplastic transport into conifer roots and where in the symbiosis between a fungus and conifer the symplastic movement of solutes is controlled.

Figure 1. A digital image of a white spruce seedling root system half way through the first post-germination growing season. The root system is heterorhizic. Many long lateral roots are white (not visible on the image) some distance above root tips. Note the presence of the unbranched, pioneer-type root portions, multibranched roots, and forked tap root (labeled on the figure).



Figures 2-5. Median longitudinal sections through paraffin-embedded root tips of long laterals of white spruce seedlings.

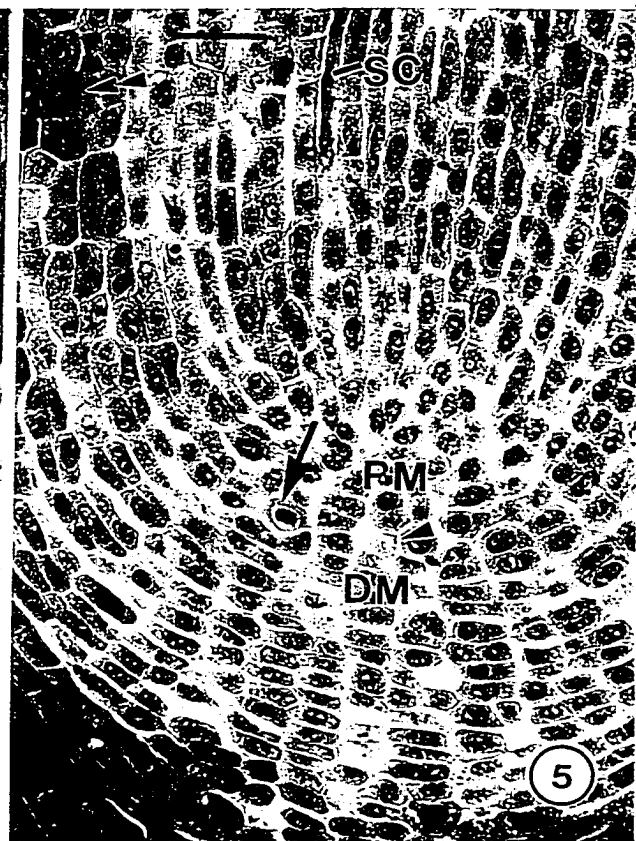
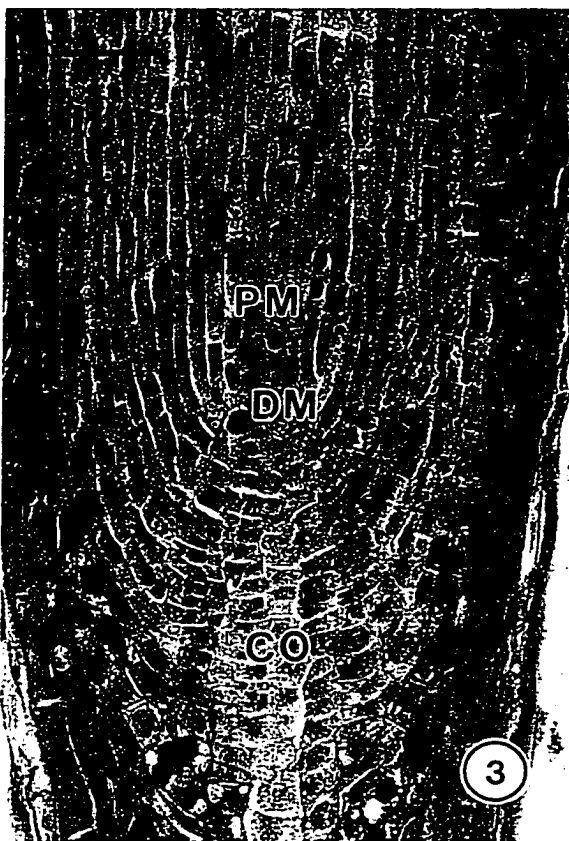
Figure 2. A section through the tip of an elongating, white lateral root collected in May, two months after germination. Root cap (RC) is long and massive, the column (CO) is well discernible below the root meristem (arrow). Outer limits of the future stele (S) can be approximately distinguished from the cortex (C). Bar = 130 μ

Figure 3. An enlarged portion of the root apex from Fig. 2 showing in greater detail the proximal (PM) and distal (DM) parts of the root apical meristem. There is no distinct quiescent region between the PM and DM. No phenolized cells occur above the meristem within the portion included on the photo. Bar = 60 μ m

Figure 4. A section through a root tip of a yellowish- brown lateral root collected in summer. The root did not appear to be elongating. Root cap and column are reduced compared the white, elongating root shown on Fig. 2. Note the presence of dark phenolized cells (arrowheads) 3-4 layers wide on both sides of the undifferentiated stele. Also note that two innermost layers of the phenolized cells become dark further basipetally (arrow) than the outer dark cells. The inner cells are likely the differentiating pericycle and proendodermis. (Phenolized, necrotic cells of the cortex form a continuous sheath of the rhizoderm (arrows) together with root cap cells extending around the root tip. Phenolics present in this layer of crushed cells cause the browning of the root surface.

Bar = 80 μ m

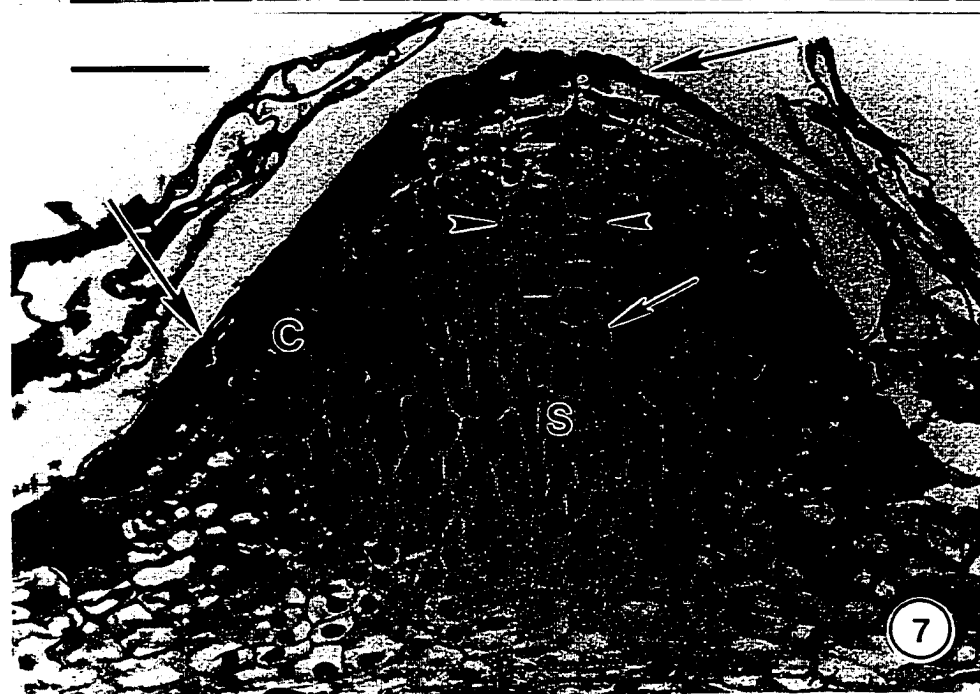
Figure 5. A more detailed view of the meristematic region of the root from Fig. 4. The apical meristem appears to have a quiescent centre (arrowhead) with lightly staining nuclei. The origin of phenolized cells above the meristem (double arrowheads) can be traced back to the upper flank of the distal meristem (large arrow). The phenolized cells shown on this figure are cortical cells. The pericycle, derived from the proximal meristem becomes phenolized further up the root as it was shown in Fig. 3. Long secretory cell (SC) is present. Bar = 50 μ m



Figures 6-7. Light micrographs of lateral root initiation in white spruce seedlings (paraffin-embedded material).

Figure 6. Lateral root primordium developing from the outermost cells of the stele. The lateral root originated from the pericycle (PC). The stele (S) is located below the pericycle. Dark cells of the endodermis (arrow) are stretched by the developing root that pushes its way through the crushed cortex (C). A centrally located circular group of cells (between arrowheads) may represent the developing root apical meristem. Bar = 150 μ m

Figure 7. A developing lateral root that has broken through the cortex of the parent root. Vertical files of cells (arrow) extend from the proximal part of the meristematic region (between arrowheads) toward undifferentiated stele. The empty-looking cells at the root tip and the dark cells on the sides of the root form the rhizodermis (larger arrows) and do not appear to be distinct from that of the rest of the cells at the root flanks that will form the future cortex (C). Bar = 100 μ m

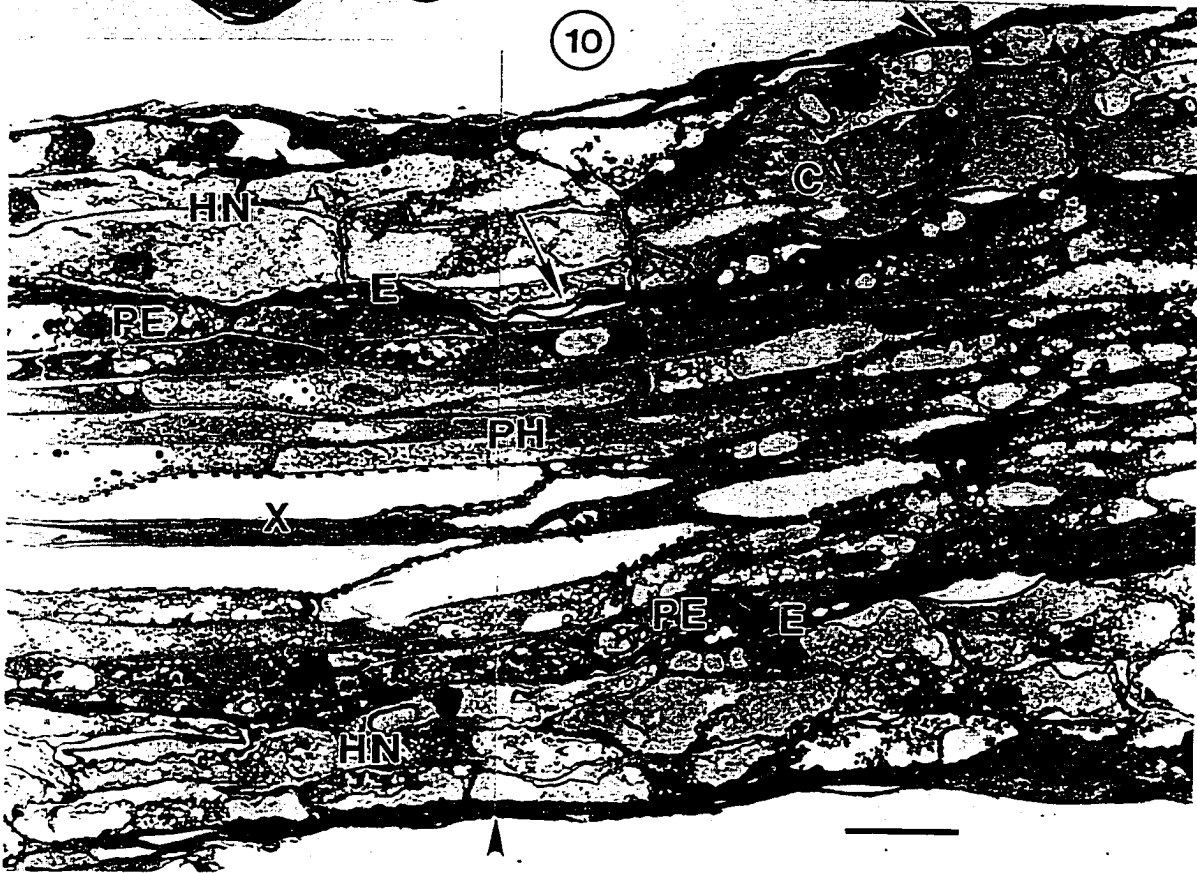
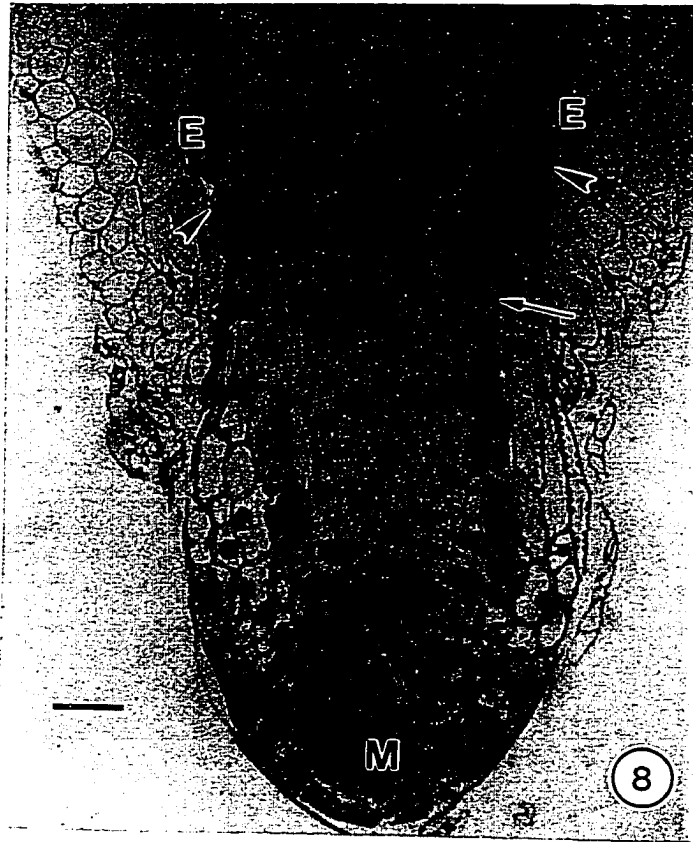


Figures 8-10. Light micrographs of elongating and non-elongating short branch roots of white spruce seedlings (resin-embedded sections).

Figure 8. A median longitudinal section through a young, elongating lateral root that emerged from a tap root. The emerging lateral has not been invaded by mycorrhizal fungi. The darker staining cells at the apex form the meristem (M) of indistinct zonation. Developing endodermis of the lateral root are the dark staining cells with small dark phenolic droplets (arrow). Arrowheads show the junctions to the endodermis (E) of the parent root. Bar = 50 μ m

Figure 9. Portion of a median, longitudinal section through a non-elongating short lateral collected in November. Dark staining cells of the endodermis and pericycle (arrows) separate cortex with Hartig net (HN) from the stele (S). Arrowheads point to the bridge-like junctions between the endodermis and metacutis enveloping the apical meristem. No Hartig net occurs in the apex isolated by the metacutis and in the stele. Open primary xylem (X). Bar = 100 μ m

Figure 10. Enlarged view of a portion of the short lateral root shown on Fig. 9, from the region with differentiated primary xylem (X) and phloem (PH). Some cells of the secondary endodermis (E) collapsed (arrow). Pericycle (PE) contains abundant phenolics. Note the thick external coat (arrowheads) of cuticle and mucilages. Hartig net is present in the cortex (C). Bar = 40 μ m



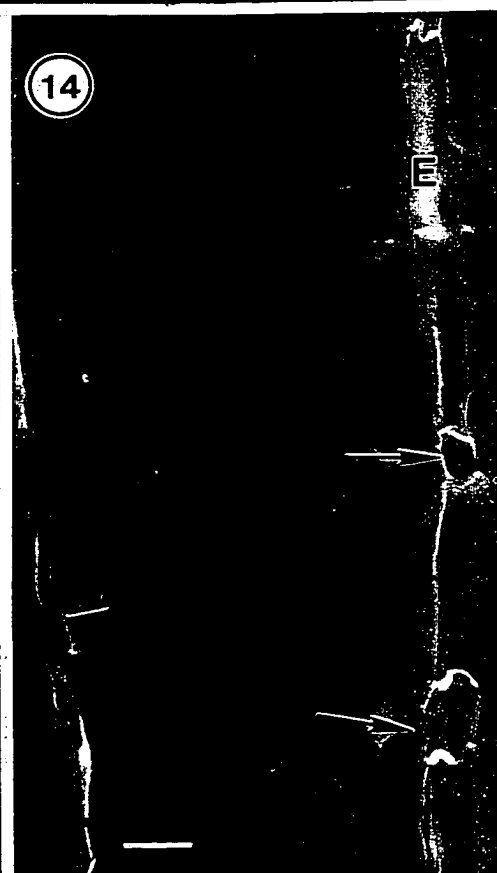
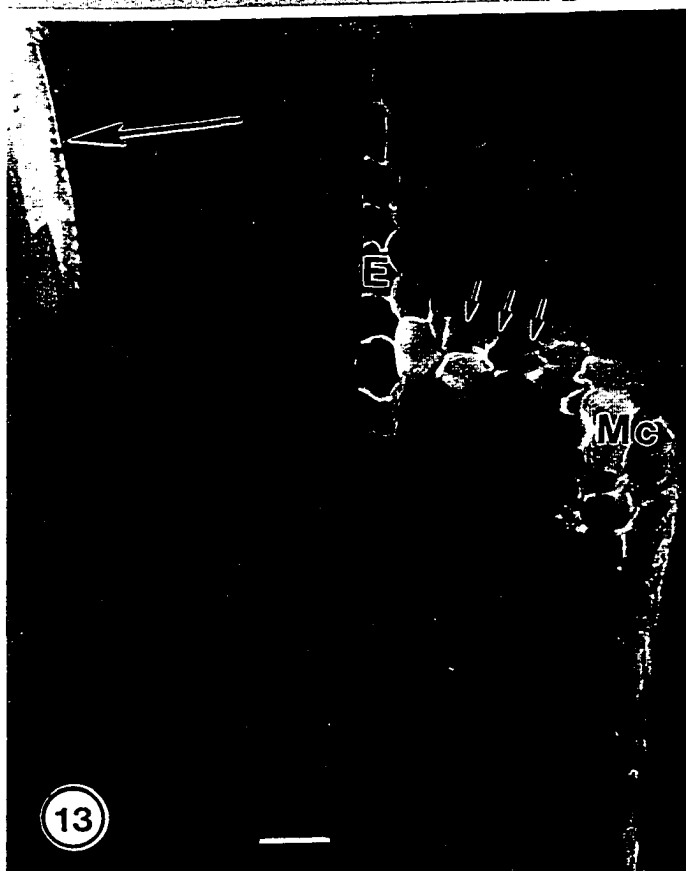
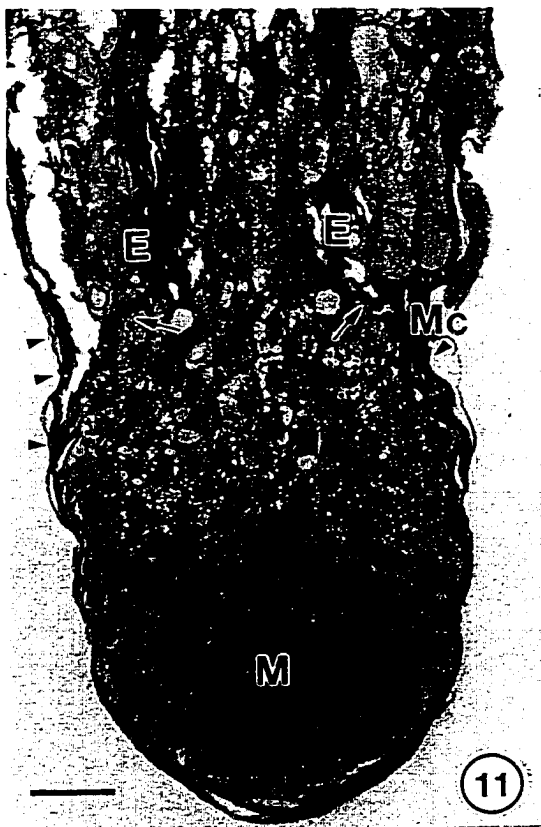
Figures 11-14. Light and fluorescence micrographs of the endodermis and metacutis in short branch roots of white spruce.

Fig. 11. An enlargement of the resin-embedded section of a root tip of the lateral root shown in Fig. 9. Secondary endodermis (E), metacutis (Mc), the bridge between the metacutis and endodermis (arrows), apical meristem (M), Note the external root layer (arrowheads) separated from the root during sectioning on one side of the root but extending on both sides of the section basipetally and connected to the metacutis. Bar = 50µm

Fig. 12. Fluorescence of the metacutis and secondary-state endodermis stained with berberine/aniline blue. Unfixed, cryostat-sectioned material. Arrows show the metacutis/endodermis bridge. Primary xylem (larger arrow). Bar = 100µm

Fig. 13. An enlargement of a portion of Fig. 12 showing details of the metacutis/endodermis bridge (arrows) in a non-elongating short lateral root. Except for the most acropetal cell, the endodermis (E) is at the secondary state as indicated by the fluorescence of its tangential walls. Differentiated tracheids of the primary xylem (larger arrow) extend acropetally almost as far as the endodermis. Bar = 50µm

Fig. 14. A portion of the endodermis away from the root apex of a non-elongating root. Secondary endodermis (E) with passage cells (arrows). The berberine-enhanced fluorescence on the left (arrowhead) is from the cutinized cells at the root surface. Unfixed, cryostat-sectioned material. Bar = 25µm



Figures 15-16. Light micrographs of resin-embedded sections through parent/lateral root junctions.

Figure 15. A glancing section through a junction between a parent root (PR) root and its short (SHT) lateral. Secondary endodermis (E) of the short lateral arches into the parent root joining to its endodermis (arrows). Cortex (C), stele (S). Bar = 50 μ m

Figure 16. A higher magnification view of the junction between a parent root and a lateral root showing the secondary endodermis of the lateral root (LE) and the secondary endodermis of the parent root (PE). Some phenolized stellar cells contain starch (St). Cortex (C), stele (S), xylem (X). Bar = 50 μ m



Figures 17-21. Light and fluorescence micrographs showing cross sections through long lateral roots at various stages of stele and endodermis differentiation.

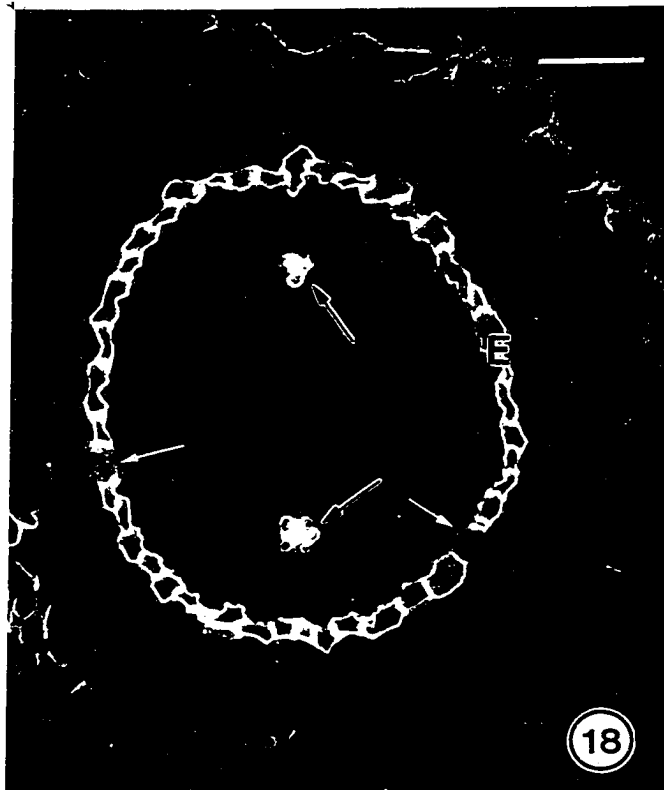
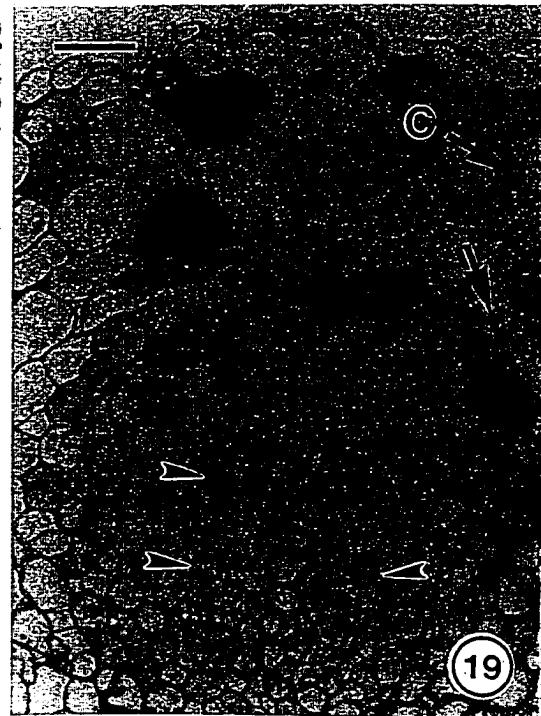
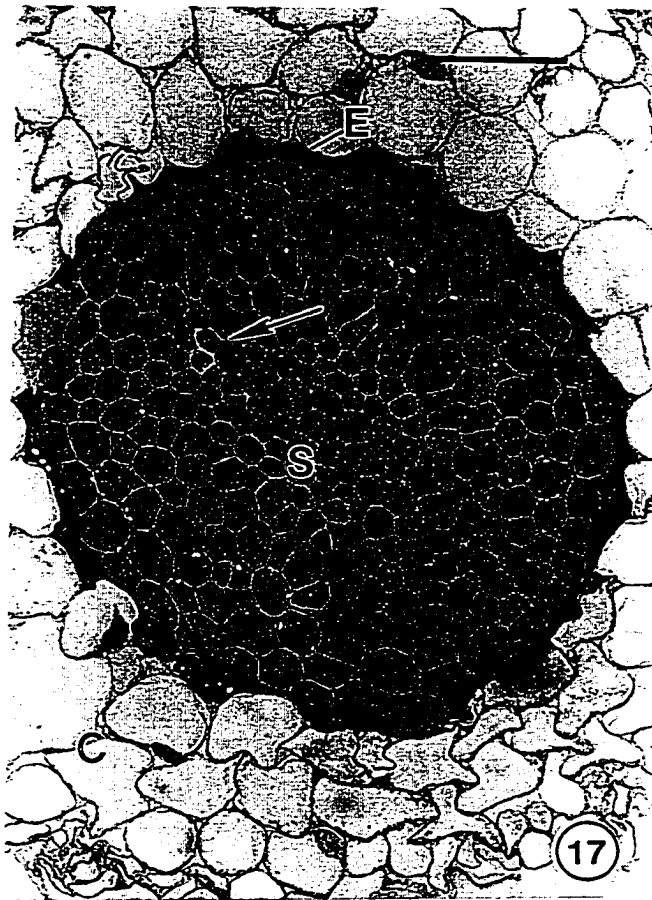
Figure 17. A central portion of a cross section of a white, elongating, resin-embedded tap root. Open primary xylem elements (arrow) have just begun to appear in the darkly stained stele (S). The endodermis (E) is in the primary state but the ongoing suberization of tangential walls was detected with an electron microscope. Cortex (C). Bar = 100 μ m

Figure 18. Cross section of an unfixed, cryostat-cut second order lateral with a browning root tip collected in summer. The section taken 15 mm above the root tip shows berberine-enhanced lignin fluorescence of cell walls of a developing diarch primary xylem (dark arrows) enclosed by the endodermis in the secondary state (E). Note strong berberine induced fluorescence of suberin in the inner and outer tangential walls of the endodermis except in passage cells (white arrows). Bar = 100 μ m

Figure 19. A cross section through a resin-embedded segment located 15 mm above the browning root tip of a second order lateral collected during summer. Secondary growth is evident (arrowheads point to the secondary xylem). Some cortical and secondary endodermal cells have collapsed (arrow). Bar = 50 μ m

Figure 20. Enlarged portion of Fig. 19 showing cells of the endodermis. The cell indicated by the arrow appears to be a passage cell with a plasmolyzed protoplast adhering to the radial but not to tangential cell walls. Bar = 20 μ m

Figure 21. A portion of a cross section through a resin-embedded, non-elongating second-order lateral collected in November. Six open primary xylem elements (arrowhead) are present 5 mm above the root tip. Secondary-state endodermis is located near the outer circumference of the root. Cortex is reduced and many of its outermost cells collapsed (arrow). Bar = 30 μ m

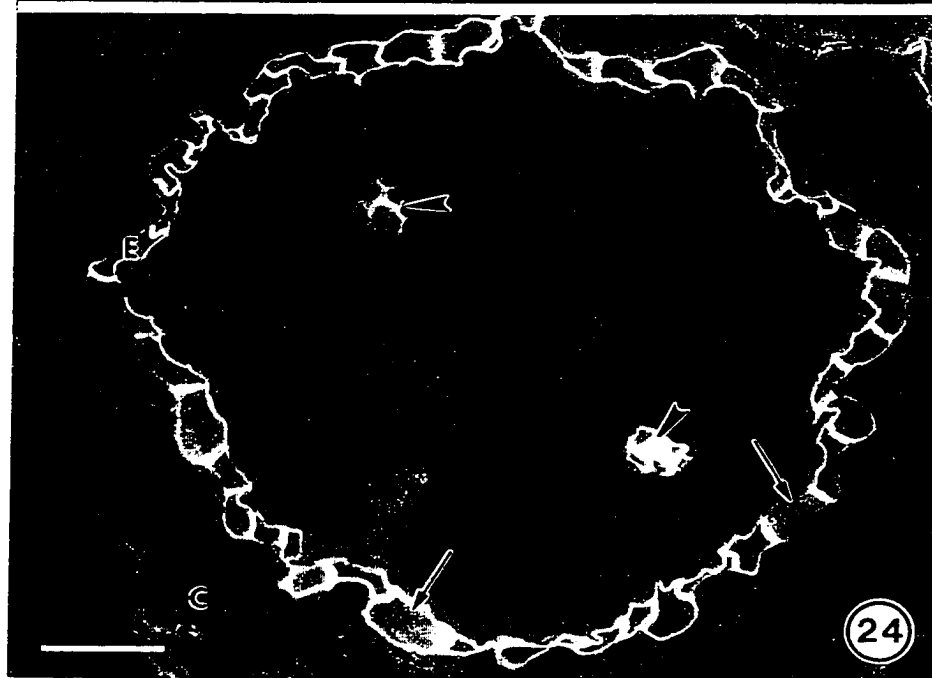
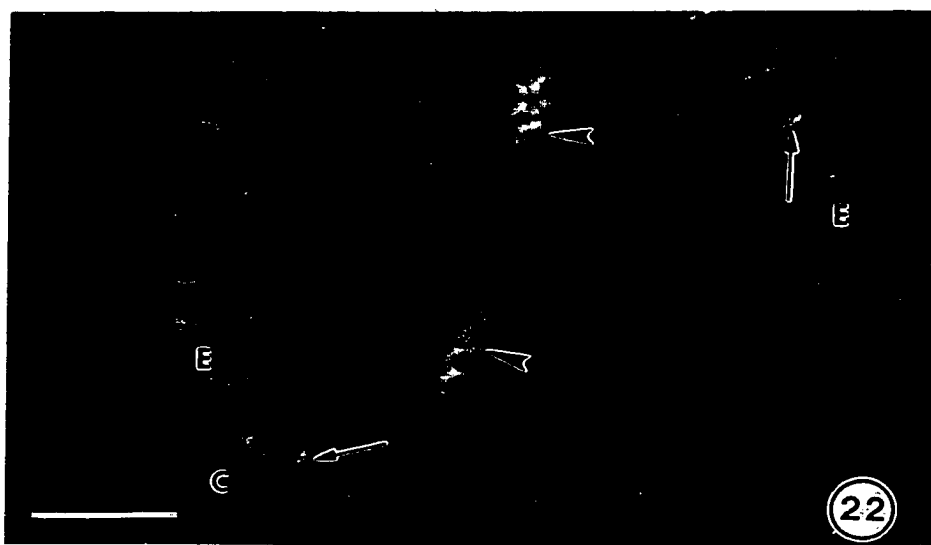


Figures 22-24. Fluorescence micrographs showing the berberine-induced fluorescence of the endodermis and the primary xylem in cryostat-sectioned short lateral roots of white spruce.

Figure 22. Primary endodermis (E) with Casparian band (arrows) enclosing the developing diarch primary xylem with 6-7 tracheids with lignified (fluorescent) walls at each pole (arrowheads). Cortex (C). The cross section was taken approximately 2 mm above the tip of an elongating but browning root. Bar = 50 μ m

Figure 23. A cross section of the same root as in Fig. 22 but approximately 0.5 mm more basipetally. The endodermis is at the secondary state with a suberin layer on the inner tangential (larger arrow) and on radial walls (white arrows). More primary xylem elements with lignified cell walls (arrowheads) are present than on the previous section. The intense fluorescence is from resin ducts (Rd). Passage cell (small arrow). Bar = 50 μ m

Figure 24. A cross section less than 1 mm behind the apex of a non-elongating root collected in November. The endodermis is at the secondary state with inner and outer tangential walls suberized except for passage cells (arrows). Note that there are fewer lignified primary xylem elements (arrowheads) than on Fig. 22. Bar = 50 μ m



Figures 25-29. Electron micrographs of the proendodermis in roots of white spruce.

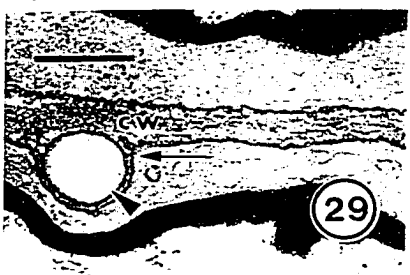
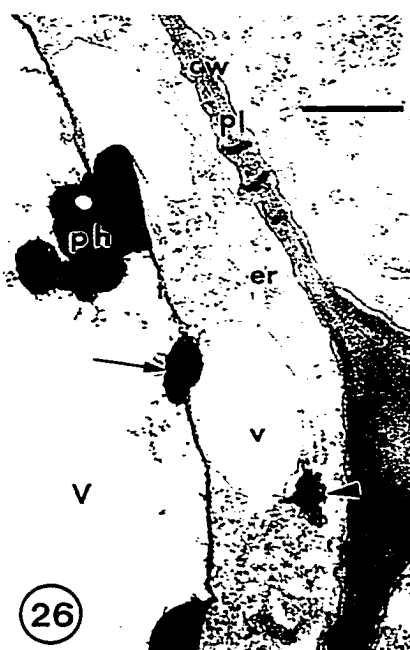
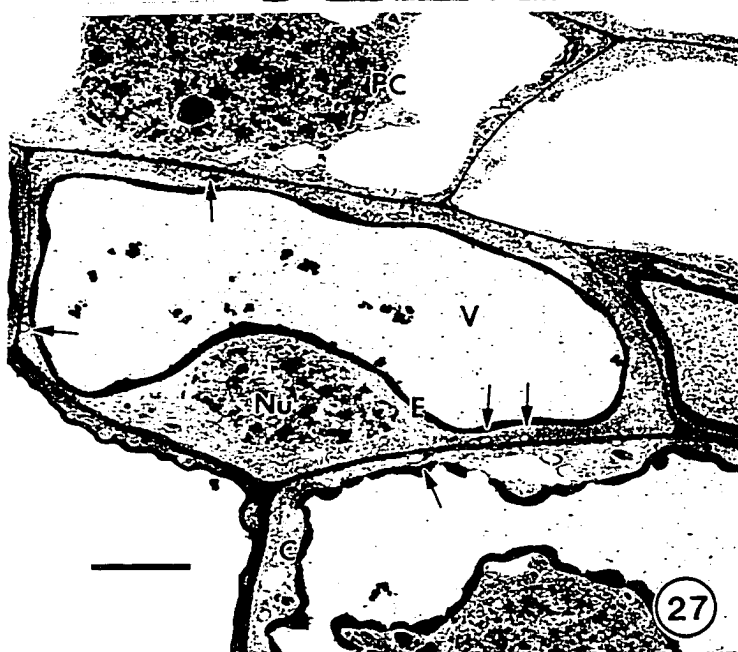
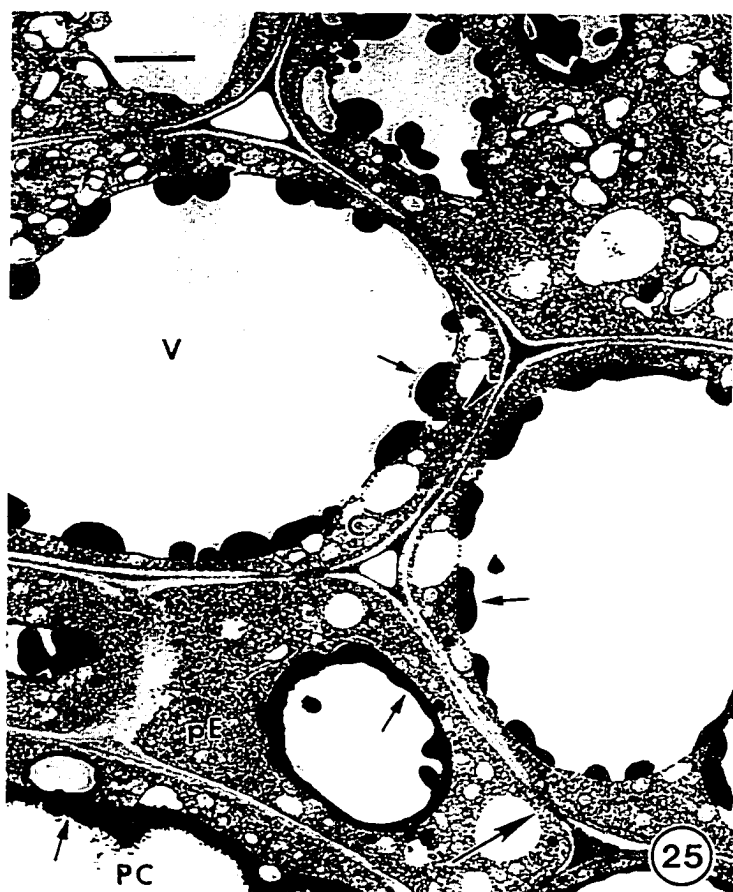
Figure 25. Proendodermis (pE) in the undifferentiated region above the root apical meristem. Phenolic deposits in the form of droplets and amorphous coats (small arrows) on tonoplasts of vacuoles (V) are visible in cortical cells (C) and in cells of the pericycle (PC). Some phenolic material is present in the cytoplasm (arrowhead). Larger arrow points to plasmodesmata in the tangential cell wall of the proendodermis that can be identified only by its position in the root. Bar = 4 μ m

Figure 26. Phenolic deposits (ph) on a tonoplast (t) of a vacuole (V) in a proendodermal cell. A droplet of phenolic material is positioned in between a larger vacuole and a smaller one (v) that appear to be fusing. Deposits of material of similar electron density to the phenolic material (arrowhead) occurs near the cisternae of endoplasmic reticulum (er). Plasmodesmata (pl) are present in the tangential cell wall (cw). Bar = 0.5 μ m

Figure 27. Large vesicles with clear contents (arrows) appear near or at the cell walls of proendodermal cells and the inner tangential walls of cortical cells. Vacuoles occupy a large portion of proendodermal cells but the cytoplasm and nuclei (Nu) are also prominent. Bar = 3.5 μ m

Figure 28. Vesicles with clear contents (arrowheads) are located near or at the tangential cell wall of a proendodermal cell. Rough endoplasmic reticulum (rer) occurs near the vesicles. Bar = 0.4 μ m

Figure 29. A large vesicle with clear contents at the cell wall of a proendodermal cell. The vesicle is located outside of the plasma membrane (arrow). Bar = 0.25 μ m



Figures 30-33. Electron micrographs of the primary-state endodermis in lateral roots of white spruce.

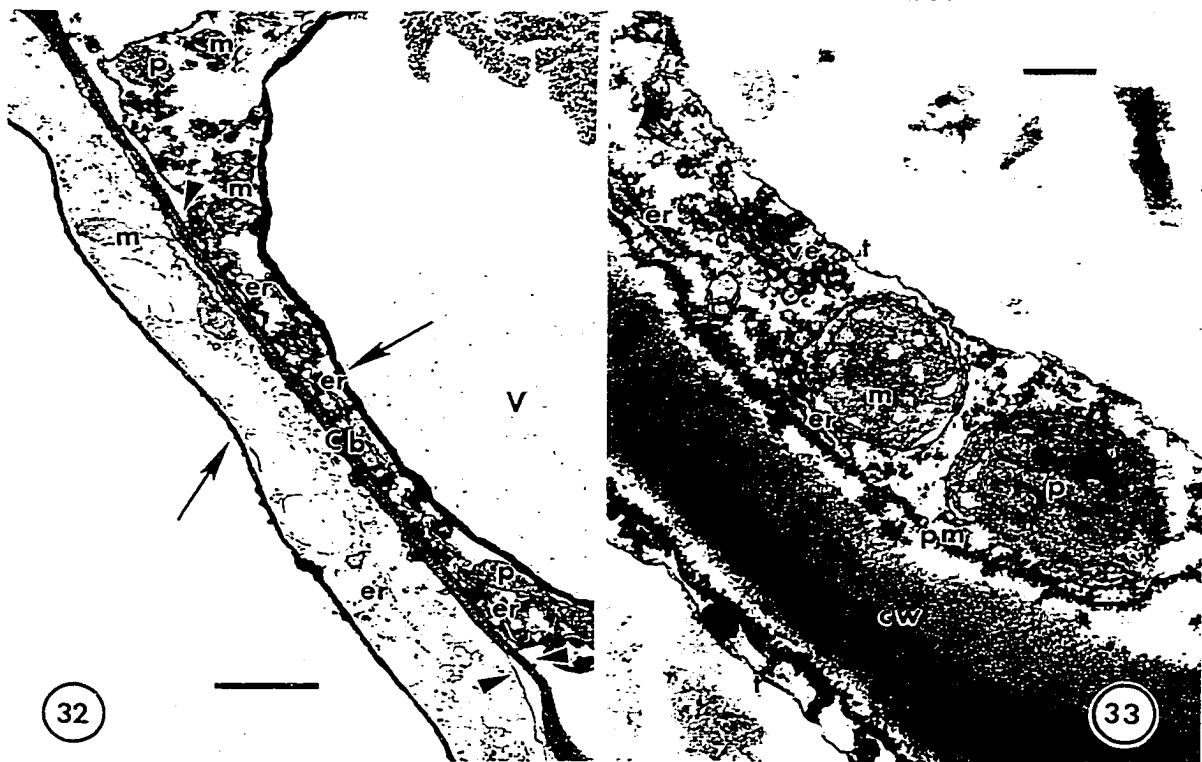
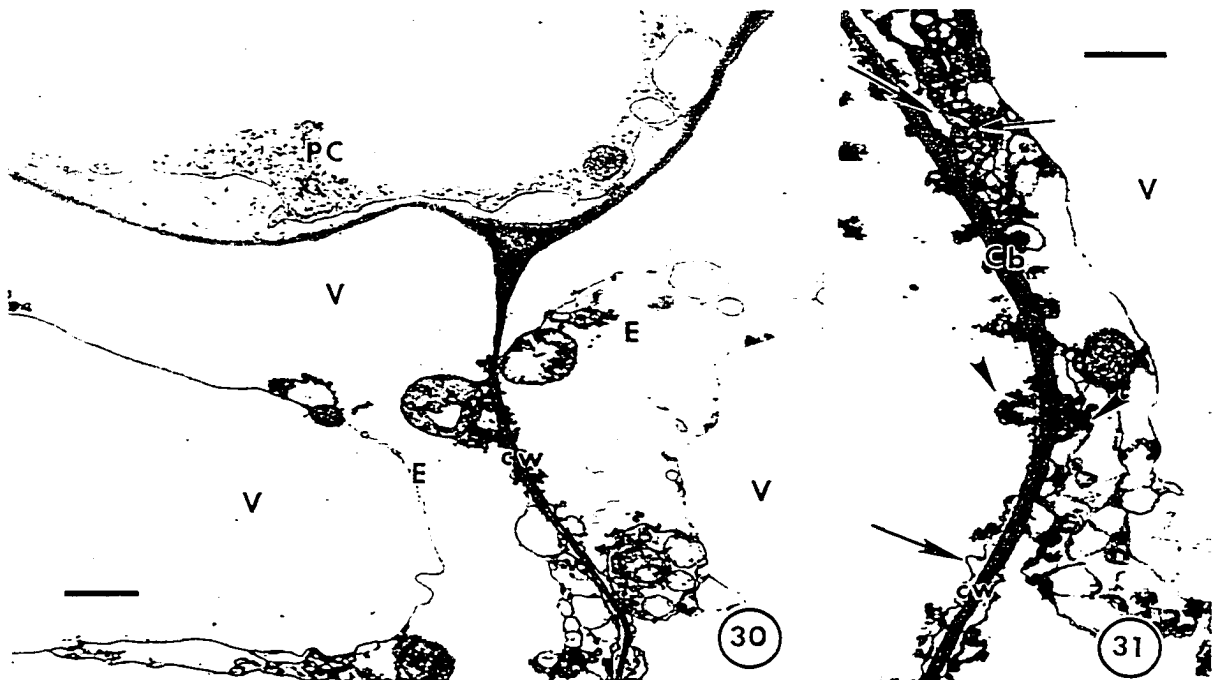
Figure 30. A low magnification view of portions of two neighbouring endodermal cells (E) and a portion of a pericycle cell (PC). Radial cell walls (cw) separating the two endodermal cells are bent and appear uniformly smooth from the presence of the Casparian band. Most of the cell volume of the endodermal cells is occupied by large vacuoles (V). The cytoplasm and organelles occur near the cell walls or in clumps dispersed throughout the cells. Note the absence of phenolic deposits on the tonoplasts of vacuoles. Bar = 1 μ m

Figure 31. The Casparian band (Cb) in radial cell walls (cw) of two endodermal cells. The plasma membrane undulates away from the Casparian band (larger arrows) but adheres tightly to the cell wall over most of the cell length occupied by the Casparian band. The cytoplasm is transparent and contains dispersed granular material (arrowheads) and vesicles (small arrow). Bar = 0.5 μ m

Figure 32. Casparian band in cell walls of another pair of endodermal cells in which the cytoplasm occurs in narrow bands along the cell walls and some phenolic deposits (arrows) are present on the tonoplasts. The plasma membrane pulls away from peripheral portions of the cell walls (arrowheads) but not from the central region occupied by the Casparian band. Mitochondria (m), plastids (p) and the endoplasmic reticulum cisternae (er) are numerous. Bar = 1 μ m

Figure 33. A higher magnification view of a portion of the cytoplasm and radial cell walls (cw) located in the cell corner where there is no Casparian band. The cytoplasm contains numerous vesicles (ve) probably of dictyosomal origin. The plasma membrane (pm) is loaded with granular material (arrowheads). Tonoplasts (t) have no phenolic deposits.

Bar = 0.25 μ m



Figures 34-38. Electron micrographs of the endodermis during the deposition of the suberin layer on the faces of the cell walls.

Figure 34. Portions of two endodermal cells (E) and a cortical cell (C) from a long lateral root. The inner face of a portion of the outer tangential cell wall of a deformed endodermal cell in the upper part of the micrograph (asterisk) is covered with well developed layer of suberin (su). The cell appears to be alive, judging by the cytoplasm and the darkly coated plasma membrane (arrowhead). The second endodermal cell has less developed suberin layer on its outer tangential wall. Its cytoplasm contains numerous strands of endoplasmic reticulum (er) and oval, vesicle-like structures probably representing transversely sectioned tubules of the er. Granular, dark material (small arrow) is present between the cell wall and the plasma membrane (pm) which is darker than that of the neighbouring cortical cell. Phenolic (ph) deposits are present in vacuoles (V) of both the endodermal and the cortical cell. Bar = 0.4 μ m

Fig. 35. The deposition of a suberin layer on the radial walls between two endodermal cells appears to be in progress. The plasma membrane has separated from the suberin layer (arrow) and it is undulating in some areas (smaller arrows). Casparian band (Cb). Bar = 0.25 μ m

Figure 36. Microtubules (mt) are present near the plasma membrane of an endodermal cell. The suberin layer on the inner face of the outer tangential wall is thin, probably at the early stage of its deposition. Granular electron-dense material occurs between the plasma membrane and the suberin layer (arrows). Bar = 0.1 μ m

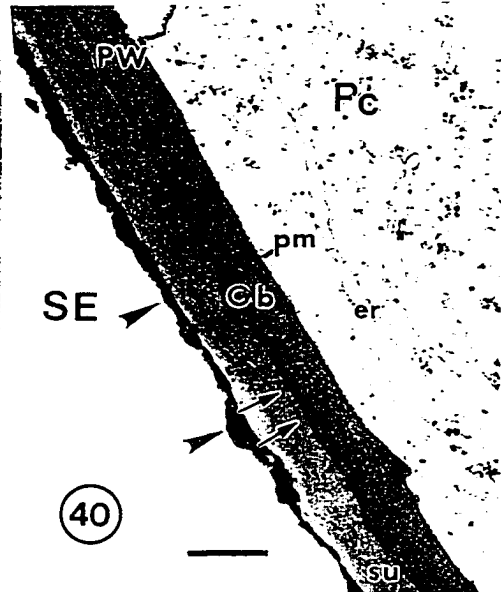
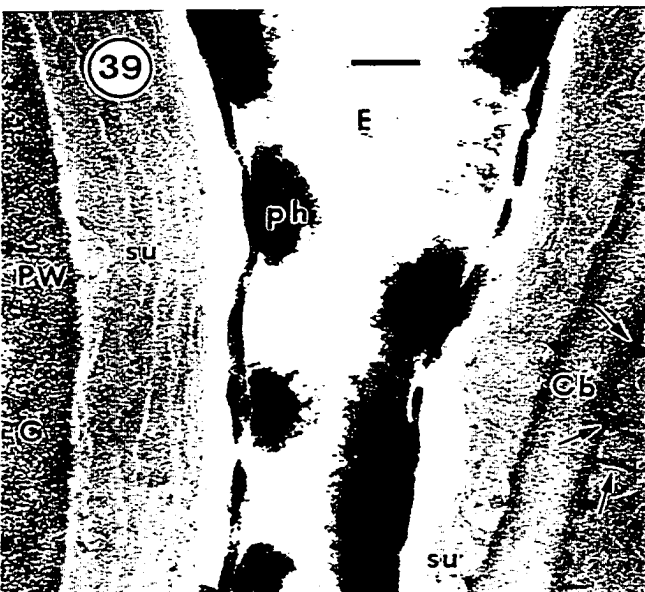
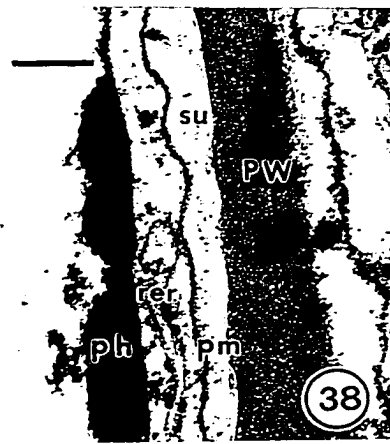
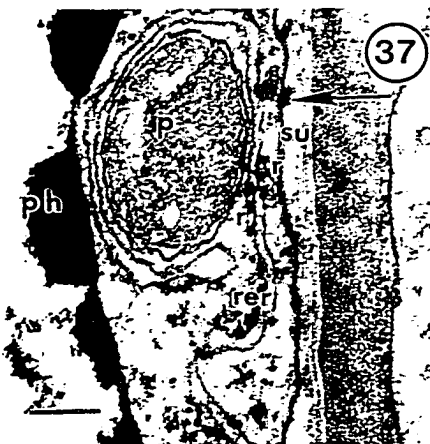
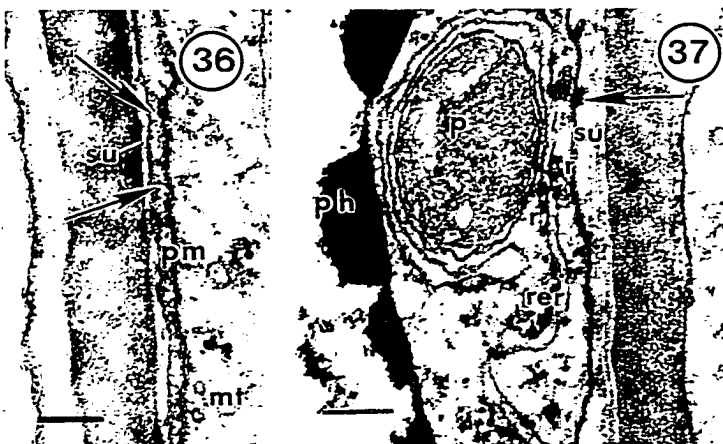
Figure 37. Strands of rough endoplasmic reticulum occur near the cytoplasm next to the developing suberin layer. Granular material (arrow) is present between the plasma membrane and the suberin layer. Plastid (p), ribosomes (r). Bar = 0.15 μ m

Figure 38. Thin layer of suberin on the inner face of the inner tangential wall of an endodermal cell. Again, rough endoplasmic reticulum (rer) occurs next to the densely stained plasma membrane. The suberin deposit on the primary cell wall (PW) varies in thickness. Bar = 0.15 μ m

Figures 39-40. Electron micrographs of the secondary-state endodermis.

Figure 39. A collapsed cell of secondary-state endodermis with massive suberin (su) layers. The cell appears dead. The plasma membrane, the cytoplasm and tonoplasts cannot be discerned. Patches of phenolic material occur along the suberin layer. Note spiked protrusions (arrows) running from the Casparian band (Cb) into the lamellar suberin. Portion of a primary wall of a cortical (C) cell is visible. Bar = 0.1 μm

Figure 40. Casparian band in the radial cell wall between an endodermal cell (SE) at the secondary state with suberin layer and a passage cell (Pc) without the suberin layer. The plasma membrane (pm) of the passage cell adheres to the cell wall along the Casparian band. The cytoplasm of the secondary endodermal cell turned into a dark amorphous deposit on the inner face of the suberin layer (arrowheads) while it appears normal in the passage cell. Spiked protrusions (small arrows) from the primary wall (PW) into the suberin layer are present (arrows). Bar = 0.25 μm



Figures 41-46. Electron micrographs of the secondary-state endodermis.

Figure 41. Collapsing endodermal cells (E) next to the pericycle cells (Pc) in the region of the root where secondary growth had begun. The endodermal cells contain darkly stained remnants of the cytoplasm and fragments of tonoplasts covered with phenolics (arrowheads). Note twisted radial wall in which the suberin layer separated from the primary wall (between the two arrows). Pericycle cells contain phenolic compounds (ph) and starch (St). Bar = 3 μ m

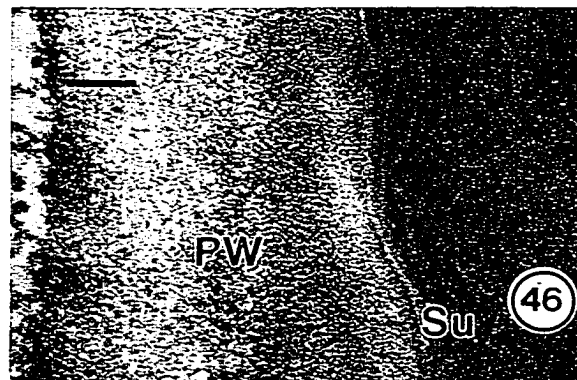
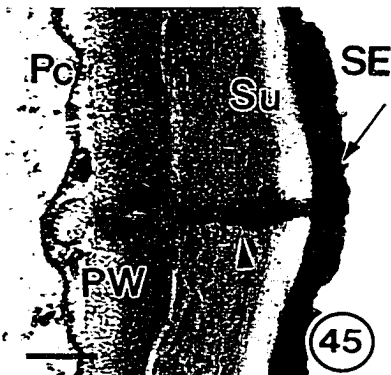
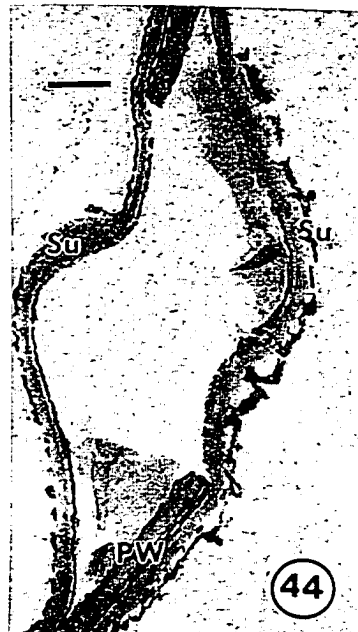
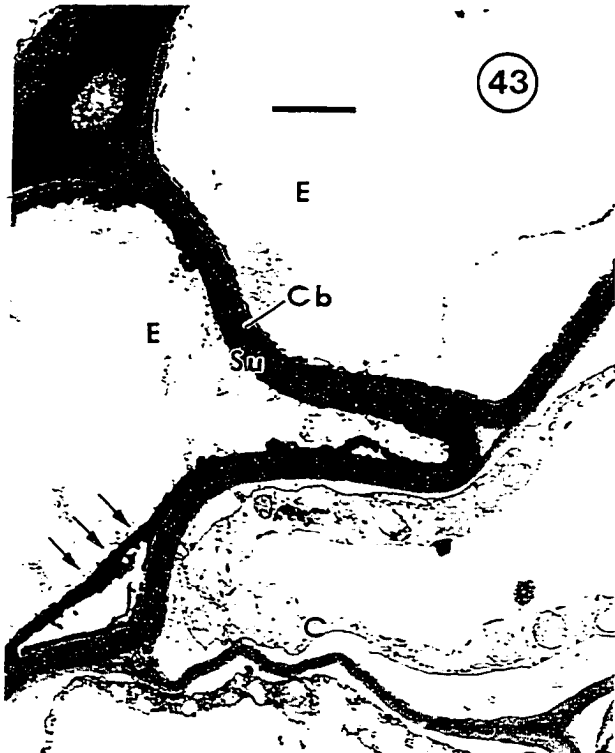
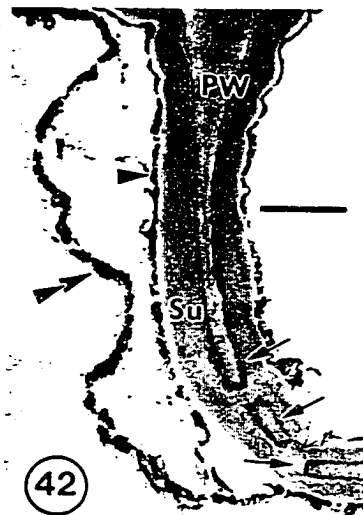
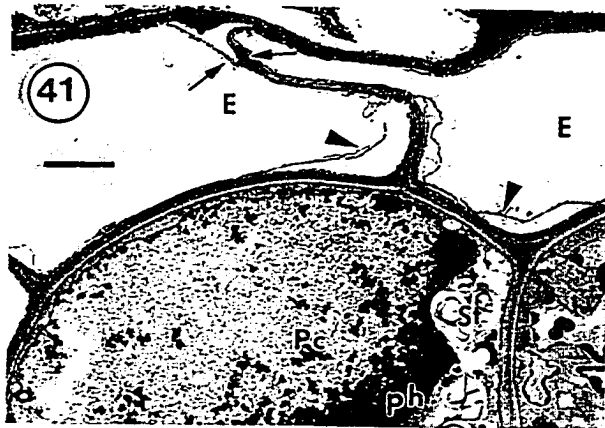
Figure 42. A portion of radial walls which shattered (arrows), stretched primary wall (PW) and an intact suberin layer (su). Cytoplasmic remnants coat the inner face of the suberin layer (arrowhead). Remnants of a tonoplast coated with phenolics (double arrowhead) are also visible. Bar = 0.5 μ m

Figure 43. Radial walls and portions of outer tangential walls of the endodermis next to cells of the cortex (C) that appear to be degenerating judging by the twisted cell walls and their plasmolyzed contents. Suberin layer is present on both sides of the Casparian band (Cb) in the radial walls and on inner faces of the tangential walls. Darkly stained remnants of protoplast of endodermal cells are visible (arrows). Cell walls of cortical cells are also twisted. Bar = 1 μ m

Fig. 44. Collapsed radial walls where pressed primary walls broke but the suberin layers have not broken. Bar = 0.4 μ m

Fig. 45. Plasmodesma (arrowhead) extending between a pericyclic cell (Pc) and an endodermal cell at the secondary state (SE). Plasmodesmata run through the primary cell walls (PW) and the suberin layer of the endodermis to the dark, granular cytoplasm (arrow) of the endodermis that does not appear to be alive. Bar = 0.15 μ m

Fig. 46. A higher magnification view of a portion of the primary cell wall that appears granular/fibrillar in structure compared to the smooth, lamellar appearance of the suberin layer. Bar = 0.1 μ m



Figures 47-51. Electron micographs of portions of short lateral roots uncolonized and colonized by mycorrhizal fungi .

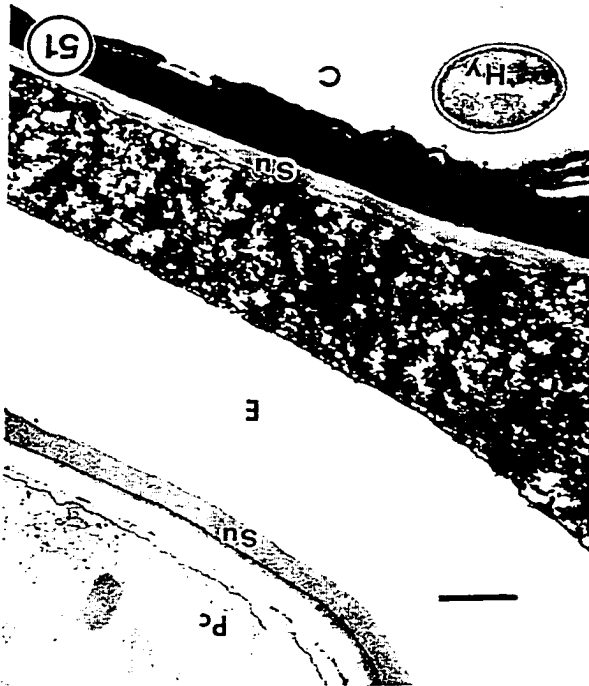
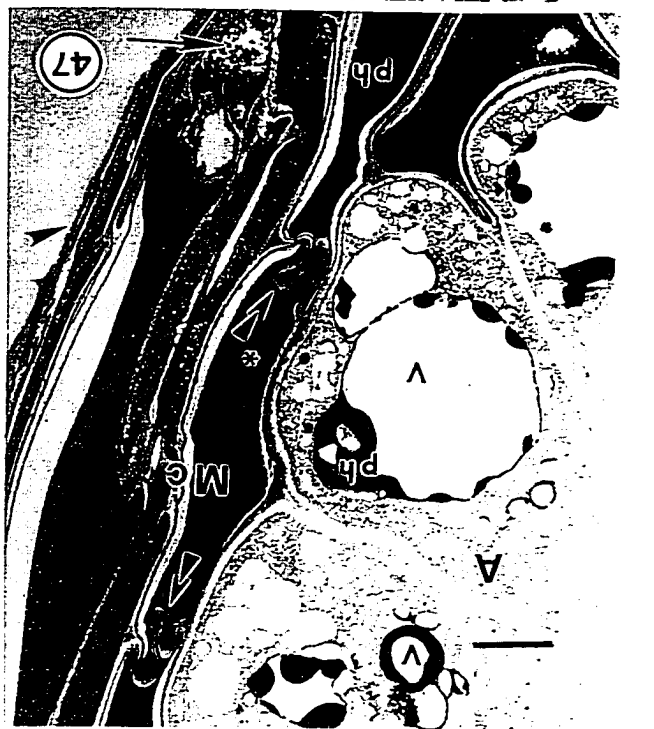
Fig. 47. A portion of a longitudinal section through the apical region of a short branched root showing the metacutis (Mc). The surface of the root apex is covered by collapsed cells. The outermost cells (arrowhead) appear to be squashed more than those in the inner cell layers. Cell walls of the surface cells in all layers are smooth and similar in appearance to Casparian bands of the endodermis (double arrowheads). Radial cell walls of collapsing cells (asterisk) are twisted (double arrowheads). Phenolic substances (ph) filling the cells differ in appearance from uniform, solid, black contents of the inner layer to lighter, grainy contents of the outer cells in some of which the phenolics appear dispersed (arrow). Apical cells (A) contain vacuoles (V) of various sizes with phenolic deposits on their tonoplasts. Fungal hyphae are not present. Bar = 2 μ m

Fig. 48. Root surface away from the root apex showing a mycorrhizal mantle (MN) formed by fungal hyphae (Hy) some of which are sectioned transversely (arrows) while others are sectioned in various oblique/longitudinal planes (double arrows). Some hyphae (double asterisks) have grown between collapsed cells of the rhizodermis (RZ). An amorphous, probably mucilagenous material (double empty arrowheads) occurs throughout the mantle. Bar = 2 μ m

Figure 49. A higher magnification view of the portion of the mantle shown on Fig. 48 showing smooth, suberized cell walls (arrow) of a collapsed rhizodermal cell filled with phenolics (ph) and clearly visible cellular contents of a cross-sectioned hypha. Bar = 0.8 μ m

Figure 50. Portion of a mycorrhizal mantle showing sectioned hyphae, one with a dolipore, parenthesome septal apparatus (arrow) indicating that the fungus is a member of Basidiomycotina. Amorphous mucilagenous material (double, empty arrowheads) is present between hyphae. Bar = 0.8 μ m

Figure 51. A section through a root showing a portion of an endodermal cell (E) at the secondary state with suberin layers (Su) on its tangential walls, cross-sectioned fungal hypha on the cortical (C) side, and no presence of hyphae at the pericyclic (Pc) side. Bar = 0.7 μ m



Figures 52-57. Confocal (false colored) and fluorescence-microscopy images of elongating long laterals of white spruce treated with apoplastic and symplastic tracer fluorochromes.

Figure 52. Confocal image of a root tip of an elongating long lateral root showing the fluorescence of an apoplastic tracer sulforhodamine G (red) staining limited to the root cap (RC) and the surface layer of root cells above the root apical meristem (M). The meristem and the undifferentiated portion of the stele (S) show only traces of the apoplastic stain Bar = 200µm.

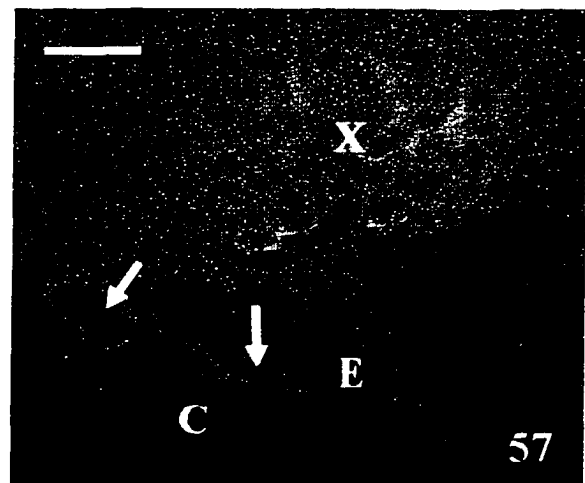
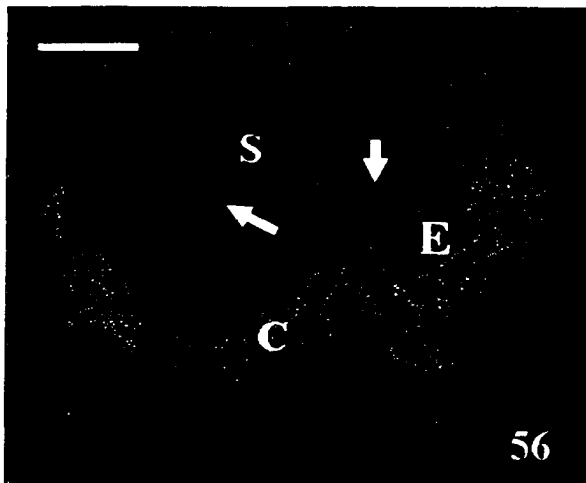
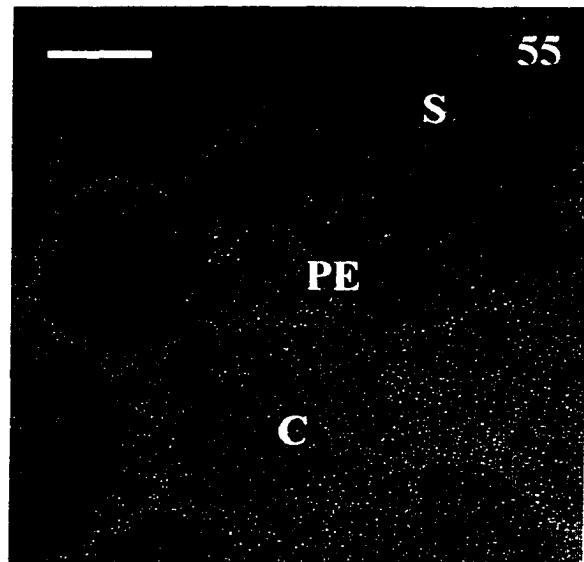
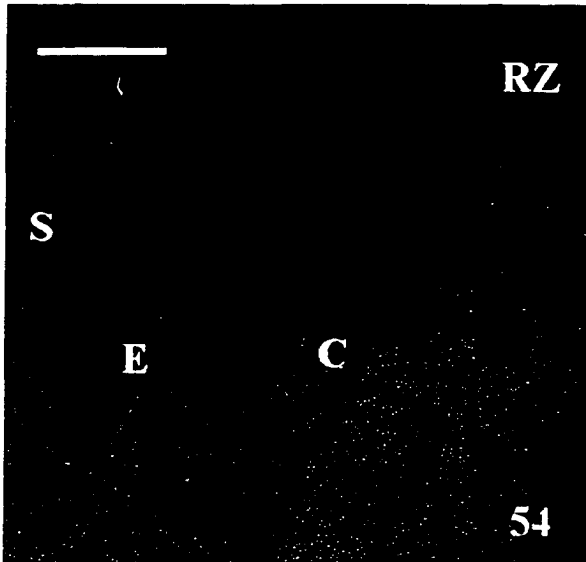
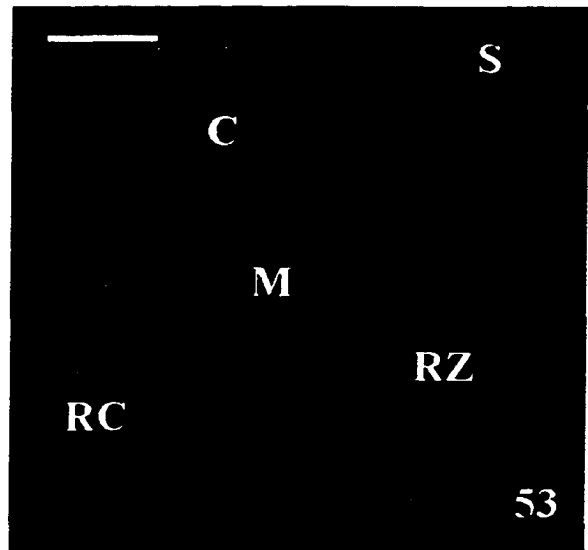
Figure 53. Confocal image of a root tip of an elongating long lateral root showing the presence of a symplastic tracer FDA (green) in most regions of the root tip except for the meristematic region. Staining is most intense in the cortex (C) but it is faint in the central region of the root containing undifferentiated stele, in the rhizodermis (RZ) and in the root cap. Bar = 200µm

Figure 54. A fluorescence-microscopy image of a free-hand cross-sectioned elongating long lateral root approximately 6 mm above the tip. No open xylem elements were present in the stele. The apoplastic tracer is present mostly in cell walls of cortical cells and accumulated (arrow) at the of the endodermis (E). Bar = 50µm

Figure 55. A fluorescence-microscopy image of a free-hand cross-sectioned root, 3 mm above the root tip. Cortical cells are heavily stained (red to intense-yellow) but there is no clear stoppage of the dye by the proendodermis (PE). Note almost no staining of cell walls in the undifferentiated stele. Bar = 25µm

Figure 56. A fluorescence-microscopy image of a free-hand cross section through an elongating long lateral root taken 60 mm above the tip showing the yellowish-red fluorescence of the apoplastic tracer. Two poles of differentiated primary xylem are visible (arrows) in the stele. The apoplastic tracer did not penetrate the endodermis (E). Bar = 150µm

Figure 57. A fluorescence-microscopy image of a free-hand cross-sectioned root 10 cm above the white tip showing the green fluorescence of the symplastic tracer FDA staining the contents of cells in the stele but not in the cortex. Contents of passage cells (arrows) are stained. Natural yellowish fluorescence of the xylem (X) is present. Bar = 100µm



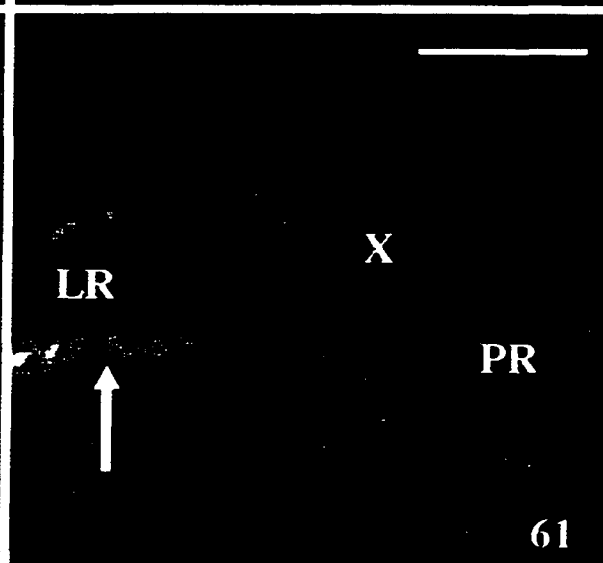
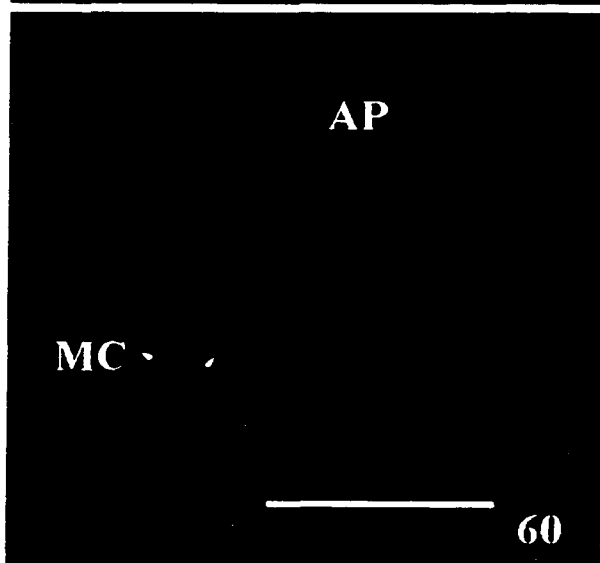
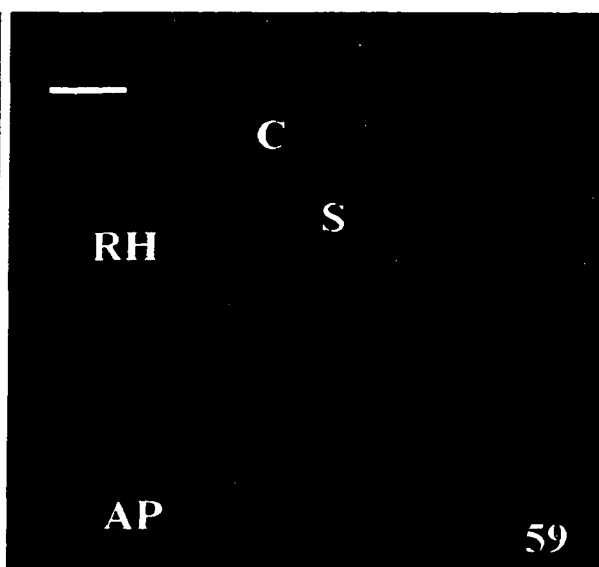
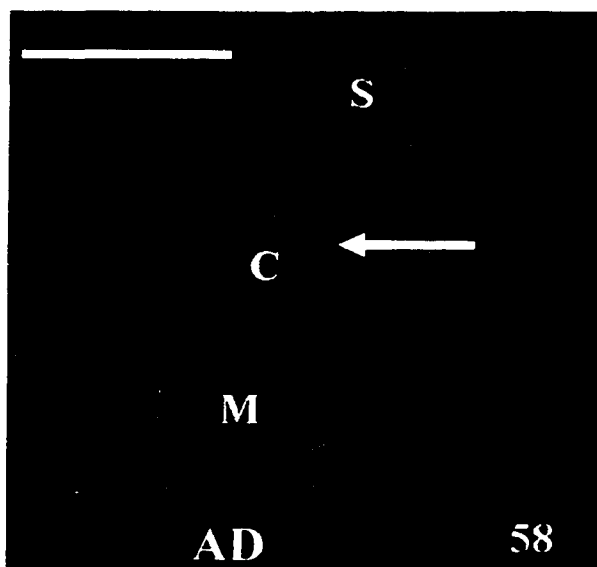
Figures 58-61. Confocal microscopy images of an elongating short root and of non-elongating roots of dormant white spruce seedlings treated with apoplastic and symplastic tracer fluorochromes.

Figure 58. Optical section through a double-stained root tip of a long lateral root with false coloring: green for the symplastic, red for the apoplastic tracer, appearing orange in regions where both tracers were present. The meristematic region (M) is not penetrated by either tracer. Both symplastic and some apoplastic tracers are present in superficial portion of the apical dome (AD). The apoplastic tracer is in cortical cells (C) but does not penetrate into the stele (S). The symplastic tracer is present in cortical cells blending with apoplastic tracer but it enters the stele short distance above the meristem (white arrow). Bar = 300µm

Figure 59. Optical longitudinal section above the apical region (AP) of a lateral root showing accumulation of the apoplastic tracer in cells of the rhizodermis (RH) and in cell walls of the cortex (C) but there is no penetration into the stele or to the apical region. Bar = 50µm

Figure 60. Optical section through a superficial portion of the root apex of a short branch root showing accumulation of the apoplastic tracer (red color) at the root apex surface covered by the metacutis (MC). Bar = 30µm

Figure 61. Optical section through the junction between a parent (PR) and a lateral root (LR) showing the presence of the apoplastic tracer in the cortex of the parent and the lateral root and intense yellow fluorescence of the stain near the endodermis-region of the lateral root (arrow). Some of the tracer is also present in the center of the parent root where the xylem is and in the stele of the lateral root. Bar = 200µm



CHAPTER 4: ROOT TRACHEIDS OF WHITE SPRUCE SEEDLINGS IN RESPONSE TO NITROGEN AVAILABILITY

ABSTRACT

This study examined how the availability of inorganic nitrogen (N) modified the anatomical characteristics of white spruce (*Picea glauca* (Moench) Voss) roots related to their hydraulic properties. Seedlings were grown for one growing season in 4 L capacity sand-filled pots under one of three N levels: low (10 ppm), medium (50 ppm) and high (125 ppm). First order lateral roots with intact tips were sampled from dormant seedlings in October. Root segments were collected from 4, 10, and 14 cm distances above the root tip for fixation and sectioning and for maceration. Additional specimens were collected from the 4 and 14 cm distances for maceration and scanning electron microscopy of xylem pits.

Root diameter and surface area occupied by the xylem on root cross sections increased basipetally in all treatments but some roots had more advanced secondary development at acropetal than at basipetal positions. Higher N-levels significantly increased root diameter and surface area occupied by the xylem. Secondary root development was more advanced close to the root tip in the two higher N treatments than in the low N treatment. There was a strong positive correlation between root diameter and cross-sectional root area occupied by the xylem (30-50% of the root cross section) but not in portions with little secondary development. Non-conducting portions within the xylem occupied 10-13% of its cross-sectional surface. Tracheids of the primary xylem were larger, with larger lumens but thinner cell walls than those of the secondary xylem. Low N treatment seedling tracheids had smaller total cross-sectional area, less lumen, and less cell wall surface area than the two other N treatments. Tracheid diameter means were 19-20 μm in the high and medium N treatments, and 15.2 μm in the low N treatment. The range was 4.5 - 51.3 μm . Tracheid length was not significantly affected by N. The average tracheid was about 1000 μm long, and the range was 110 - 3530 μm . Pit-border diameters ranged between 4.1 - 20.6 μm (10 - 11 μm at average) and were not affected by the N treatment. Pit aperture diameters were within 0.62 - 10.2 μm (average between 3 - 4 μm) and were also not significantly affected by the N treatment, although tracheids from the medium-treatment roots tended to have larger apertures. The pit border

diameter equals that of the margo while the aperture size should be similar to that of the torus of the pit membrane. If the capacity for axial water transport in spruce roots is affected by N, it would be by its impact on conduit diameter and, possibly on the pit-membrane pore sizes but not by changes to conduit length and to the size of the pit membrane surface area.

INTRODUCTION

Root tissues create the pathway for water movement in roots (Moreshet and Huck, 1991). Xylem anatomy is, therefore, an important factor affecting water flow through roots (Tyree and Karamanos 1981). While xylem development in relation to conductive capacity of roots has been extensively studied in maize (St. Aubin *et al.* 1986; Wenzel *et al.* 1989; McCully and Canny, 1988; Wang *et al.* 1991), the development of xylem water conducting capacity in conifers has not been broadly researched. Anatomical studies of Wilcox (1954; 1962; 1964) emphasized the development of primary tissues, including the xylem. The most detailed coverage of conifer root primary xylem characteristics is probably that represented by Wilcox's (1962) study on *P. resinosa* where not only the developmental pattern was described but also characteristics of the primary xylem tracheids, including their pits. Xylem differentiation in roots of *A. alba* was described by Rypacek (1976) but it did not extend beyond primary development.

There have been few reported studies on wood formation in roots. Woods (1991) noted that the common understanding of root growth is almost invariably synonymous with root elongation. In his review on cambial activity in roots, this author stated that in spite of the coverage of the subject in works by Zimmerman (1964), Fayle (1968), Philipson *et al.* (1971), and Yeoman (1976), only the general principles of applicable processes had been described while research on cambial growth in roots of individual species still remained to be carried out. Among the published reports on cambial growth and wood formation in roots there were several studies of conifer roots. These included the description of seasonal patterns of cambial growth in several species (Lobzhanidze and Kandelaki, 1972; Liphshitz *et al.* 1984), and the impact of soil moisture on that activity (Coutts, 1982b; Pompart-Dasse and David, 1974). The properties of conifer root tracheids in general and as influenced by environmental or cultural factors have been described in only a few cases. Pompart Dasse and David (1974) found pine root tracheids shorter on wet and on dry sites, compared to those produced on sites with moderate soil moisture. Manwiller (1972) compared the dimensions of root and stem tracheids of southern pines finding the former longer, larger in cell and lumen diameter, but having thinner cell walls (Woods, 1991).

Unlike the properties of wood in roots, stem wood characteristics have been studied frequently both in relation to the technical quality of wood and to its water conducting capacity. The size and shape of xylem conduits have been found to be influenced by genetic factors (Burdon and

Low, 1992; Ford, 1993;) tree age (Dinwoodie, 1963; Connor and Lanner, 1990; Pande *et al.* 1995) , the season of initiation, the growth rate (Dodd and Fox, 1990; Antal and Micko, 1991; Burdon and Low, 1992; Tavita, 1992), position on the bole (Dinwoodie 1961; Tavita 1992; Saranpää, 1994), environmental factors (Larson, 1960; Wodzicki, 1960; 1964; 1971; Antonova and Stasova, 1993), spacing (Yang and Hazenberg, 1994; Persson *et al.* 1995;), nutrition (Harvey and van den Driessche, 1997), variation in the size of the tree crown (Funada *et al.* 1990), and the level of toxic trace elements in the plant's environment (Hagemeyer *et al.* 1994).

Anatomical properties of wood conduits relate to their hydraulic efficiency (Tyree *et al.* 1994). In the xylem of some plants, crosswalls increase the resistance to axial flow (Sanderson *et al.* 1988; St. Aubin *et al.* 1986; Clarkson, 1991). Passioura (1988) stated that large, open xylem elements would offer little resistance to water flow. McCully and Canny (1988) considered the diameter of the largest open xylem element(s) in a given root segment to be the most important anatomical characteristic related to the hydraulic conductivity of roots. This property would change with the distance from the root tip as a result of the progressing xylem maturation. Conifer tracheids, although empty a short distance above the root tip (Wilcox, 1954, 1962), are closed by cell walls at both ends and tracheid to tracheid communication is through pits in their side walls (Bailey and Tupper, 1918; Esau, 1977). Because tracheid length is between 1-3 mm, water has to pass through up to 1000 tracheids per meter of wood length (Tyree *et al.* 1994). Short tracheids decrease wood hydraulic conductivity (Pothier and Margolis 1989). Wide conduits are more efficient in water transport than narrow conduits (Zimmerman, 1983). However, they are also more prone to dysfunction by the breakage of the continuity of water column under increased tension and to embolism-caused cavitation (see the discussion in Zimmerman 1983, and Tyree *et al.* 1994). Recently, the loss of hydraulic conductivity in response to raised xylem tension or cavitation in conifers has been attributed more to the anatomy of the tracheid pit membrane than to the conduit size. The variation in tracheid diameters is apparently small (Cochard 1992; Sperry and Sullivan 1992). The decline in hydraulic efficiency of xylem under high tension can result not only from cavitation but also from plugging pit apertures by pressure-displaced toruses (Sperry and Tyree 1990). This subject is discussed in depth by Tyree and Sperry (1989), Sperry and Tyree (1990) and Tyree *et al.* (1994). Pit membrane elasticity determines its functioning under increased and relaxed tension. The size of margo pores reflects on its water permeability affecting hydraulic conductivity of tracheids and their vulnerability to cavitation. Although cavitation has been reported to occur mainly in the stem xylem (Crombie *et al.* 1985; Tyree and Dixon, 1983; Jackson *et al.* 1995), it may also occur in the roots (Byrne *et al.* 1977).

The purpose of this work was to examine the properties of root xylem in white spruce seedlings grown under different levels of nitrogen availability. Xylem development relative to the position of the examined segment on the root was studied and the relationship between root diameter and area occupied by the xylem was determined. Projected cross-sectional tracheid surface area was partitioned into the area of cell walls and lumens and their ratio was calculated. The length and width of tracheids were measured as well as the diameter of pit apertures and pit borders. The characteristics listed above were analyzed to determine how they were affected by the level of nitrogen available.

MATERIALS AND METHODS

Plant material and fertilizer regimes

A single local white spruce seed source (British Columbia Ministry of Forests registered seed lot number 6866, Central Plateau, elevation 940 m) was used throughout the study. In early March 1996, seeds were single-sown into over two hundred Spencer-Lamaire containers filled with peat/vermiculite mix and germinated in a heated greenhouse equipped with an overhead watering/fertilizing system. More than 90% of the seeds germinated and 60 germinants uniform in appearance were randomly selected for the study. The Spencer Lamaire container sides could be taken apart, thus the germinants were gently removed from the containers together with the growing medium soon after germination. The germinants were transplanted into forty five 4 l capacity pots (approximately 150 x 150 x 180 mm, with bottom drainage holes) containing unsterilized masonry sand. All seedlings remained in a common greenhouse culture until May 5, 1996. At that time N-treatments began and overhead watering and fertilization was terminated. Fifteen seedlings were randomly assigned to each of three N-treatments: 1) low N ($5\text{--}10\text{ mg l}^{-1}$), 2) medium N (50 mg l^{-1}), and 3) high N (125 mg l^{-1}) applied as NH_4NO_3 at pH 5.2. All other macronutrients and all micronutrients were supplied at the same concentrations required to prevent their potential deficiency (R. van den Driessche, 1996, person. comm.). Nutrient stock solutions were mixed and diluted to desired concentrations at the time of application to prevent precipitation. Nutrient applications were made twice a week until early-September. Seedlings received 500 ml of the nutrient solution in every application. Additional watering was carried out as necessary and every 2-3 weeks pots and boxes were flushed with water to dripping to minimize the build up of salt residues. In July, N-deficiency symptoms occurred in the low N treatment and N concentration in this treatment was increased to 10 mg l^{-1} . Seedlings were allowed to harden in a cool greenhouse between mid-September and mid-October with no more fertilizer application and watered only as needed to prevent drought.

Sampling and measurements

In October 1996, three seedlings from each treatment were randomly sampled and their root systems were washed clean. Five first-order laterals longer than 15 cm with intact, unpruned root tips were sampled from each seedling. Three sampling positions along the root length were defined: 140 mm (**position 'a'**), 100 mm (**position 'b'**) and 40 mm (**position 'c'**) above the root tip. Two approximately 6 mm long root segments were collected from each sampling position - one extending acropetally, the other one basipetally from the defined sampling position. The former segments were individually fixed in formalin-acetic-acid fixative (FAA), dehydrated in an alcohol series, and embedded in paraffin (Johansen 1949), then sectioned transversely at 6 μm thickness on a rotary microtome and stained with safranin-hematoxylin. The latter segments were placed into individually marked vials and macerated for 24 h in 50:50 v/v mix of 10% chromic and 10% nitric acids (Johansen, 1940). Following several rinses in distilled water, the segments were stained for 1 h with 1% aqueous safranin and rinsed again. External tissues were removed with fine needles and the woody centre of each segment was placed on a glass slide in a drop of Farrant mounting medium. If the samples were of large diameter and contained abundant secondary xylem, only a narrow portion extending from the sides through the center of the root was used. The macerated tracheids were gently spread over the slide and more Farrant medium was added to secure a glass cover slip.

Light microscopy

Root cross sections were placed under a compound microscope equipped with a video camera and measurements were taken from a display screen using the Optimas 3 (Biomax) software. Depending on the size of sections, x 1, x 4 and x 10 objectives (with appropriate calibration of the display) were used to view the sections. The smallest and largest section diameters, section surface area and that occupied by the xylem were measured by tracing the cursor on the display monitor. When it was visually estimated that the primary xylem occupied more than a quarter of the total xylem area, separate measurements were made of the area occupied by the primary and by the secondary xylem. Otherwise, the total area occupied by the xylem was measured without distinguishing the two types of xylem. Resin ducts and very wide rays (on sections with advanced secondary development) were deducted from the xylem-occupied area.

Cross sections of individual tracheids were also measured on the calibrated video display but under a x 40 objective. On sections where the primary and secondary xylem areas were separately measured, two sampling areas were defined within the primary xylem, each by enclosing about half of the primary xylem beginning in the middle of the section and extending towards a primary xylem pole. This was done by free-hand drawing roughly around but within the area occupied by the primary xylem and closing the beginning and the end of the curve. In two cases, the area occupied by the primary xylem was very small and only one sampling area was established within the primary xylem enclosing it almost entirely. Two more sampling areas were made within the secondary xylem, one on each side of the long axis of the area occupied by the primary xylem. On sections with more advanced secondary xylem development usually three sampling areas were established in the secondary xylem only in positions roughly corresponding to the two, six and ten o'clock positions if related to a clock dial. There were some instances where only two sampling areas could be made if portions of the section suffered from imperfections such as compression from sectioning or broken cell walls. Sizes of the sampling areas were delimited by the interaction between section size and the size of the display screen and usually varied between 8,000-10,000 μm^2 , less (5,000-6,000 μm^2) for sections with little secondary xylem. Surface areas were automatically determined by the software and saved to a file.

Tracheids within the sampling areas were measured by tracing and drawing with a mouse around the outer cell perimeter, then around the inner perimeter. Again, surface areas enclosed within the drawn lines were automatically calculated and the measurements saved to a file. Thus, the total area of each cell section and lumen area were determined and the difference between the two equaled the area occupied by the cell wall. When only a portion of a cell was contained within the sampling area, only that portion was measured and such cell portions were marked by an identifying character so they could be distinguished from whole-cell sections. Cells other than tracheids were not measured

Tracheid radius (TR) was calculated for each cross-sectioned tracheid from tracheid surface area (TA) measurements as follows:

$$TR = \frac{\sqrt{\frac{TA}{3.14}} + \frac{\sqrt{TA}}{2}}{2} \quad (1)$$

and lumen radius (LR) was calculated identically but lumen area was used instead of TA. This formula was developed to account for cross-sectional shape variation (rectangular to elliptical) of tracheids.

Average cell wall thickness was calculated for each tracheid as:

$$TR - LR \quad (2)$$

Tracheid widths (walls included) were measured on macerated wood under a compound microscope equipped with a calibrated eyepiece (x10) insert and a x100 (oil) objective by scanning slides from a random point left to right, then down and right to left and so on until 50 tracheids were measured on each slide. Each tracheid appearing in the view field was measured in three places (avoiding its tapered ends) and a mean of the three measurements was used as an average width. Tracheid length was measured under x4 or x10 objectives, depending on the length of measured tracheids. Slides containing macerates were scanned as described above and intact, uncut tracheids crossing the zero mark on the eyepiece insert were measured to a total of 30 per slide.

Scanning electron microscopy

An additional three roots were sampled from three seedlings per treatment but only from positions 'a' and 'c'. These samples were macerated as described above, gradually dehydrated and transferred from 100% alcohol into hexamethyldisilazane (a substitute for critical-point drying). After three changes of this liquid, the external parts of each segment were peeled off and the woody centre spread over the surface of an aluminum stub covered with adhesive silver paste. The hexamethyldisilazane was then allowed to evaporate and dry tracheids were sputter-coated with gold. The prepared specimens were viewed with a JEOL JSM-35 scanning electron microscope.

Statistical analyses

The acquired data were analyzed using the SAS⁵ 6.12 statistical package. All but one analysis of variance (ANOVA) were performed according to a split-plot design with the nitrogen treatment (N) being the main plot factor. Individual trees (T) were replicates of N and were treated as

⁵SAS Institute, Carry, North Carolina, the U.S.A.

experimental units for the main plot. Roots were subsampling units and were nested in the higher order factors such as trees and N. Either, the sampling position on the root or the xylem development class (see the Results section for explanation) were used as split-plot factors. Experimental units for the split-plot factor varied depending on the response variable of interest and the method in which it was measured. In some cases the split-plot was subsampled while in others it was not. When differences between the primary and secondary xylem were considered, the xylem type was a split-split plot factor and it was subsampled. Complete analysis of variance tables are shown in Appendices 4.1 - 4.10. Multiple comparisons were examined using the Least Significant Difference method; a Bonferroni correction was also used to control the overall experimentwise type I error rate (Milliken and Johnson, 1992). Diagnostic checking was conducted with the Univariate Procedure in SAS using studentized residuals (each residual divided by its standard error). The underlying assumption of normality was confirmed when: (i) the distribution of the studentized residuals was bell-shaped and centered near zero, (ii) both estimated skewness and kurtosis had values between 2 and -2, and (iii) more than 95% of the studentized residuals fell between -2 and 2. When these conditions were not fulfilled, the data were transformed using the Rank Procedure in SAS and the diagnostic tests were repeated (Peter K. Ott, 1996-98; personal communications⁶).

⁶Peter, K. Ott, biometrician, British Columbia Ministry of Forests, Research Branch, Victoria, B.C.

RESULTS

Root sections

Five cross sections were of poor quality and had to be discarded (see Table 4.1 for the distribution of missing sections). The remaining 130 sections varied in advancement of the secondary xylem development. To account for this variation, four classes of xylem development were arbitrarily established: 1) the primary/secondary xylem class when the primary xylem occupied more than half of the total xylem area, 2) the secondary/primary xylem when 50 - 75% of the xylem was secondary, 3) medium-advanced secondary xylem, and 4) advanced secondary xylem with many layers of cells. Classes 1 and 2 were distinguished on the basis of a precise measurement while classes 3 and 4 were distinguished subjectively. Examples of specimens representing each class of the secondary xylem development are shown in Figures 4.1 - 4.4. The secondary xylem developmental classes are referred to by the above shown numbers throughout this text.

The distribution of the secondary xylem-development classes across the N-treatments and sampling positions on the root is shown in Table 4.1. The class 4, representing the most advanced development of the secondary xylem, was most frequent in the medium and high N treatments. In these two treatments, the class 4 occurred mainly at the basipetal sampling position 'a' and in many sections sampled from the position 'b'. In the low N treatment, class 3 was most frequent occurring mainly in the positions 'a' and 'b'. The classes 1 and 2 occurred mostly at the acropetal sampling position 'c' in all three treatments. Generally, the distribution of secondary xylem development classes indicated a slower cambial activity in the low N treatment than in the other two treatments. In most roots, the advancement of secondary xylem development progressed in the basipetal direction but exceptions were found. In the low N treatment, there were two roots with little secondary xylem (class 1) at the basipetal position 'a', the more advanced class 3 at the position 'b', and the most advanced class 4 at the acropetal position 'c'. There was also one root where position 'b' had the most advanced secondary development (class 3) while the basipetal and acropetal positions both had less advanced class 2 xylem. One root in the high N treatment also had little secondary xylem (class 1) at the basipetal position 'a' while class 3 occurred at the position 'b' and class 2 at the position 'c'. These observations indicated that the rate of the secondary development of long lateral roots in spruce

could vary along the root length sometimes resulting in its greater advancement closer to the root tip than in more basipetal locations.

The variation in root diameter among the segments sampled from different trees, roots, and sampling positions was large. The diameters ranged from about half a millimeter to over three millimeters. Seedlings grown under the high N treatment had the thickest roots (Fig. 4.5). All three treatments differed significantly from each other at $\alpha = 0.05$ with roots in the low N treatment being about half the size of those from the two other treatments. Root diameter increased basipetally and the three sampling positions differed significantly from each other (Fig. 4.5). This was a general rule but exceptions were noted and some segments closer to the root tip were thicker than those further away. Using the xylem development class in the ANOVA instead of the sampling position showed a significant effect of the xylem development class on root diameter. Class 4 root segments had the largest diameter (Fig. 4.5) but only class 4 differed significantly from the other classes. Thus, early initiation and differentiation of the secondary xylem did not cause an immediate significant increase in root diameter. The xylem development class effect on root diameter was stronger than that of the sampling position (compare Appendices 4.1 and 4.2). There was no significant interaction effect of the xylem class with the N treatment while such an effect was detected for the sampling position and the N treatment. This was due to the variation in the distribution of the xylem classes at the same sampling positions in different treatments (Table 4.1).

Not surprisingly, there was a strong positive correlation between root diameter and the area occupied by the xylem on root cross sections. The strongest correlation was found at the position 'a' (Pearson correlation coefficient 0.98), and at 'b' (0.97), while at 'c' this correlation was somewhat weaker (0.85). It was also very strong in the xylem classes 4 (Pearson correlation coefficient 0.98) and 3 (0.93) but much weaker in the classes 2 (0.47) and 1 (0.67). The lowest correlation was found for xylem type 2 in the 'c' parts (0.33). The greatest xylem areas were in roots of the high N treatment seedlings and the smallest in the low N treatment (Figure 4.6). All three treatments differed significantly from each other at $\alpha=0.05$ and so did the sampling positions (means shown on Fig. 4.6). There was a significant variation among the trees and a significant interaction effect of N and sampling position ($p = 0.008$) explainable by the earlier shown variation in root growth and development in different treatments. The xylem class 4 specimens had strikingly more xylem than specimens from the three other classes (Fig. 4.6). Generally, the area occupied by the xylem constituted less than half of the total cross sectional root area. This percentage was unaffected by N (Fig. 4.7). There was a general trend for an

increasing proportion of the xylem in root cross sectional areas with increasing distance from the root tip and with an increasing development of the secondary xylem (Fig. 4.7).

N-treatment significantly affected the size of transverse sections of individual tracheids and areas occupied by the cell walls and by lumens on these transverse sections. Tracheids in the low N-treatment were significantly smaller in terms of their cross sectional area, lumen area, and cell wall area than those in the other two treatments (Table 4.2). However, N did not significantly change the proportion between areas occupied by cell walls and lumens. On average, cell walls and lumens occupied about the same proportion of the tracheid transverse surface area. The shape of these sections and that of the lumens could be best described as intermediate between elliptical and square or rectangular. Cell walls were thicker in tracheid corners.

Tracheids from plants grown under low N had significantly thinner cells walls and smaller lumen radii than plants grown under medium and high N (Table 4.2). There were significant interactions of N and sampling position for both these variables. However, cell walls were generally thicker in specimens from basipetal positions and in those from xylem classes 3 and 4 than from acropetal positions and xylem classes 1 and 2. This indicated that cell wall thickness increased with progressing development of secondary xylem. Lumen radii were significantly greater at the 'b' and 'c' sampling positions than at the 'a' position while there was no difference among the xylem classes in this respect (Table 4.2) and no significant interaction effect of N and the xylem class.

Xylem areas occupied by non-conducting space ranged between 5-30% of the total xylem area with a large variation among the replicates on the same root cross section. Because of this variation, a mean of all replicates per section was calculated and these means were used to obtain the proportion of the area of conducting elements to the total area occupied by the xylem for each individual root section. The non-conducting areas occupied averages of 10%, 11% and 13% of the total xylem area in the low, medium and high N treatments, respectively, with no significant treatment effect. There was also no significant treatment effect on the area occupied by lumens (approximately 45% of the area subsampled from a root cross section) and by cell walls (about 42-44%). The sampling position and its interaction with N had no significant effect on any of these variables.

On average, cells of the primary xylem had much larger transverse areas than those of the secondary xylem (Fig. 4.8). The ANOVA used unbalanced data while comparing the primary and secondary xylem because the primary xylem was measured on many more sections in the low N

treatment that in the other two N treatments and the same was true for the part 'a' and 'b', compared to part 'c'. Cell wall areas were somewhat greater in tracheids of the primary xylem but lumens were much larger in the primary than in the secondary xylem (Figure 4.8). These data explain the great difference in the proportion of the cell wall surface to the total tracheid cross sectional surface (Figure 4.9). Thus, the primary xylem cells were larger, had larger lumens, but thinner cell walls than the secondary xylem cells as the area occupied by the cell walls was stretched around a greater circumference.

Root macerations

One root segment was destroyed during specimen handling so the total of 134 were examined. The shortest measured tracheid in the data set was 110 μm long while the longest was 3530 μm . Effects of a tree and of a root on tracheid length were very strong. Judging by the means presented in Table 4.2, a significant N-treatment effect on the tracheid length could be expected. However, such an effect was not detected (if $\alpha=0.05$) when the sampling position was used in the model while it was significant when the xylem class was used in the model. It can be said that tracheids in the medium N treatment tended to be longer than those in the other two treatments. Tracheids at the sampling position 'a', were shorter than those at 'b' and 'c' (Table 4.2) and this difference was statistically significant. The xylem development class did not have a significant effect on the length of tracheids while its interaction with the N treatment did. In the medium N treatment, the longest tracheids were in the xylem class '1' containing the greatest percentage of primary-xylem tracheids. In the other two treatments tracheids in the class '1' were the shortest. There is no obvious explanation of this situation.

The width of measured tracheids varied somewhat even among the three measurements taken from single tracheids as their shapes were not always regular. The smallest recorded individual tracheid width (cell wall included, a mean of three width measurements) was 4.57 μm while the widest measured tracheid had 51.3 μm . They both occurred in the low N treatment specimens. While extreme differences occurred in all three N treatments, most of the tracheids from the same sample tended to be of similar width. Mean widths of tracheids in the high and medium N treatments were greater than those in the low N treatment (Table 4.2) and a significant treatment effect on tracheid width was detected. The sampling position on the root also had a significant effect on the width (Table 4.3). The xylem development class effect was also significant with the class 4 being the only one significantly different from the other three, having wider tracheids (Table 4.2). While this is not what would be expected, judging by the results of the analysis with

the sampling position in the model, the design became much more unbalanced when the xylem class was introduced into the model because of the distribution of these classes in different treatments. Also, some observations had no corresponding xylem class (recall that five sectioned specimens were discarded so their xylem class was not identified while the maceration of corresponding specimens was successful) and these observations had to be deleted from the analysis.

The pits

When viewed under the light microscope, the pit-halves were reasonably round in shape of borders and apertures (Figure 4.10). However, the pits were very small even at the high magnification of the light microscope. Therefore, pit characteristics were measured using the scanning electron microscope. The maceration technique used in this study separated tracheids by dissolving the middle lamellae thus causing the cells to separate. In this process, most of the primary walls, including areas where they formed pit membranes, were also lost exposing pit borders and pit apertures (Figures 4.11 - 4.12; Figure 4.14). The images shown on these figures represent halves of bordered-pit pairs or the tracheid halves of the half-bordered pits. Occasionally, more or less intact pit membranes were observed (Figs 4.13 and 4.15) and these showed great variation in pit-membrane pore sizes.

Scanning electron microscope images of pits and pit apertures suffered from distortion caused by the viewing angle dependent on the tilt of the tracheid relative to the position of the electron detector. It would be the same as the distortion of a round object viewed at an angle by the eye causing it to appear elliptical. Assuming that the pits were perfectly round, the largest observed diameter of the ellipse representing a distorted view of a round object would equal the true diameter of that object. Thus, only these dimensions were measured and analyzed. The smallest measured width of a pit border was 4.1 μm while the greatest was 20.6 μm . The smallest pit aperture was 0.62 μm while the greatest was 10.2 μm . Although many pits on adjacent tracheids were of similar sizes (Figure 4.11), there was a considerable variation in pit sizes both within the same tracheid and among tracheids, with the pits located near the tips being often much smaller than those in the mid-tracheid portion, as shown on Figure 4.12. While the sources representing the background variation (tree, root, tracheid, and their interactions with other factors) had a significant effect on the measured pit characteristics, the N treatments and the sampling position did not. Although the N treatment did not affect the pit border and pit aperture dimensions significantly, the p values were not very far from the arbitrarily chosen $\alpha=0.05$ level. There was

little difference in pit border means among the treatments (11.7, 11.8, and 10.2 μm , in the high, medium, and low N treatments, respectively). The pit aperture mean was greater in the medium N treatment than in the other two (4.2, 3.7, and 3.3 μm , in the medium, high, and low treatments, respectively). The correlation between pit border and pit aperture dimensions was not strong (Pearson's correlation coefficient = 0.48).

DISCUSSION

This study has described the characteristics of water conducting tissues from roots of young white spruce seedlings. Effects of nitrogen availability on these characteristics were examined as well as changes associated with the position of these tissues along the root length and the advancement of the secondary root development. The results reported here may contribute to the improvement of the understanding of the growth and functioning of conifer roots and the mechanism underlying the efficiency of water transport through the root xylem. Anatomical details of the secondary xylem were compared for roots and stems by Patel (1965) but only older trees of angiosperm species were included in that report. Vessel diameters reported in that study were determined from sectioned material. The wide array of studies on conifer xylem from stems, discussed in the introduction to this chapter, represented a variety of technical approaches to these measurements. Tracheid lengths and widths were measured on either sectioned or macerated specimens. In estimations of tracheid cross-sectional characteristics, a diameter measurement was usually reported and cell wall thickness determined by linear rather than area measurements. The study of Antonova and Stasova (1993) represented a different approach and included measurements of cross sectional surface area.

There are some important differences and potential problems related to the technical approaches used in the estimation of tracheid dimensions. Maceration appears to be a technique suitable for measurements of tracheid widths and lengths as the tracheids are separated along their entire length and the individual variation in their thickness (width) can be estimated. In the current study, a mean of three measurements was taken to estimate the average tracheid diameter. It was noted that some tracheids were more regularly shaped than others, some had both ends tapered but more commonly one end was pointed while the other end was blunt. Although it is unlikely, swelling or shrinkage of cells could be introduced by the macerating solutions, the staining solution or by dehydration of the specimens (if it is done). It is obvious from viewing sectioned material that tracheids are neither perfectly oval or square in their cross sections. Their shape is usually more or less rectangular and, depending on which side the tracheid is placed on the slide, its wider or narrower side is exposed to the view. One would expect that more tracheids would fall on their wider side so diameter estimates made on macerated specimens could be overestimating the actual average diameter. The area occupied by the xylem constituted usually less than half of the total root cross section area. In roots with more advanced secondary development, the correlation between these variables was very strong so estimates of the

approximate partition of the root cross sectional area to the xylem could be accurately estimated from external root dimensions. Such estimates would be much less accurate for roots with less advanced secondary development.

Measuring cross-sectioned specimens introduces a different problem caused by the taper of the tracheid ends. It is not known whether one has measured the diameter of a narrow tracheid or a narrow end of a wide one unless serial sections are also examined. The same is true for measurements of cross sectional areas. When lumens are measured, the same problem is encountered as the lumens taper when the tracheid tapers while the cell wall thickness does not change much. Consequently, the proportion of the cell wall to lumen area and the cell wall to tracheid cross sectional area is greater at the tapered ends than in the middle. Perhaps such measurements should be made on longitudinal sections to avoid some of these problems. However, longitudinal sections through thin roots are difficult to interpret. The proportion of tracheids from the primary and secondary xylem seen on different longitudinal sections could vary. The primary xylem tracheids are shown by this study to be larger than those of the secondary xylem so their proportion on different longitudinal cross sections could impact on the results of measurements.

The problems discussed above pertained to this study as much as to the other reported studies. However, comparing the mean tracheid widths measured on macerated specimens with tracheid cross sectional areas measured on sectioned specimens showed similar effects of the N treatment on tracheid size. The effect of the sampling position on tracheid sizes was also similar, whether measured by the width or by the cross sectional area and the effects of the xylem development on the size were not dramatically different in each measuring method. The area measurement appeared to increase the magnitude of differences caused by different factors, compared to the width measurements. Mean radius of macerated tracheids can be roughly calculated by deducting double thickness of the cell wall from the mean tracheid width. When these radii are compared to those calculated from transverse sections, the former means are greater than the latter by 0.25, 1.22, and 1.02 μm in the low, medium, and high N treatments, respectively. This underestimation of lumen radii could be related to some tracheids being sectioned through their narrow, tapering ends while in measurements on macerated specimens these narrow ends were avoided.

Tracheid lengths measured in this study generally corresponded to the range listed by Tyree *et al.* (1994) which was 1-3 mm. The mean lengths were near 1 mm, regardless of the factors by which they were calculated. Sometimes, tracheids about 3.5 mm long were observed but there

were many more tracheids shorter than 1 mm than those longer than 3 mm. There was a large background variation in the size of tracheids, regardless of whether it was the width, the length, or cross sectional area that was measured. These characteristics varied among roots and among trees, indicating that they could be related to the growth behavior of individual roots and to the genetic factors peculiar to individual trees.

Considering the fact that over seven thousand tracheids were measured in the total (not counting those only partially enclosed in the subsampled area), it could be expected that the effects of the imposed treatments, if strong, would override the error caused by the sectioning through some tapering parts of the tracheids. Mean diameters of tracheids in roots of one-year-old spruce seedlings were greater than the effective lower limit on conduit diameter in trees which, according to Tyree *et al.* (1994) would be about 10 μm . They were, at average, almost twice that size but that included the cell wall. Some much larger tracheids were observed and it was obvious that the size of these conduits increased mainly due to the increasing lumen diameter while the cell wall thickness increased much less. The proportion between the areas occupied by the cell wall and the lumen was not significantly affected by the N treatments. The larger the tracheid's external dimension, the larger its potential conductive capacity would be and the less of its diameter would be taken by the cell wall. Large in transverse dimensions tracheids of the primary xylem had much thinner cell walls than the usually smaller cells of the secondary xylem. Tracheids with thicker cell walls tended to occur in the more advanced xylem development classes 3 and 4.

Results of this study indicated that the cambial activity of roots in white spruce seedlings was stimulated by the availability of inorganic nitrogen. This was expected and confirmed the results reported by Coutts and Philipson (1976) and Philipson and Coutts (1977) for *P. sitchensis*. The observed increase in root diameters and cross sectional surface areas, both total and occupied by the xylem, was achieved by both an enlargement of the tracheid size, that is its diameter and cross sectional area, and by an increased number of cells produced by the vascular cambium. This was demonstrated by an increased number of root sections with advanced secondary xylem development in the two higher N treatments, compared to the low N treatment, at all sampled distances from the root tip. However, the difference in cambial activity and tracheid enlargement was significant only when a near-deficiency treatment was compared to the approximately optimal N supply (considered to be the 'medium', 50 mg l^{-1} N-treatment, R. van den Driessche, personal communication, 1997) and much less or not at all when the medium and high N treatments were compared. The limit for the effective stimulation of radial root growth in white spruce is not much above the 50 g l^{-1} N supplied twice a week. The effects of N treatment on the

size of tracheids in the primary xylem were not compared as there were few primary xylem tracheids measured in the two higher N treatments.

The distribution of sections representing different stages of the secondary xylem development in roots of young white spruce seedlings suggested that there is a general trend for increasing number of secondary xylem cell layers in basipetal direction which results in a basipetally increasing root diameter. This is not surprising. However, the fact that less advanced segments were sometimes found at more basipetal locations than in those located acropetally showed that the regulation of cambial activity could vary along the root length and could be independent of the position of the cambium relative to the root tip. This supports the statements of Woods (1991) regarding the behavior of the root cambium in older trees where annual growth rings did not correspond to the real age of the root and differed in numbers in different root segments, not necessarily in the manner corresponding to that observed in the growth of stems. This irregularity of the growth was, thus, shown to occur in roots of very young seedlings over a single growing season resulting in root cross sections that would unlikely show the first annual growing ring for that season or show false rings probably resulting from the reactivation of growth after some period of inactivity. The fact that less secondary xylem could occur further away from the root tip than near the root tip could be interpreted that in young roots in which there are numerous open primary and secondary xylem elements the size of the conducting area may not cause a serious limitation to the axial transport of xylem sap. This, though, needs to be proven. The large dimensions of the primary xylem elements might represent a developmental adjustment enhancing the conductive capacity of root segments closer to the root tip where the secondary xylem development had not yet occurred at any substantial level. It should be kept in mind that the examined roots were collected from dormant seedlings and lacked any white root tips.

Addoms (1946) observed absorption of weak aqueous dye solutions by older, suberized roots of yellow poplar, sweet gum, and shortleaf pine. She observed that these roots did not appear to absorb unless the younger branch roots were excised and the wounds sealed. This suggested that the absorption by older roots would occur only if the necessity arose, perhaps due to increased xylem tension caused by the inability to feed the transpirational stream by the normally absorbing branch roots that had been removed. Kramer (1946) found that roots that varied from 3.2 to 17.0 mm in diameter absorbed water even though many had thick layers of cork. He remarked that water absorption through non-elongating, older roots must be of vital importance to overwintering trees. Chung and Kramer (1975), supported this conclusion by quantifying the absorption of water and radioactive phosphorus by loblolly pine roots. Krasowski *et al.* (1995)

reported an efficient absorption and transport through the brown, non-elongating roots of dormant white spruce seedlings at soil temperatures barely above freezing and low foliage water content indicative of high tension in the xylem. Thus, the anatomical observations reported in the current study support the notion of high capacity for axial transport of water in dormant roots beginning at short distances above the root tip of brown roots.

Peterson and Steudle (1993) calculated the hydraulic conductivity of early metaxylem vessels in *Zea mays* L. They postulated that the radial movement of water through the non-pitted portions of the cell wall could be substantial and that it should not be ignored in detailed analysis of water flow through roots. However, their calculations included a number of assumptions that could introduce an error that might account for the calculated 8-31% contribution of the transport through the wall to the total lateral transport through vessels. Even more critical is the fact that the method of this calculation cannot be readily applied to evaluate the lateral hydraulic conductivity of conifer tracheids. This is because a single tracheid can be laterally connected to a number of other tracheids and parenchyma cells which makes it difficult to estimate how many pits conduct water into the tracheid and how many conduct it out of that tracheid. A large variation among different tracheids can be expected in this respect.

Techniques used in this study did not allow to determine N treatment effects on the pit-membrane pore size. However, the distance between pit borders is that occupied by the margo. Since the torus must fit the pit aperture, it can be presumed that its size is closely related to the size of the pit aperture. N treatments did not significantly alter these properties, although the pit aperture sizes tended to be greater in the optimal medium N treatment than in treatments where N supply was either in surplus or deficient. Results of the study reported here could be of use in work concerned with modeling the axial flow of xylem sap in the roots.

Table 4.1 The distribution of root cross sections categorized according to the advancement of xylem development (xylem category) in specimens collected at different distances (root parts) from root tips of first order laterals of white spruce seedlings grown under different nitrogen (N) levels.

		N - Treatment									
		Low			Medium			High			
Root part		a	b	c	a	b	c	a	b	c	Total
Xylem											
Category											
1		2	0	4	0	1	2	1	0	2	12
2		1	3	8	0	1	11	0	3	8	35
3		6	8	0	0	4	1	0	1	2	22
4		5	3	2	14	9	0	14	11	3	61
		14	14	14	14	15	14	15	15	15	130

Note: Xylem development categories are in progressing order from (1) primary-secondary, (2) secondary-primary (3) medium-developed secondary, and (4) advanced secondary xylem (see the text and Figure 2 for class definition). Root parts are: (a) 140 mm above the root tip, (b) 100 mm above the tip, (c) 40 mm above the root tip. N-treatments are: low (5-10 ml/L⁻¹); medium (50 ml/L⁻¹ and high (125 ml/ L⁻¹) applied bi-weekly during the 1996 growing season.

Table 4.2 Morphological characteristics of individual tracheids from first order lateral roots of white spruce seedlings and their standard errors (in brackets). Length and width were measured on macerated specimens, other characteristics on root cross sections.

Variable	Nitrogen treatment			Sampling position			Xylem development class			
	Low	Medium	High	a	b	c	1	2	3	4
Length (μm)	1019a (11)	1155a (12)	991a (11)	946 * (10)	1117 ** (11)	1106** (11)	1039de (31)	1110c (13)	1069cd (15)	1023e (9)
Width (μm)	15.2b (0.10)	19.8a (0.11)	19.6a (0.12)	17.6* (0.09)	19.1** (0.13)	17.7* (0.13)	17.6d (0.27)	17.4d (0.13)	16.8d (0.16)	18.2c (0.09)
Transverse area (μm^2)	199b (9)	284a (9)	290a (8)	226* (9)	300** (9)	248* (9)	239cd (12)	258c (10)	219d (10)	257c (8)
Cell wall area	100b (3)	136a (3)	138 a (1)	119* (3)	140** (3)	115* (1)	111d (6)	120cd (4)	111d (3)	129c (4)
Cell wall thickness (μm)	2.26b (0.04)	2.58a (0.04)	2.56a (0.04)	2.53** (0.04)	2.57** (0.04)	2.29* (0.04)	2.37d (0.09)	2.38d (0.05)	2.47c (0.07)	2.58c (0.05)
Lumen area (μm^2)	96b (7)	148a (7)	153a (6)	107 * (7)	159*** (6)	133** (7)	128c (12)	138c (10)	107d (8)	128c (6)
Lumen radius (μm)	5.09b (0.15)	6.10a (0.15)	6.22a (0.14)	5.24* (0.15)	6.33*** (0.14)	5.83** (0.15)	5.69c (0.33)	5.99c (0.18)	5.90c (0.26)	5.50c (0.17)

Means with the same letters or symbols are not different from each other at $\alpha=0.05$ (within the group by which they were tested).

Cell wall thickness and lumen radius were calculated from measured transverse areas of tracheids as explained in the text.

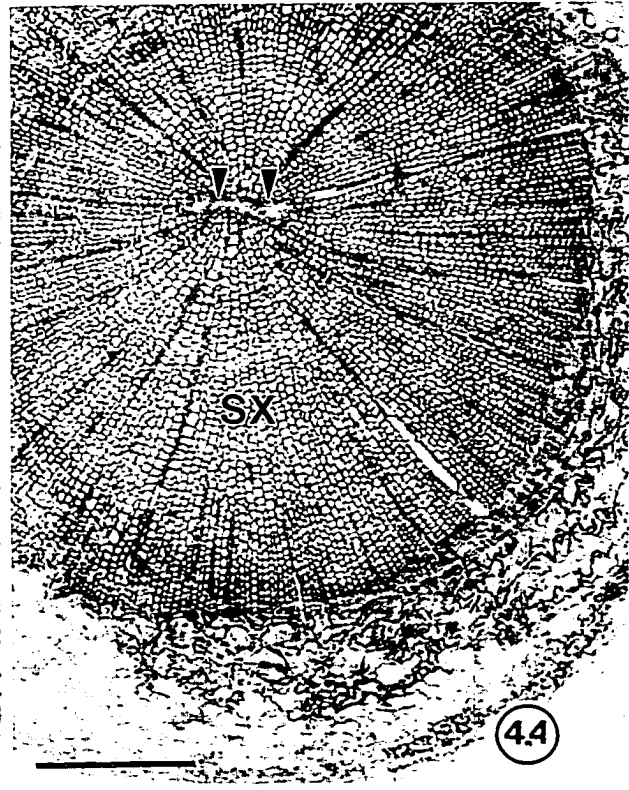
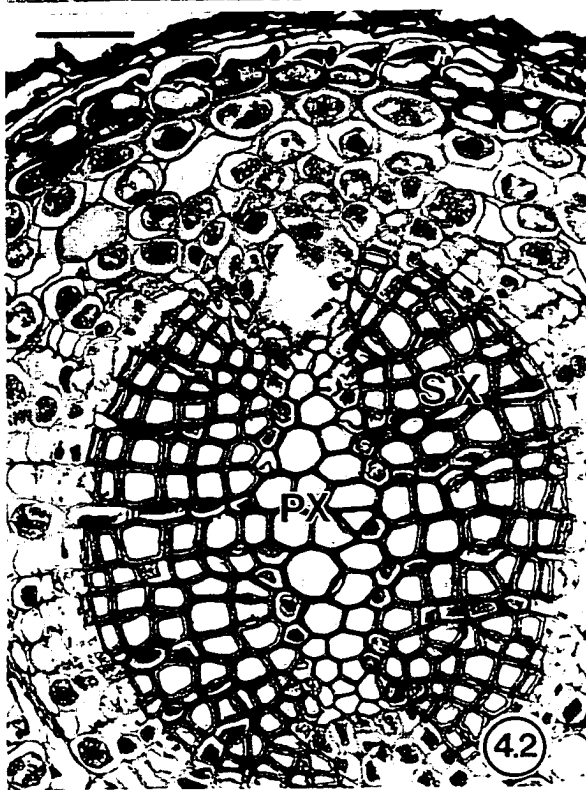
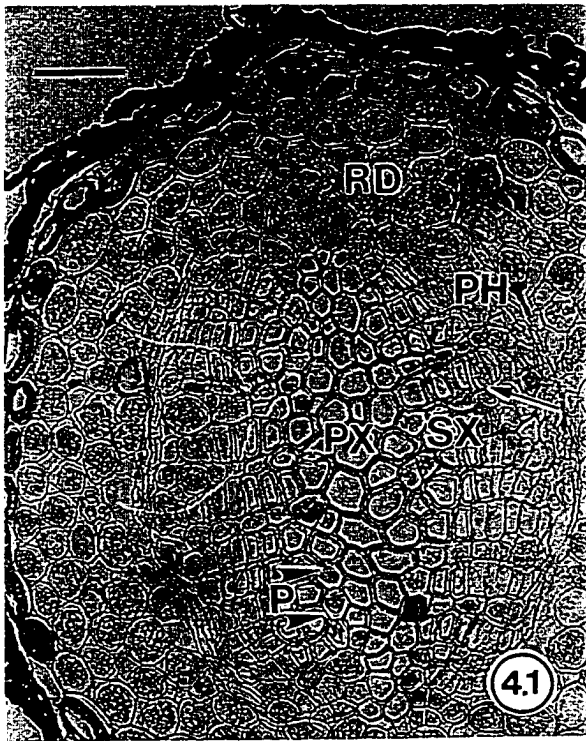
Figures 4.1-4.4. Cross sections of long lateral roots of white spruce seedling. The sections represent the four arbitrarily established classes of the advancement of secondary xylem development.

Figure 4.1. The primary/secondary xylem development class referred to as class 1 in the text. The figure shows large cells of the primary xylem (PX) and smaller cells of the secondary xylem (SX) arranged in files on both sides of the primary xylem. Primary xylem occupies more than half of the total xylem area. Secondary phloem (PH) is separated from the secondary xylem by vascular cambium (arrow). Also shown are: a resin duct (RD) and parenchyma (P). Bar = 50µm

Figure 4.2. The secondary/primary xylem development class referred to as class 2 in the text. The secondary xylem occupies more than half of the total xylem area. Bar = 50µm

Figure 4.3. The medium-advanced secondary xylem development class referred to as class 3 in the text. The files of the secondary xylem contain many more cells than those shown in the previous figure. The primary xylem still occupies a significant portion of the xylem but less than a quarter of its total area. No separate measurements of the primary and secondary xylem were made for specimens in this class. Bar = 50µm

Figure 4.4. The advanced secondary xylem development class referred to as class 4 in the text. The primary xylem located in the center of the section (arrowheads) constitutes only a small portion of the total xylem area and was not separately measured. Note the variation in secondary xylem cell sizes and the deceptive appearance of its central portion that could be mistakenly identified as an annual ring while the root was collected from a one year-old seedlings. Bar = 10µm

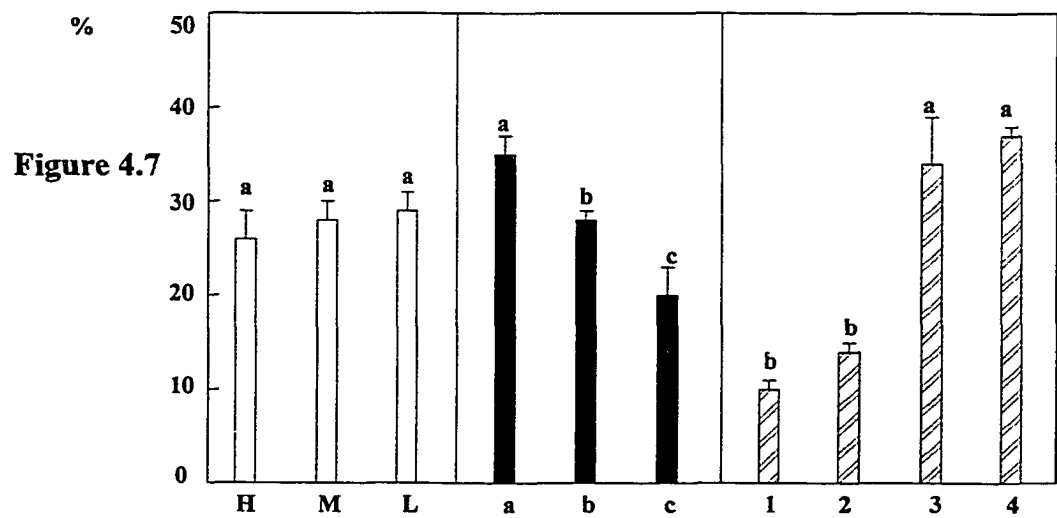
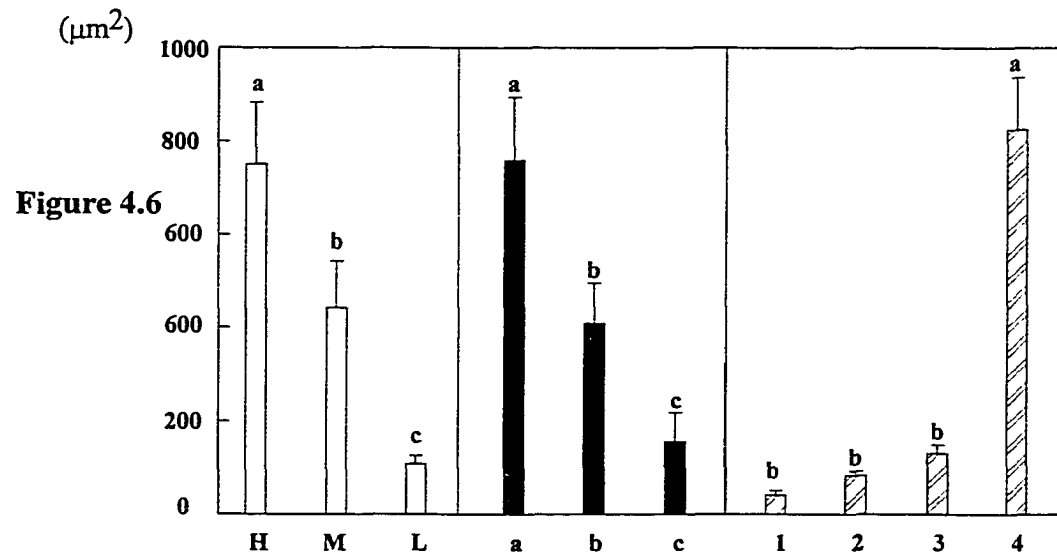
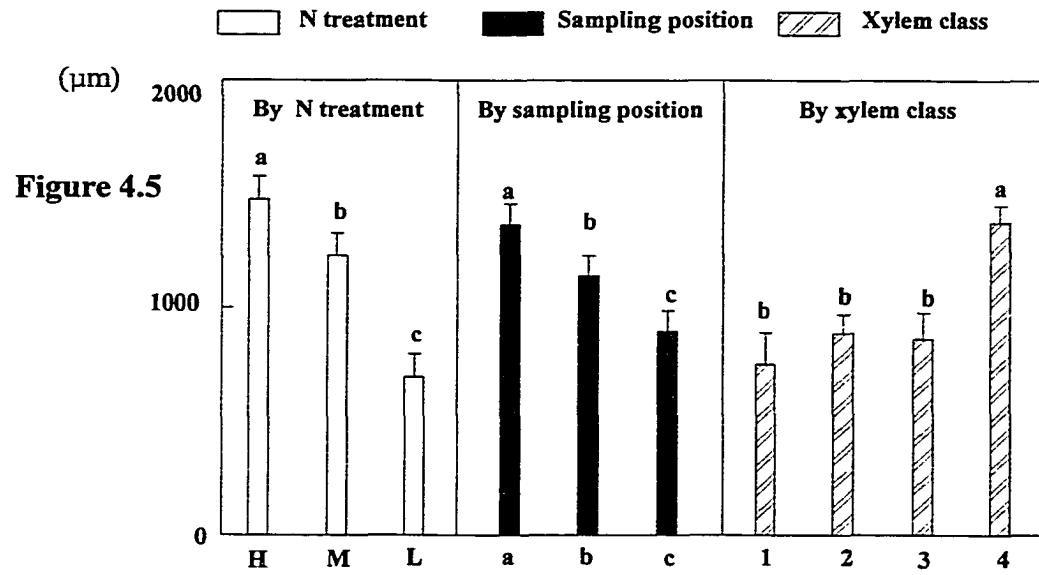


Figures 4.5-4.7. Bar graphs showing mean root diameters, mean xylem surface areas on root cross sections and the mean proportions between the xylem surface area and the total cross sectional root area in roots of one-year-old white spruce. The means are shown by N treatment (H=high N, M=medium N, L=low N treatments), sampling positions on the root (a=14 cm, b=10 cm, and c=4 cm above the root tip), and by the xylem development class (1-4 as shown on Figures 2.1-2.4 and explained in the text). Capped bars represent standard errors of the means.

Figure 4.5. Mean root diameters measured on cross-sectioned specimens. For each specimen, the greatest and the smallest root diameter of each cross section was measured and the mean of the two was recorded as the diameter of the root cross section for each specimen.

Figure 4.6. Mean xylem surface areas measured on cross sectioned root specimens.

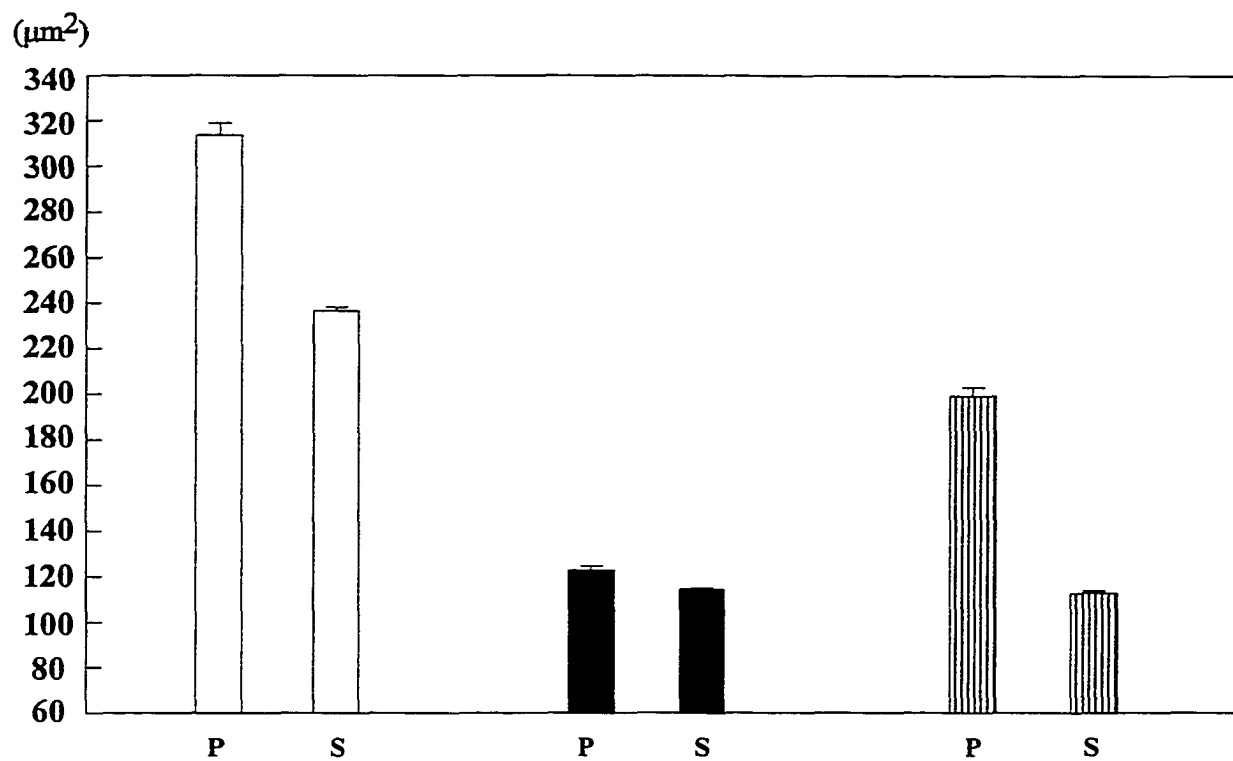
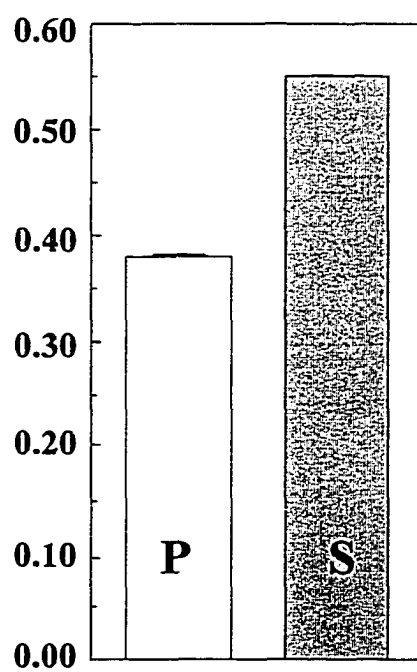
Figure 4.7. Mean percent of the total cross sectional root area occupied by the xylem.



Figures 4.8-4.9 Mean cross sectional tracheid areas, their mean cell wall and lumen areas, and the proportion of the cell walls in primary and secondary xylem of cross sectioned tracheids from the roots of one-year-old white spruce seedlings. Capped bars represent standard errors of the means.

Figure 4.8 Mean cross sectional tracheid areas (blank bars), their mean cell wall (black-filled bars) and lumen (line-patterned bars) areas of primary and secondary tracheids. P = the primary, S = the secondary xylem.

Figure 4.9 Mean proportions of cell walls in tracheid cross sections of the primary (blank bar marked 'P') and the secondary (shaded bar marked 'S') xylem.

**Figure 4.8****Figure 4.9**

Figures 4.10-4.15. Light and scanning electron micrographs of bordered pits and pit membranes of tracheids from macerated segments of the roots of one-year-old white spruce seedlings.

Figure 4.10. Phase-contrast view of the pits in tracheid cell walls showing pit borders (arrowheads) and pit apertures (small circles in the centre of the bordered regions).

Bar = 50µm

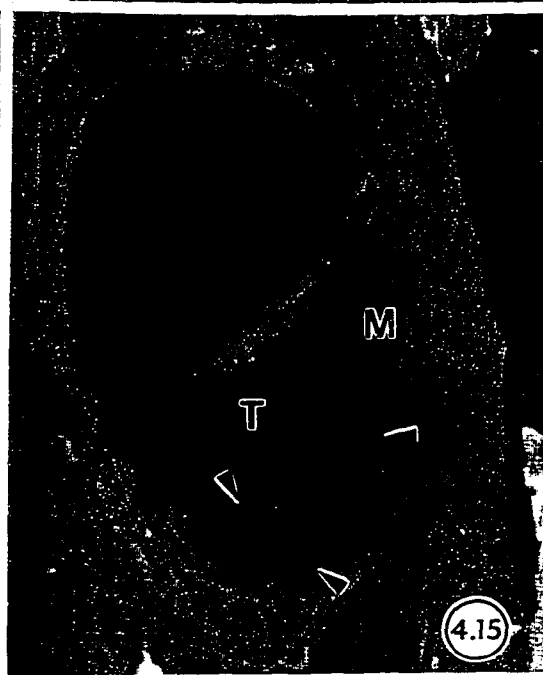
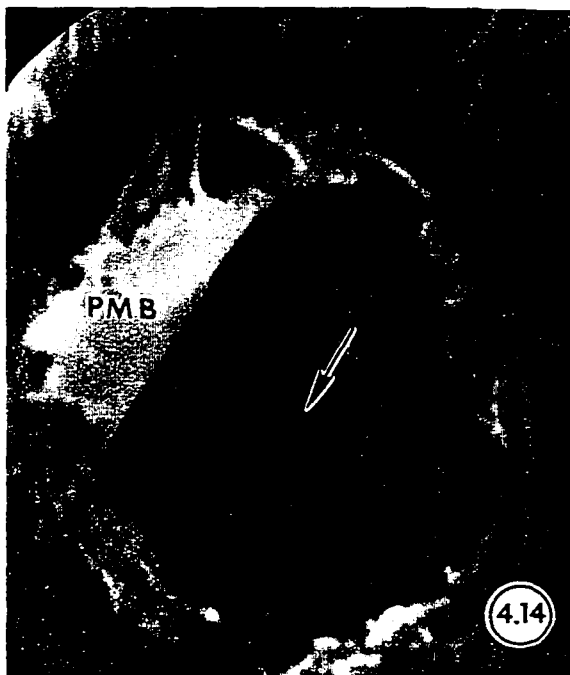
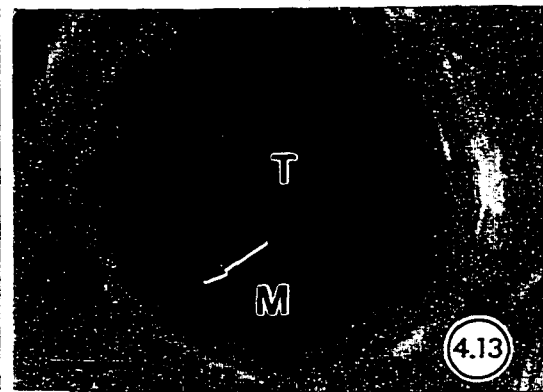
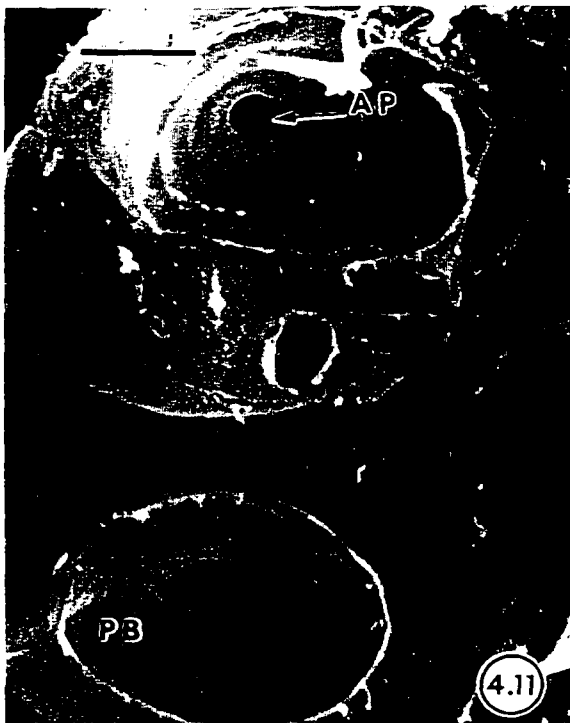
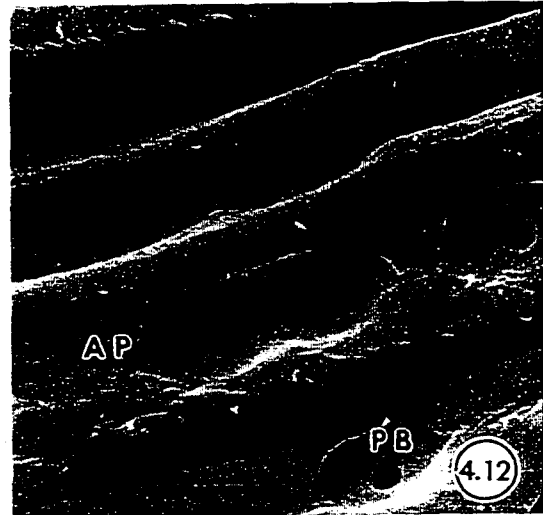
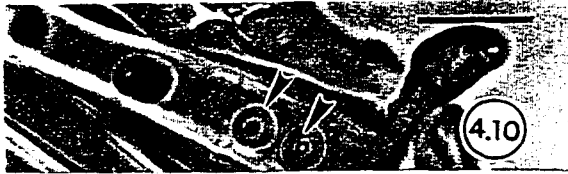
Figure 4.11. Scanning electron micrograph of tracheids showing uniform size pit borders (PB) and pit apertures (AP). Note the elliptical shape of the borders and apertures, a distortion caused by the viewing angle. Bar = 5µm

Figure 4.12. Scanning electron micrograph of tracheids illustrating the range of pit border and pit aperture sizes in tracheids from the same region of the same root. Bar = 10µm

Figure 4.13: Scanning electron micrograph of a pit membrane showing the torus (T), and the margo (M) with pores (arrows) of various sizes formed by the cellulose microfibrils of the primary wall. Bar = 150µm Bar = 2µm

Figure 4.14. Scanning electron micrograph showing remnants of the pit membrane (PMB) almost completely removed by specimen maceration and an unobscured view of the aperture (arrow). Note that the specimen is tilted relative to the viewing angle and the thickness of the cell wall on the side of the pit aperture can be discerned. Bar = 2µm

Figure 4.15. Scanning electron micrograph showing a partially torn pit membrane with torus (T) and margo (M) with pit membrane pores (arrowheads) of various shapes and sizes. Bar = 2µm.



CHAPTER 5. ABOVE AND BELOW-GROUND GROWTH OF WHITE SPRUCE SEEDLINGS WITH ROOTS DIVIDED INTO DIFFERENT SUBSTRATES WITH OR WITHOUT SLOW- RELEASE FERTILIZER.

ABSTRACT

One year old white spruce (*Picea glauca* Moench. Voss) seedlings were grown outdoors for one season in 35 L pots buried in the soil. The pots were vertically split in half. One compartment was filled with mineral soil (sand). The bottom of the other compartment was filled with sand to a depth of 10 cm and topped with 15 cm of organic substrates. Two seedling types, polystyrobloc - grown (PSB) and peat-board grown with mechanical root pruning (Vapo) had their root systems split approximately in half into each of the vertical compartments. Controlled-release (26-12-6 N-P-K) fertilizer was added to one or to none (control) of the compartments. Above-ground characteristics were all positively affected by the applied fertilizer, regardless of the compartment in which it was placed. Nutrient content of the foliage was greater in fertilized than in unfertilized seedlings, N and P concentrations were significantly increased. Bud reflushing occurred more frequently in fertilized than in unfertilized seedlings. Fertilized seedlings had less injury attributed to frost desiccation than unfertilized seedlings and Vapo had less damage than PSB seedlings. Unfertilized PSB seedlings had few roots in either soil compartment. Unfertilized Vapo seedlings had significantly more root length, root surface area, and root tips in mineral than in organic compartments. They also had more P in current-year leaves than did unfertilized PSB seedlings. Fertilizer added to mineral compartments significantly affected root growth in these compartments only, whereas fertilizer added to organic compartments affected root growth in both compartments. In Vapo seedlings, the number of roots was reduced by fertilizer but their elongation and especially radial growth increased. In PSB seedlings, root proliferation was stimulated by fertilizer and this increased total root length and root surface area. Of the five ectomycorrhizal morphotypes identified, E-strain was the most abundant. *Mycelium radicis atrovirens* (MRA) was often found on older, senescing roots which occurred in more PSB than Vapo seedlings, especially if unfertilized. *Thelephora* and *Amphinema* were also present as well as *Cenococcum* which occurred at low levels and in mineral rather than in organic soil. Except for *Cenococcum*, all morphotypes were present in nursery stock prior to planting. *Thelephora*, *Amphinema*, and MRA were most abundant on PSB

seedlings which had little E-strain before planting. In contrast, E-strain was most abundant on Vapo which also had moderately abundant *Thelephora* but no *Amphinema* and MRA. Changes in distribution of morphotypes after planting appeared related to root condition rather than directly to applied fertilizer.

INTRODUCTION

Slow growth (growth check) during the first post-planting years has been reported for various forest species including *P. glauca* and its hybrids with *P. engelmannii* (Mullin, 1964; Sutton, 1972; Vyse 1981; Burdett *et al.* 1984). Growth check occurs even on sites where resource supply does not appear to be growth limiting (Hallsby, 1995). A positive effect of the presence of surface organic matter on growth of planted seedlings has been recognized (Nyland *et al.* 1979; McMinn 1982) and site preparation treatments that incorporate organic soil into the planting microsite have been successfully tried (McMinn, 1982; Hawkins *et al.* 1995; Sutton, 1993; Hallsby, 1995). Methods of organic matter treatment that would optimize seedling establishment are yet to be developed (Hallsby, 1995). Rather than the mineral soil, Balisky *et al.* (1995) considered the forest floor as an appropriate rooting medium. They proposed that root morphologies and planting strategies would have to be site-specifically modified to promote the development of roots into the organic/mineral soil interface. These authors suggested that planting into microsites consisting of rotten wood or duff might be advantageous to seedling establishment while planting into mineral soil according to the Forest Service recommendations (Anonymous, 1990) could be detrimental. Planting strategies in British Columbia still remain controversial.

Organic horizons of forest soils are an important reservoir of mineral nutrients, especially of nitrogen (Salonius 1972). However, organic material must be decomposed and its elements mineralized to be available for uptake by plants. Adding fertilizer to organic matter may increase its decomposition rates (cf. Salonius, 1972). Different chemical forms of the same element supplied in the fertilizer can variously affect the microbial activity (Salonius, 1972). Several types of slow-release fertilizers are now available for application at planting. Carlson and Preisig (1981) reported shoot and root growth stimulation of Douglas fir seedlings by controlled-release fertilizer for two years after its application at planting. The fertilizer caused two flushes of shoot elongation. Coutts and Philipson (1976) reported increased shoot extension in Sitka spruce grown under high nitrogen levels. The second flush of growth caused this increase in shoot elongation.

In colder climatic zones and at high elevations, tree planting can be limited by the longevity of snow cover and the short growing season. Commonly in central British Columbia, spring planting of higher elevation sites begins in mid-May and extends into June. On these sites fertilizing at planting could increase stem diameter and thus reduce the risk of snow-press

damage. Tall, vigorous plants could compete with other vegetation. However, it is uncertain whether conifer seedlings can substantially benefit from slow-release fertilizer when planted late in the season.

A British Columbia review of trials with slow-release fertilizers (Anonymous, 1995) reported an average 7% decrease in first-year survival of fertilized seedlings compared to unfertilized controls. This decline was attributed to preferential browsing by rodents, succulent shoots subsequently damaged by frost and desiccation, and to fertilizer burn. Growth performance of seedlings treated with fertilizer at planting was inconsistent although some trials established in the province showed promising results (van den Driessche, 1988). The identification of causes for inconsistent growth performance of fertilizer-treated seedlings from various types of planting stock was recommended (Anonymous, 1995).

This study evaluates growth responses of two types of white spruce planting stock to root placement in different growing media and to the application of slow-release fertilizer into organic or mineral rooting substrates. The two tested stock types differed from each other by their root system morphology resulting from different nursery treatments. The end-of-season morphology of the above-ground seedling portion and of the root system were studied in detail. The abundance and diversity of ectomycorrhizal fungi, as affected by the rooting substrate in combination with fertilizer level, were also determined. Experiments with divided root systems of individual plants have been found to be well suited for studying effects of differing root environments (Coutts, 1982a). This approach was used to assess the two different rooting media. Results of a small trial using the two stock types planted in an old nursery bed with or without slow release fertilizer are also presented.

MATERIALS AND METHODS

Plant material

A single full-sib white spruce seed source of central British Columbia origin was used. Seedlings were grown during 1996 at Red Rock Research Station in Prince George, British Columbia. Two types of planting stock were produced: 1) polystyrobblock - grown PSB 415B⁷ seedlings and, 2) peat - board⁸ grown, mechanically root pruned seedlings (Vapo). Four hundred seedlings of each stock type were grown. Vapo seedlings were mechanically pruned three times over the growing season (last time one week before lifting to separate individual seedlings) using an electric knife run through slots in the side of the plastic tray containing the peat boards. Both stock types were fertilized similarly during their nursery growth, although the peat boards had been pre-fertilized while manufactured. Seedlings were lifted in October 1996, sorted (the majority of seedlings were uniform and of acceptable size), packaged, and stored frozen until June, 1997.

Planting trial

On June 16, 80 randomly chosen seedlings of each stock type were planted into sandy soil of an old nursery bed currently used as a planting-trial space at the research station. Planting was in approximately 2 x 2 m spacing. Half of the seedlings in each stock type were randomly selected to be given fertilizer at planting. The fertilizer - Silva-Pack⁹ was applied at the time of planting, according to manufacturer's recommendations, approximately 2-3 cm away from the outermost roots and in the bottom of the planting hole. The controlled-release fertilizer (26-12-6, N-P-K, 5 g per package) was contained within a tea bag - like paper package. Most of the N (total of 26.33%) was in the form of urea (23.79%) while the rest (2.54%) was in the form of ammonia. The only other element listed on the package was sulfur (6.01%). Seedling height and

⁷Beaver Plastic Limited, Edmonton, Alberta

Note: Manufacturers are mentioned for reference purposes only. This mention does not constitute endorsement and the study should not be viewed as an evaluation of any brand - name products.

⁸ Kekkilä OY, Vapo Group, Tuusula, Finland

⁹Reforestation Technologies International, Monterey, CA, USA.

stem diameter above the root collar was measured with a ruler and electronic caliper, respectively, after planting and again after completion of the first year growth. Winter damaged foliage was assessed visually in the spring of 1998 and estimated as a percent of total foliage for each individual seedling, similarly as described in Krasowski *et al.* 1996.

Divided root system experiment

Another 16 seedlings of each stock type were carefully washed to expose the roots. Each seedling was individually planted into a plastic pot divided vertically in half (Fig. 5.1) by a sheet of aluminum sealed to the pot with silicone caulk. A small notch was cut in the middle of the top edge of the aluminum sheet to allow placing a seedling with its root collar below the soil surface. Each seedling had its roots split approximately in half into each of the two compartments separated by the metal sheet. In each pot, one compartment contained non-sterilized sand taken from the nursery bed and this compartment is referred to as the mineral compartment. The second compartment, further called the organic compartment, had the bottom 10 cm filled with non-sterilized masonry sand topped with 15 cm of commercially available organic mix containing compost, peat, and decomposing ground bark¹⁰. Samples of the three soil types were analyzed for N, P, and K content. Seedlings of each stock type were then randomly assigned to one of three fertilizer treatments: 6 pots to the control treatment (no fertilizer added), 5 pots to fertilizer into the organic compartment, and 5 to the fertilizer into the mineral compartment treatments. A fertilizer - containing package (the same as in the planting trial) was placed about 2 cm away from the outermost roots at the depth of about 12 cm. The 32 pots were buried to their rims into the soil of the nursery bed, watered once just after planting, then left at ambient conditions until October when they were excavated.

Seedling measurements and ectomycorrhizae assessment

Seedlings were removed from pots and their shoots and roots were separated at the root collar. Shoots were individually packaged and labeled for identification. Subsamples of foliage from the nursery year and current year shoots were taken for scanning and determination of projected leaf surface area¹¹, then the subsamples were dried to steady weight and the relationship between

¹⁰ The Answer Garden Products, Ltd., Abbotsford, B.C., Canada

¹¹ WinNeedle software, Regent Instruments, Quebec City, Qc, Canada

projected leaf area and dry weight was determined for each year-portion of each seedling. The remaining foliage and stems were also dried to steady weight and determined separately so the total leaf projected area of each year of growth of each seedling could be calculated from the ratios derived from the subsampled foliage. The subsamples and the rest of the foliage were then combined and sent for nutrient analysis to the analytical laboratory of the British Columbia Ministry of Forests (BCMoF). Roots from different pot compartments were separated and individually labeled. The roots were gently washed, only to remove the adhering soil. Roots from each pot compartment were subdivided to smaller portions to reduce overlapping, scanned on the Hewlett-Packard C2521B scanner and digitally analyzed¹². Immediately after scanning, the roots were packed in plastic bags and sent to the University of Northern British Columbia (Prince George Campus) for ectomycorrhizae assessment. Lateral roots from each split compartment were examined to determine mycorrhizal status. Characterization and confirmation of each type of ectomycorrhiza was done using bright field optics and the percent of root colonization by each morphotype was visually estimated. The identification of morphotypes was based on key-factor descriptions developed by Dr. H. Massicotte of the University of Northern British Columbia in Prince George.

Samples of the three soil types filling the pot compartments as well as foliage collected from the trees grown with split roots were analyzed at the BCMoF analytical laboratory. Most procedures were based on methods described by Carter (1993) and Kalra and Maynard (1991). For most nutrient elements, ground plant tissue samples were strong acid/microwave digested and analyzed with ARL 3560 ICP spectrometer. Plant tissue nitrogen and carbon were determined by combustion analysis on the Fisons NA-1500 NCS elemental analyzer. Soil exchangeable cations (Ca, Mg, K, P, B, Mn, and S) were determined by a 0.1M barium chloride extraction. Available ammonium-N and nitrate-N were extracted from soil samples with 2N KCl and measured with the Technicon colorimetric auto-analyzer. Available P was determined by the Bray - P1 extraction method followed by colorimetric analysis for ortho-phosphate on the UV-visible spectrophotometer. Finely-ground soil subsamples were used to determine total N and C with Fisons NA-1500 NCS elemental analyzer and sulfur with the Leco SC - 132 sulfur elemental analyzer. Soil pH was determined on a 1:1 water slurry except for the organic substrate where 1:2 water slurry was used.

¹²WinRhizo software, Regent Instruments.

Experimental design and statistical analysis

The planting trial was established as a completely randomized design and the data were analyzed by one-way analysis of variance (ANOVA) using SAS 6.9¹³ statistical package. The divided root experiment also used a completely randomized design and all variables not separated by pot compartments or years of growth were analyzed by one-way ANOVA using the mean square (MS) of a tree nested in fertilizer treatment and stock type as an error term for testing effects of all sources. For variables determined by pot compartment (e.g. root length in each compartment) the design was treated as a split-plot with fertilizer treatment and stock type being the crossed main plot factors and the compartment the split-plot factor. The same was done for foliage nutrient content analysis, with foliage year of origin being the split-plot factor. Effects of the stock type, fertilizer treatment and the interaction between these two were tested with MS of a tree nested in stock type and fertilizer treatment. All other effects were tested with MS of the compartment (or the year) nested in all higher factors. The same model was used when analyzing root length and root surface area distribution by multivariate analysis of variance (MANOVA). Diagnostic statistics were performed using the Univariate Procedure of SAS. No data transformations were necessary. Results of MANOVA on root length, root surface area distribution, and on the composition of ectomycorrhizae are given in Appendices 5.1 - 5.3.

¹³The SAS Institute, Carry, N.C., USA.

RESULTS

Planting trial

At planting, there were no significant differences between the two stock types in their height and stem - base diameter. Seedlings treated with slow release fertilizer had mean height growth of 70 mm during the first growing season compared to only 48 mm in untreated seedlings ($p=0.0001$). The mean stem diameter increment over that growing season was 1.25 mm in fertilized compared to 0.75 mm in unfertilized seedlings ($p=0.0001$). Seedlings of both stock types began setting buds in mid-July, whether fertilized or not. Reflushing (lammas growth) occurred in 20% of fertilized seedlings but not in unfertilized seedlings. Two-thirds of the reflushed seedlings were of the Vapo stock type.

The 1997/98 winter was mild and there was little snow. An assessment of foliage damage, presumably due to winter desiccation, showed significant differences in foliage injury between the two stock types ($p=0.0001$) and fertilizer treatments ($p=0.0003$) but no significant interaction between the two factors. Mean foliage injury of the Vapo stock was 10.9% while it was 18.4% in the PSB stock. Unfertilized seedlings had mean foliage damage of 20.3% while the fertilized seedlings had 8.9%.

Divided root system experiment

Shoot growth

At planting, Vapo seedlings used in this part of the study were significantly taller (mean height 133 mm) and thicker at the stem base (mean diameter 2.7 mm) than PSB seedlings (113 mm and 2.4 mm, respectively) ($p=0.02$). However, at the end of the 1997 growing season, planting stock had no significant effect on seedling height and stem diameter nor on any other morphological variable listed in Table 5.1. Only the fertilizer treatment significantly affected the final 1997 seedling height ($p=0.0001$), the height increment ($p=0.001$), the final 1997 stem - base diameter ($p=0.0001$) and its increment ($p=0.0001$), total dry weight of stems ($p=0.04$), dry weight of the 1997 leaves ($p=0.02$), projected area of the 1997 leaves ($p=0.04$) and dry weight of the root

system (both pot compartments combined) ($p=0.0001$). Means of all impacted variables were always greater in fertilizer-treated seedlings than in untreated controls. However, pot compartment choice for placement of fertilizer did not affect shoot growth (Table 5.1). In summary, fertilized seedlings had more shoot growth than unfertilized seedlings. Almost half of the seedlings in pots reflushed buds in mid-summer but only two were from unfertilized plants - both of PSB stock type. Among the dozen fertilized plants that reflushed, eight received fertilizer into the mineral compartments and both stock types were equally represented.

Root growth

Unfertilized PSB seedlings had few root tips in either soil (Fig. 5.2). Unfertilized Vapo seedlings had copious root tips in the mineral soil and few in the organic soil (Fig. 5.2). Fertilizing PSB seedlings in mineral compartments stimulated root proliferation in these compartments only. Fertilizer placed into organic compartments significantly stimulated root proliferation in this compartment in both types of planting stock. Fertilizing Vapo seedlings in either compartment significantly reduced root tip numbers in the mineral soil. In both stock types, fertilizing mineral compartments had no marked effect on root numbers in organic compartments (Fig. 5.2). The differences in root tip numbers between the stock types, pot compartments, and fertilizer treatments resulted in a significant three-way interaction of these three factors (Table 5.2).

Root length and root surface area were analyzed using the same analytical model as for root tip numbers (Table 5.2). Surprisingly, results of these ANOVAs did not show a significant three-way interaction effect on root length ($p=0.17$) and root surface area ($p=0.22$). However, pair-wise comparisons of least square means of different three-way combinations indicated that such interaction effect should be expected for both these variables. Because of this discrepancy, least square means of all three-way combinations are shown for root length (Fig. 5.3) and root surface area (Fig. 5.4). Significantly different means for both variables were separated on the basis of calculated least significant differences.

Root length and root surface area of unfertilized PSB seedlings were low in both compartments (Figs. 5.3-5.4). Fertilizing PSB seedlings in mineral compartments increased root tip number, root length, and root surface area in these compartments only (Figs. 5.2-5.4). Fertilizing organic compartments increased the length and surface area of PSB roots in both compartments, compared to unfertilized seedlings (Figs. 5.2-5.3). Root length and root surface area of unfertilized Vapo seedlings were much greater in mineral than in organic compartments.

Although root number of fertilized Vapo seedlings declined in fertilized mineral compartments, root length was not reduced and root surface area significantly increased (Figs. 5.3-5.4). Vapo seedlings responded to fertilizing mineral compartments by elongating and thickening their roots and reducing root proliferation. In spite of this reduction, there were still more roots than in unfertilized organic compartments of Vapo or PSB seedlings. Fertilizing organic compartments of Vapo seedlings stimulated root proliferation in these compartments and increased the length and surface area of the roots (Figs. 5.2-5.4). Responses of roots in mineral compartments to fertilizing organic compartments were different in the two stock types. In PSB, root tip numbers rose correspondingly in both compartments (Fig., 5.2). Root length and root surface area in mineral compartments also increased (Figs. 5.3-5.4). In Vapo, root numbers in mineral compartments declined the same as in response to their direct fertilizing but root length and surface area did not change, compared to unfertilized seedlings (Figs 5.2-5.4).

Experimental effects on radial growth of roots were further examined by MANOVA using root length and root surface area distribution divided into three root diameter classes: 1) less than 0.4 mm, 2) 0.4 to 0.8 mm, inclusive, and 3) more than 0.8 mm. Results of these analyses, performed on absolute values and on percent of root length and root surface area in these classes are shown in Appendices 5.1-5.2. Outcomes were similar for both root length and root surface area and differed little whether absolute values or percents were analyzed. Roots and root parts thinner than 0.4 mm constituted more than half of the total length of root systems, regardless of the stock type, compartment, and fertilizer treatment (Fig. 5.5a-b). Roots thicker than 0.4 mm were the main contributors to the root system surface area (Fig. 5.6a-b). Differences to root surface area distribution due to fertilizer treatments were more conspicuous than the differences in the distribution of root length. Both stock types, when unfertilized, had the greatest percent of their root length (Fig. 5.5a) and root surface area (Fig. 5.6a) in the smallest root diameter class, especially the Vapo seedlings. Fertilizer application shifted more root length and root surface area to the two greater root diameter classes in both stock types, however more so in Vapo than in PSB seedlings and more in mineral than in organic compartments (Figs. 5.5a and 5.6a). Note that these shifts occurred in roots not only in fertilized compartments but also, to some extent, in unfertilized compartments.

Soil nutrients, foliage nutrient concentration and nutrient content

The organic soil had a higher C/N ratio than did either of the two mineral substrates (Table 5.3). It had more N in the form of ammonia whereas the two sands had more N in nitrate. The total

exchangeable cations was greater in the organic mix than in the mineral substrates. The nursery-bed sand (mineral compartment) had more available phosphorus than either the organic mix or the sand used for filling the bottom of the organic compartment, possibly due to prior use of the nursery bed for seedling production and related fertilizer applications. All three soil substrates had neutral pH (Table 3).

Regardless of foliage age and fertilizer treatment, concentrations of macronutrients and micronutrients in leaves of seedlings in the divided - root experiment were adequate according to Chapin and Van Cleve (1989). Concentrations of macroelements contained in the fertilizer were highest in leaves of fertilized plants, regardless of which compartment was fertilized. However, differences existed among N, P, and K concentrations in foliage of different age, and/or between the stock types. N concentration following fertilizing was increased by approximately two-fold in nursery foliage and by 2.5 times in new, current-year foliage, compared to unfertilized seedlings that had 1.1% (percent of dry weight) N concentration, regardless of leaf age. This was reflected in foliage N content (concentration x dry weight) as shown in Table 5.4. Significant interaction effects between fertilizer treatment and foliage age were detected for N concentration ($p=0.0001$) and N content ($p=0.0076$). No significant differences were found between stock types.

Unfertilized PSB seedlings had low P concentration (0.12%) compared to unfertilized Vapo seedlings (0.28%) ($p=0.02$). Vapo seedlings had P concentrations similar to that of fertilized seedlings. Table 5.4 shows P contents. Fertilized and unfertilized Vapo seedlings had significantly greater concentration of K (0.71% and 0.82%, respectively) than unfertilized PSB seedlings (0.52%) but not fertilized PSB seedlings (0.75%). Different age leaves of Vapo seedlings had similar K concentrations while new leaves of PSB seedlings had 0.13% higher K concentration than nursery foliage. These differences caused interaction effects of stock type with fertilizer treatment ($p=0.0005$) and stock type with foliage age ($p=0.02$) on the concentration of K. Contents of K in foliage are shown in Table 5.4.

Concentrations of other elements were lower in new leaves than in nursery leaves but their contents were greater in new foliage due to its greater biomass (Table 5.4). Iron, whose content was also much lower in new than in nursery leaves, was an exception.

Ectomycorrhizae and root condition assessment

Except for *Cenococcum*, all morphotypes found on roots from the divided-root experiment were present in nursery stock prior to planting. *Thelephora* was the most abundant morphotype that colonized PSB seedlings during nursery growth. *Amphinema* and *Mycelium radialis atrovirens* (MRA) were common on PSB seedlings but E-strain occurred at low levels. In contrast, E-strain was the most abundant nursery morphotype on Vapo seedlings which also had moderately abundant *Thelephora*. *Amphinema* and MRA did not occur on Vapo roots prior to outplanting.

Compositional MANOVA (see Appendix 5.3) on percentage of *Picea glauca* roots colonized by different ectomycorrhizal fungi and percent of non-colonized roots showed an uneven distribution of morphotypes ($p=0.0001$ for the intercept). Significant effects were detected for fertilizer treatment interacting with pot compartment ($p=0.0007$) and of the stock type ($p=0.0005$) on the composition of ectomycorrhizae.

Five ectomycorrhizal morphotypes were characterized on roots from divided-root experiment and a small number of roots on two seedlings were colonized by unidentified fungi. The most abundant ectomycorrhizae was E-strain. It was absent on the entire root system of only one seedling and on roots from the organic compartment of another. Generally, E-strain was less abundant on seedlings with poorer (with many shriveled roots) root systems and these occurred more often in PSB, especially in unfertilized seedlings (Fig. 5.2-5.4). E-strain abundance was analyzed by ANOVA and significant effects of the pot compartment ($p=0.01$) and of stock type ($p=0.0001$) were found. More roots were colonized by E-strain in mineral (75%) than in organic (61%) compartments and in Vapo (85%) than in PSB (51%) seedlings.

Amphinema was the second most common ectomycorrhiza, although much less abundant than E-strain. It occurred in 42 of 64 pot compartments, more frequently in PSB (29 compartments) than in Vapo seedlings, and more abundantly in PSB than in Vapo (17.8% versus 1.9% of colonized root tips, respectively). *Thelephora* and MRA occurred at similar lower levels, rarely occupying more than 5% (*Thelephora*) or 1% (MRA) of roots in compartments. Both appeared to associate more frequently with older roots, such as those in the root collar area. MRA also seemed to associate with root systems in poorer condition, often those on which E-strain levels were low. *Thelephora* occurred in 56 of 64 compartments. MRA occurred in both soil types but in 29 PSB compartments compared to only 14 Vapo.

Cenococcum occurred at very low levels, often on less than 1% of the root system. It occurred on roots of six PSB and seven Vapo seedlings and in all but two seedlings only in the mineral compartment. Percent of non-colonized or lightly colonized root tips was greater in organic (22%) than in mineral (6%) compartments. This difference occurred in PSB (32% and 7% for organic and mineral compartments, respectively) but not in Vapo seedlings (approximately 10% for each compartment), producing a significant stock type and pot compartment interaction effect ($p=0.02$) and contributing to the interactions involving the pot compartment detected by the compositional MANOVA.

DISCUSSION

Slow-release fertilizer stimulated growth of white spruce seedlings of both stock types in spite of late planting. Austin and Strand (1960), Rothacher and Franklin (1964), and Carlson and Preisig (1981) obtained similar results with different fertilizer formulas and with different conifers. Our study covered only the first growing season and may not indicate long-term trends in growth performance of fertilized seedlings. One reason for inconsistency of planting stock performance treated with slow release fertilizer (Anonymous, 1995) may be the variation among different trials in fertilizer formulas, stock types, and site, especially soil characteristics.

Fertilized seedlings sustained less foliage injury during overwintering than did unfertilized seedlings. Winter desiccation of exposed foliage apparently caused these injuries, a risk reported in the literature (Anonymous, 1995; Brockley, 1988). The susceptibility to frost and desiccation injury was allegedly related to delayed cessation of shoot elongation and the tendency to bud reflushing (lammas growth) in fertilized seedlings resulting in succulent shoot tips. In the planting trial reported here, reflushing occurred only in fertilized seedlings but unfertilized seedlings suffered more winter injury, even though they did not reflush. Three times as many Vapo than PSB seedlings reflushed their buds but Vapo seedlings had significantly less winter injury than PSB seedlings. Fertilizer and root pruning definitely increased the resistance to winter injury in white spruce seedlings. Seedlings capable of prolific root production, especially near soil surface rather than those with deep, vertically oriented root systems, were recommended for reforestation of areas where winter desiccation could cause severe seedling damage (Krasowski *et. al.* 1995; 1996; Krasowski, 1996). The current study supports this recommendation. The effectiveness of slow release fertilizer in reducing the severity of winter - related injuries should be further field tested. Slow release fertilizer may also modify seedling resistance to growing season drought stress. Apparently, excessive concentrations of N reduce drought tolerance of conifer seedlings while moderate concentrations improve drought resistance, over low N concentrations (van den Driessche, 1991).

Frequent bud reflushing in potted seedlings might have resulted from the confinement of the soil in pots causing concentration of fertilizer around the roots. Fertilizing mineral compartments caused most of the reflushing. Differences between the compartments in moisture fluctuation would not be surprising and these could modify the timing or rates of fertilizer release. The contribution of lammas growth to the current - year shoot extension was so minimal that it was

not separately measured. Coutts and Philipson (1976) showed a negligible effect of high N concentration on shoot elongation from the first flush of growth of Sitka spruce seedlings, but a very significant effect on shoot growth from the second flush. However, in their study, a long and constant photoperiod was used that sustained shoot elongation after the second flush. The slow-release fertilizer used in my study stimulated shoot elongation enough to cause numerous bud flushes in mid-summer but not enough to sustain the resultant shoot elongation under ambient, increasing night length.

The study reported here accounted only for nutrients in the foliage and not for translocation of nutrients between different parts of the plant or for losses due to leaching. Nevertheless, total nutrient pools of all elements other than apparently unavailable Fe were greater in fertilized than in unfertilized seedlings. Nutrient pools reflect the plant's nutrient uptake (Chapin and Van Cleve, 1989) which must have been greater in fertilized than in unfertilized plants. Only N accumulated in fertilized plants at a level that significantly increased its concentration in leaves. However, fertilizer N was in much greater proportion than P and K. The effect of stock type on the uptake of the latter two elements by unfertilized plants is especially interesting and it could be the only evidence of a direct relationship between root growth and nutrient uptake obtained in this study. The most striking difference between root growth of unfertilized PSB and Vapo seedlings was the great proliferation of roots of the latter in mineral compartments. Vapo seedlings had significantly higher percent of their roots colonized by mycorrhizal fungi than did PSB seedlings. They also had fewer roots in poor condition. A reduced root colonization by E-strain fungus was particularly common in unfertilized PSB seedlings. It is suspected that these factors were reflected in the documented differences in P and K uptake from unfertilized pots between the two stock types. While the growth of both stock types benefited from the applied slow-release fertilizer, it appears that the fertilizer was much more critical to the improved growth of PSB than Vapo roots.

Studies on Sitka spruce (Coutts and Philipson, 1976; Philipson and Coutts, 1976) showed that nutrients, particularly N, stimulated radial growth of that part of a divided root system to which they were applied. There was little growth stimulation of unfertilized portions of Sitka spruce roots even though the supplied nutrients were apparently translocated to these roots. In white spruce seedlings examined in this study, fertilizer had localized effect on root proliferation, elongation, and radial growth mainly when it was placed into mineral compartments. When applied to organic compartments, the fertilizer effect was no longer localized but the growth of roots was differently affected in the two stock types. Localized proliferation of roots could be viewed as an adaptive mechanism of root systems allowing them to exploit local concentrations

of nutrients in the soil (Drew, 1975; Drew and Saker, 1975; Robinson, 1994; van Vuuren, 1996). This would explain responses of PSB seedlings to fertilizer addition and the response of Vapo seedlings to fertilizing the organic compartments. In the absence of localized nutritional stimulus in the soil, root proliferation and root growth of white spruce seedlings depended on the soil type and on properties of root systems resulting from different nursery cultures. Based on results of this trial and on results obtained by Coutts and Philipson (1976) and Philipson and Coutts (1976), it might be expected that the symmetry of root systems treated with localized supply of fertilizer could be altered. However, Carlson and Preisig (1981) reported no such alterations in Douglas fir supplied with slow - release fertilizer at planting. An assessment of root system symmetry in white spruce seedlings used in the field - planting portion of this study will be made at a later date.

Differences in root growth between the two unfertilized stock types in mineral soil were not reflected in corresponding differences to the shoot growth of these stock types. However, in another portion of these studies (Chapter 6), significant differences were found between PSB and Vapo planted into large boxes containing unfertilized sand (the same as that in the mineral - soil pot compartments in this experiment) topped with a thin layer of organic soil. In that experiment, shoots and roots of Vapo grew significantly more than PSB seedlings.

The poor root growth in organic soil was reversed by adding fertilizer. Stimulation of all aspects of root growth in the organic soil must have been mediated by the addition of at least one of the elements contained in the fertilizer. We did not examine root growth in natural forest soils but this study indicates further work is needed before following recommendations for planting seedlings with roots located in organic layers of forest floor (Balisky *et al.* 1995). To increase root proliferation during the establishment phase, slow release fertilizer could be recommended for white spruce, especially for container-grown seedlings. Seedlings planted in organic soil and not supplied with fertilizer may have underdeveloped root systems.

Coutts (1982a) reported stimulation of root growth in wet soil but poor growth and dying of some roots in dry soils. These responses were independent of the soil type (sandy loam or peat were used). Soil moisture was not monitored in the currently reported study but differences in moisture fluctuation may have existed between the mineral and organic soils. Surface organic soil may dry excessively on warm days (Sutton, 1991) and this may have occurred in my study. However, the stimulation of root growth in organic compartments by fertilizer suggests that nutrient availability rather than soil moisture limited root growth in organic compartments. It is unknown whether the improved root growth in organic compartments by fertilizer was related to

changes in organic matter decomposition rates. Salonijs (1972) suggested that the acidity and shortage of decomposable organic material would be more limiting to the microbial activity in the soil than the availability of N, P, and K. In the current study, the pH of the organic soil was neutral and organic material was abundant, therefore limitations to microbial decomposition of organic substrates were unlikely.

Crowley *et al.* (1986) reported decreased colonization of root systems of *P. echinata* seedlings by *Pisolithus tinctorius* (Pers.) Coker & Couch with increasing rates of fertilizer release. Reductions in the degree of root colonization by fungi could be related to the suppression of fungal development by high, toxic concentrations of fertilizer salts in the soil (Maronek *et al.* 1981; 1982). This was not evident in the current study indicating that there were no toxic effect of the applied fertilizer on mycorrhizal fungi. The differences in the composition and abundance of ectomycorrhizae appeared to be related more to root health status than to the different root-growth media and fertilizer treatments. Since root assessment revealed more poor and dying roots in unfertilized than in fertilized seedlings and in PSB than Vapo seedlings, interaction effects involving stock type and fertilizer treatment on ectomycorrhizae composition could be indirect. It is not known why PSB seedlings had more shriveled and dying roots than Vapo. In unfertilized PSB seedlings, the proliferation of lateral roots appeared suppressed. This was consistent with observations made in other studies. Excavated root systems of white spruce PSB seedlings often resembled the expansion of their nursery configuration (Krasowski *et al.* 1996). These root systems had numerous long, vertically oriented laterals, and fewer branches than “fibrous” root systems of Vapo seedlings (Krasowski, 1996).

Dahlberg (1990) found no evidence that the presence of organic layer in forest soils could affect the establishment and development of ectomycorrhizae in planted seedlings. The only mycorrhizal fungus that seemed to prefer one type of soil was *Cenococcum*. It was more common in roots located in mineral than in organic soil, even though its abundance was extremely low. Since organic compartments had sand in their bottom portions, it is possible that the two occurrences of *Cenococcum* in organic compartments were in sand rather than in organic soil. Inoculation of Sitka spruce seedlings with *Cenococcum* did not improve growth of seedlings planted on clearcuts in Alaska (Shaw *et al.* 1987; Loopstra *et al.* 1988). Resident fungi provided better nutritional benefits to the host plants than inoculated species of three fungi, including *Cenococcum* (Sidle and Shaw 1987).

Slow-release fertilizer used in this study did not reduce colonization of roots by mycorrhizal fungi. Declining root colonization by *Laccaria bicolor* with increasing levels of N was reported

for container-grown Douglas fir seedlings (Gagnon *et al.* 1995). *Thelephora*, a common morphotype during nursery growth, was partly replaced by other fungi after planting. Low levels of *Amphinema* and MRA on roots of Vapo may reflect the absence of these fungi on nursery roots of this stock type. The fact that seedlings came from greenhouse culture and were growing outdoors for only one season may account for the absence of other mycorrhizal fungi. The apparent reverse relationship between the abundance of E-strain on healthy root systems and MRA on poorer root systems is interesting. Numerous reports have proposed that MRA may display semi-pathogenic behaviour on weak or senescent roots (Livingston and Blaschke 1984; Haug *et al.* 1988; De la Bastide and Kendrick 1990).

This study shows that successful planting of white spruce seedlings and future growth may depend on the attributes of seedling's root systems and on the soil substrate in which these roots are placed. Planting seedlings into different types of organic matter can yield varying results. Sitka spruce seedlings planted with roots in undisturbed duff grew better than those planted into rotten wood (Shaw *et al.* 1987; Loopstra *et al.* 1988). The currently reported study also shows that slow release fertilizer improves the growth of shoots and roots of white spruce seedlings in spite of the short growing season resulting from late planting. Stress resistance of spruce seedlings was improved by the fertilizer. Testing the effectiveness of slow-release fertilizer on growth of shoots and roots of seedlings of forest species planted into natural and varied forest soils would be a logical expansion of the reported study.

Table 5.1. Morphological characteristics (least square means) of seedlings grown with their root systems split into organic and mineral compartments with or without slow release fertilizer.

Variable	Fertilizer placement		
	None	In mineral	In organic
Height (mm)	182b	230a	228a
1997 height increment (mm)	65b	109a	97a
Stem-base diameter (mm)	4.09b	5.96a	5.81a
1997 stem diameter increment (mm)	1.43b	3.42a	3.27a
Dry weight (g) of:			
all leaves*	2.08a	3.04a	2.84a
all stems	1.57b	2.36a	2.57a
new (1997) leaves	1.06b	2.08a	1.77a
root system (compartments combined)	1.02b	1.96a	2.01a
New (1997) leaves projected surface area (mm ²)	6136b	11132a	9334a

*1996 (nursery-grown) + 1997 foliage

Note: Means of the same variable followed by the same letters are not significantly different from each other at $\alpha = 0.05$.

Table 5.2. Results of analysis of variance on root tip numbers of white spruce seedlings grown with root systems divided into organic and mineral compartments with or without slow-release fertilizer.

Source	DF	MS	F	p>F	Error term
STK	1	3662099	0.7	0.4255	MS Tree (STK Fert)
Fert	2	23128947	4.1	0.0275	MS Tree (STK Fert)
STK*Fert	2	62611690	11.2	0.0003	MS Tree (STK Fert)
Tree (STK Fert)	26	5585536	1.6	0.1033	MS Error
Comp	1	70123100	20.8	0.0001	MS Error
Comp * STK	2	29650960	8.8	0.0064	MS Error
Comp * Fert	2	20380424	6.0	0.0070	MS Error
Comp*Fert*Stk	2	14244234	4.2	0.0259	MS Error
Error	26	3378566			

STK = planting stock, Fert = fertilizer treatment, Comp = divided-pot compartment, DF = degrees of freedom, MS = mean square.

Table 5.3. Soil nutrient elements in the three types of root growth media used in the divided-root experiment with white spruce seedlings.

Element	Units	Nursery-bed sand (mineral compartment)	Soil organic mix	Sand on bottom of organic compartment
Total C	%	0.86	9.94	0.19
Total N	%	0.05	0.19	0.01
N(NH ₄ ⁺)	ppm	0.32	1.21	0.25
N(NO ₃ ⁻)	ppm	6.90	0.44	2.31
C/N ratio		18.97	52.99	15.88
Available P	ppm	162.2	111.4	57.7
Exchangeable K	cMoL +/-kg	0.19	0.45	0.21
Exchangeable Fe	cMOL+/kg	0	0	0
Exchangeable Ca	cMOL+/kg	2.76	10.47	3.25
Exchangeable Mn	cMOL+/kg	0.01	0	0
Exchangeable Mg	cMOL+/kg	1.05	1.74	1.39
S	%	0.0055	0.0199	0.0036
Effective CEC*	cMOL+/kg	4.10	12.8	4.90
pH		6.10	6.93	7.38

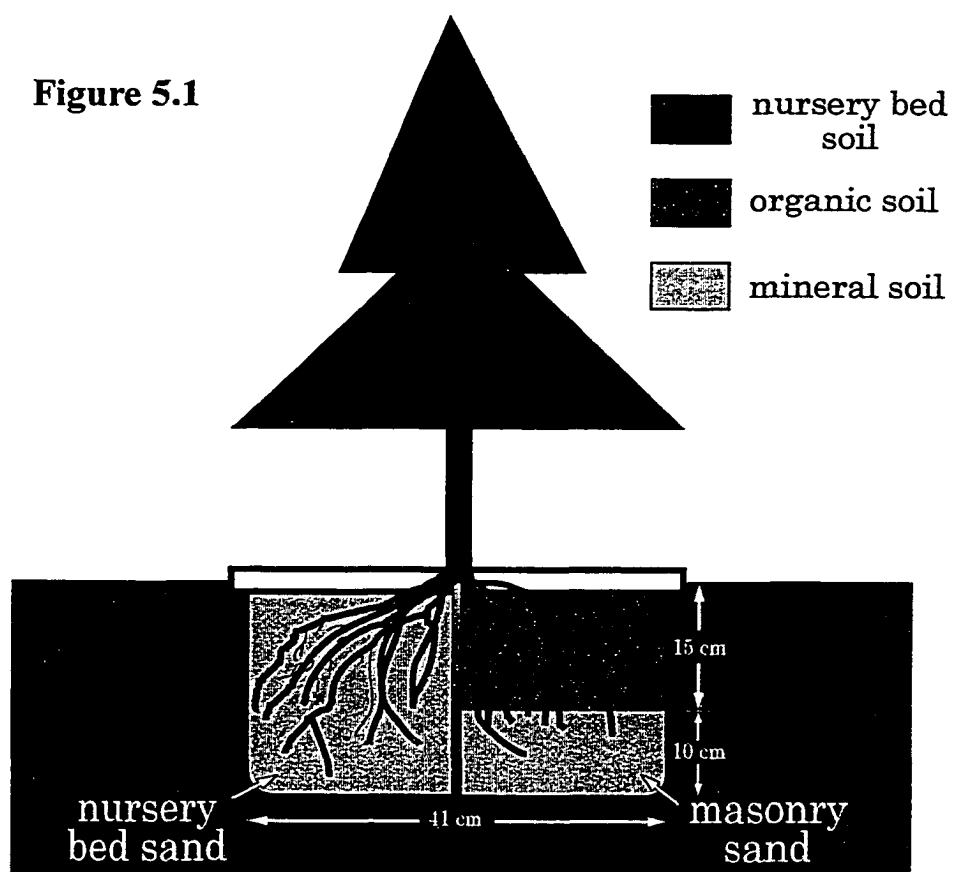
*Effective exchangeable cations (CEC) are expressed as the sum of seven cations (Ca, Mg, K, Mn, Fe, Al, and Na) % = percent of dry weight.

Table 5.4. Nutrient content of white spruce seedling foliage of plants from the divided root experiment.

Fertilizer treatment		None		In mineral		In organic	
Foliage produced in		1996	1997	1996	1997	1996	1997
Element	Units						
C	gx10 ⁻¹	0.5244b	0.5193b	0.4581b	1.064a	0.540b	0.941a
Ca	gx10 ⁻¹	0.0090ab	0.0051b	0.0083ab	0.0122a	0.0108a	0.0130a
K	gx10 ⁻¹	0.0072b	0.0071b	0.0060b	0.0162a	0.0081b	0.0161a
Mg	gx10 ⁻¹	0.0020b	0.0016b	0.0019b	0.0030a	0.0020b	0.0022ab
Mn	gx10 ⁻⁶	438.8cd	245.4d	494.6bc	843.7a	563.3bc	800.6ab
N	gx10 ⁻¹	0.0076c	0.0076c	0.0176b	0.0512a	0.0121bc	0.0532a
P	gx10 ⁻¹	0.0021b	0.0023b	0.0022b	0.0058a	0.0030b	0.0060a
S	gx10 ⁻¹	0.0013b	0.0011b	0.0017b	0.0031a	0.0021ab	0.0029a
Foliage dry weight	gx10 ⁻¹	1.02c	1.06c	0.96c	2.07a	1.10c	1.77b

Note: Means of the same variable followed by the same letters are not significantly different from each other at $\alpha = 0.05$.

Figure 5.1. Schematic drawing (not to scale) of the arrangement of soil substrates in pots used in the divided-root experiment.

Figure 5.1

Figures 5.2-5.4. Mean numbers of root tips (Fig. 5.2), root lengths (Fig. 5.3), and root surface areas (Fig.5.4) of white spruce seedlings grown with root systems divided into two compartments of different soil types and with or without slow-release fertilizer. The clear panels on the left show responses of roots located in mineral compartments whereas the shaded panels on the right show responses of roots in organic compartments. Bars marked with the same letters show means not significantly different from each other at $\alpha = 0.05$.

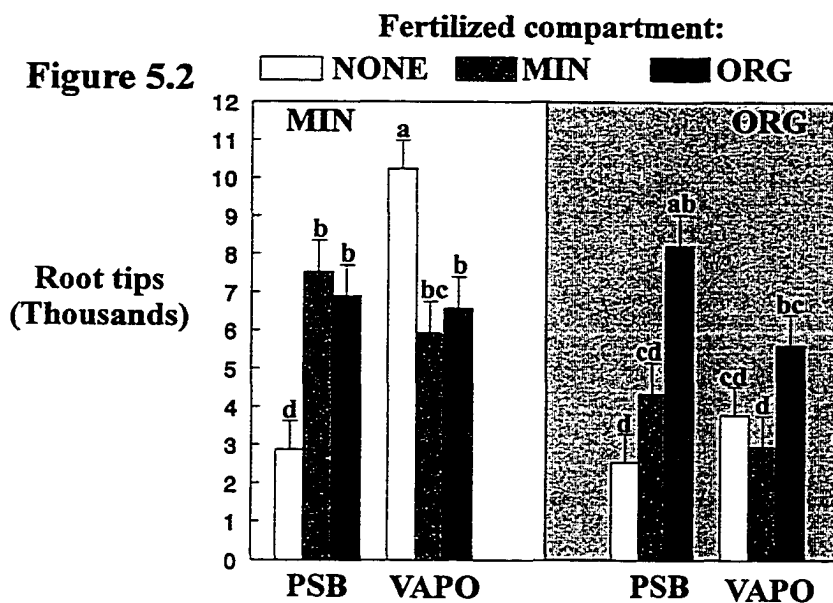
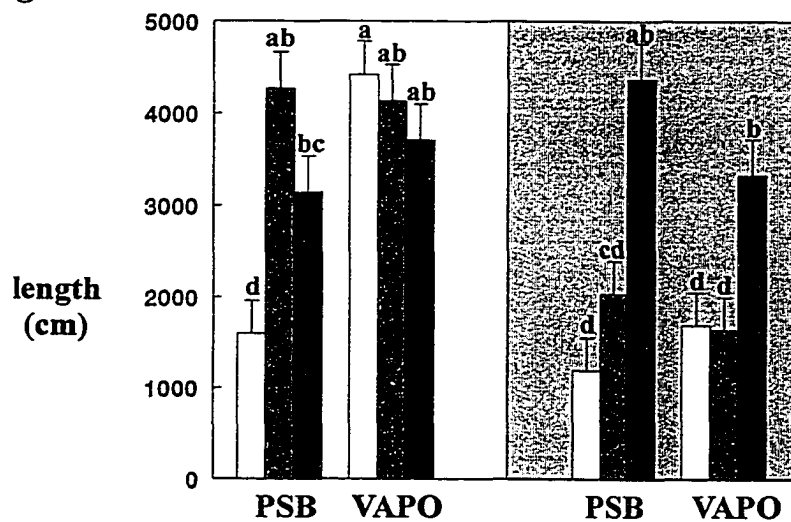
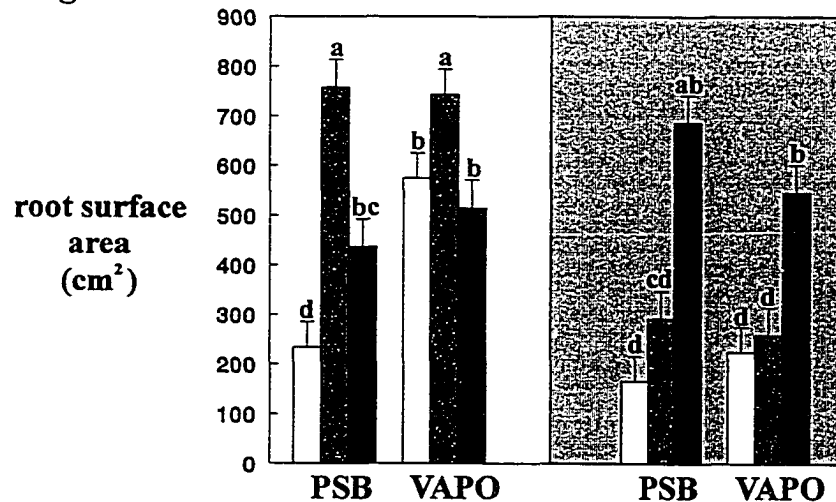
**Figure 5.3****Figure 5.4**

Figure 5.5a-b. Distribution of root length (in percent) into three root diameter classes in seedlings of white spruce grown with root systems divided into two compartments of different soil with or without slow-release fertilizer.

Figure 5.5a shows root length distribution by stock type and fertilizer treatment.

Figure 5.5b shows root length distribution by the soil compartment and fertilizer treatment.

Figure 5.5a

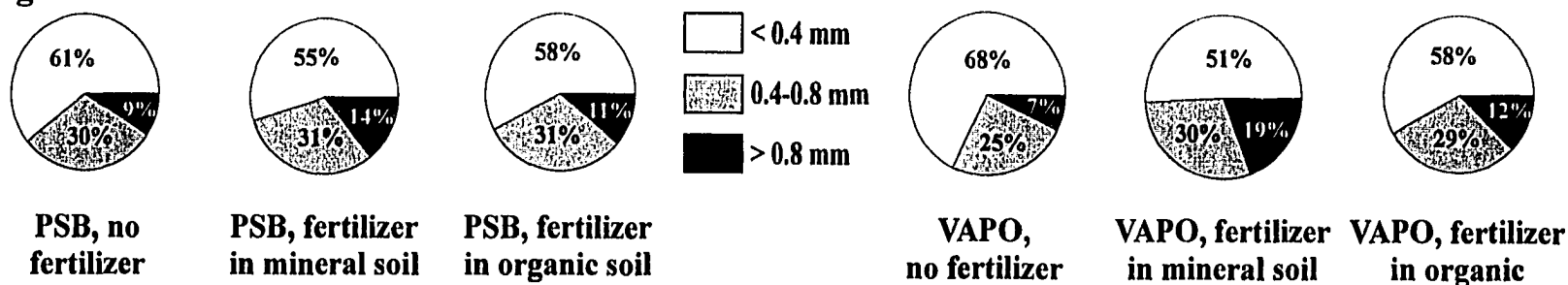


Figure 5.5b

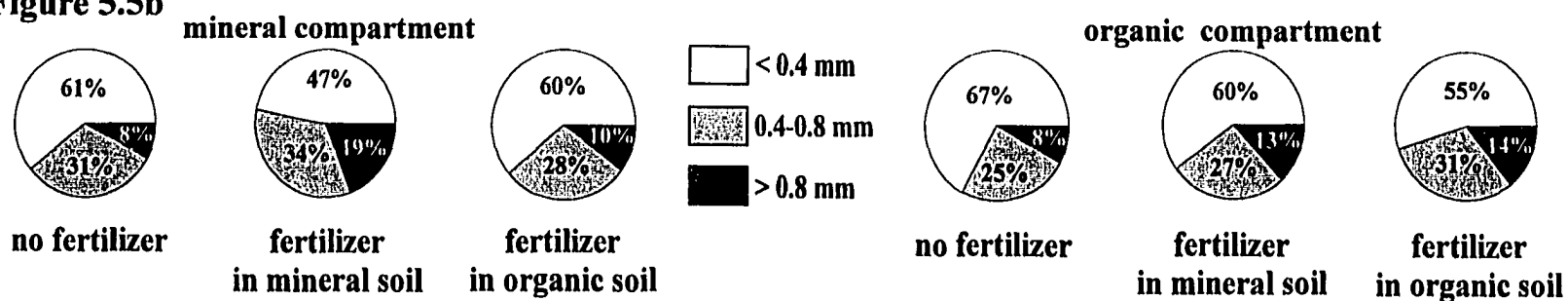


Figure 5.6a-b. Distribution of root surface area (in percent) into three root diameter classes in seedlings of white spruce grown with root systems divided into two compartments of different soil with or without slow-release fertilizer.

Figure 5.6a shows root surface area distribution by stock type and fertilizer treatment.

Figure 5.6b shows root surface area distribution by the soil compartment and fertilizer treatment.

Figure 5.6a

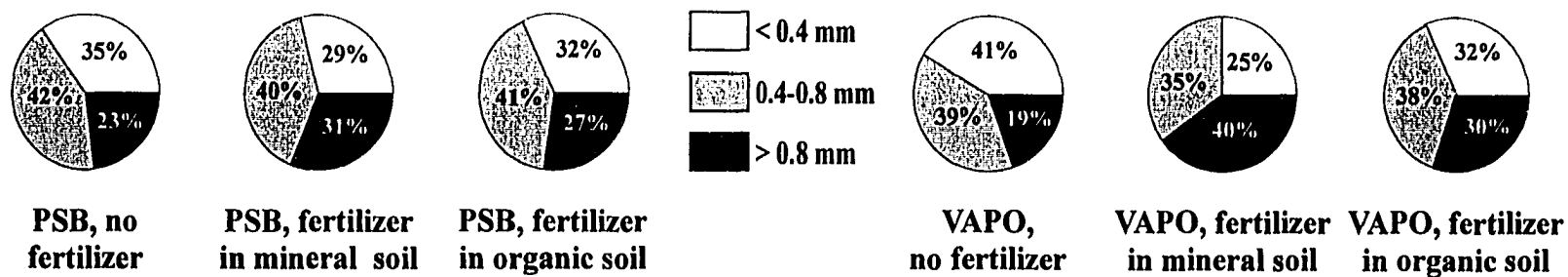
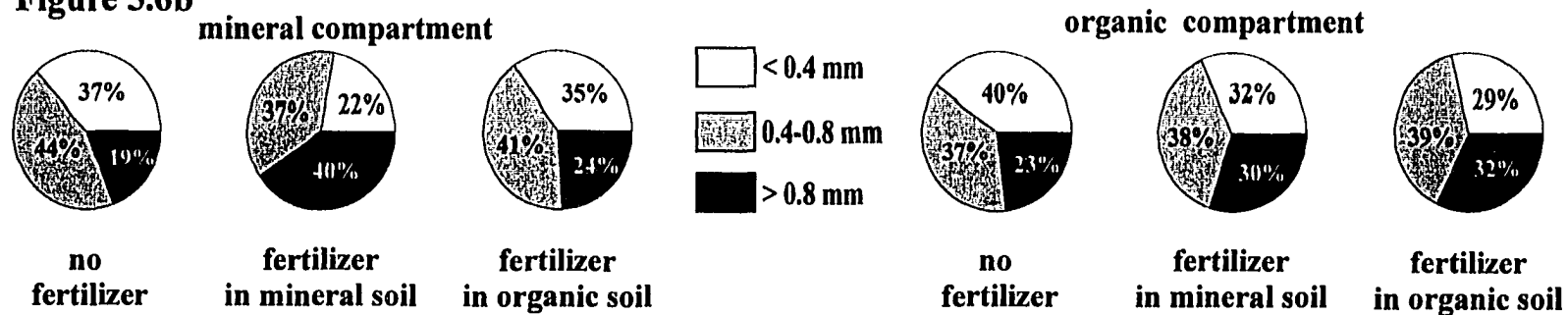


Figure 5.6b



CHAPTER 6: MODIFYING ROOT SYSTEM MORPHOLOGY AT THE NURSERY ALTERS THE GROWTH OF WHITE SPRUCE SEEDLINGS AFTER PLANTING AND AFFECTS THE MORPHOLOGY AND HYDRAULIC CONDUCTIVITY OF THEIR ROOT SYSTEMS.

ABSTRACT

White spruce [*Picea glauca* seedlings (Moench) Voss] seedlings with root systems treated differently at the nursery were planted on two forest sites and into large boxes buried in the soil at ambient conditions but without vegetative competition. On both forest sites, stem diameter growth was significantly better in mechanically-pruned (box-pruned) seedlings than in container-grown seedlings, whether chemically root-pruned or not. The box pruned seedlings planted into 64 L boxes very significantly outperformed the polystyrobloc - grown seedlings in all measured aspects of shoot and root growth. Hydraulic properties of the three seedling types were measured with pressure probes and related to changes in root morphology between the second post-planting week and 6-8 weeks after planting. The box pruned seedlings had the smallest root systems to start with and their roots also grew the least between these two periods. The variation in hydraulic conductivity and water flux through root systems was large, thus the differences among the tested seedlings were rarely statistically significant. Water flux through root systems of the box pruned seedlings was the lowest one week after planting but it increased very significantly 6 - 8 weeks after planting while it decreased or did not change in the other seedlings. Root pressure recorded at both studied periods, particularly shortly after planting, was much greater than ever reported for conifers and close to that of broadleaf seedlings. It is concluded that root system size at planting and its early post-planting expansion have little to do with root hydraulic efficiency. The rate of root growth early after planting did not accurately predict the growth performance of white spruce seedlings assessed at the end of the growing season.

INTRODUCTION

Root pruning of forest tree seedlings is a wide-spread practice used for controlling the size of root systems and inducing root proliferation (Rook 1971; Bacon and Bachelard, 1978; Duryea 1984). Seedlings with smaller root systems are easier to lift without damaging their roots. They are also easier to handle and to plant (Sutton, 1980). Various practices by which root system size can be controlled include undercutting, wrenching, pruning of lateral roots, mechanical "box pruning", chemical pruning, and air pruning. Undercutting, wrenching, and lateral root pruning have been commonly used in bare root nurseries. Various fixed and reciprocating blades are used to cut roots to the required depth or lateral spread (van Dorsser and Rook 1972; Hobbs *et al.* 1987). These treatments often stop shoot growth and enhance root fibrosity (proliferation of small diameter roots), yielding seedlings with smaller shoot : root dry weight ratios (Tanaka *et al.* 1976; Edgren, 1981; Duryea, 1984). Sutton (1980) stated that the timing of root excision may have great effect on the results and cited the work of Riedacker (1976) who showed that some species required root system excision to be carried out shortly after germination in order to modify root morphogenesis. On the other hand, Hobbs *et al.* (1987) found that the morphology of Douglas fir seedlings at lifting was more affected by undercutting versus not undercutting than by the timing, the depth, and the number of applied treatments. Donald and Simpson (1985) reported no significant effects of shallow conditioning (undercutting and wrenching at 10 cm depth) on the survival and growth of seedlings of several conifer species, compared to standard undercutting and wrenching at 20 cm depth.

Many studies have reported improved performance of undercut and wrenched bare root seedlings after planting (van Dorsser and Rook 1972; Tanaka *et al.* 1976). In Douglas fir, field survival and performance of wrenched seedlings depended on the date of lifting (van den Driessche, 1983). Others reported no difference in the performance of wrenched and undercut Douglas fir, compared to untreated controls (Duryea and Lavender, 1982; Stein, 1984; Hobbs *et al.* 1987). Edgren (1981) demonstrated improved survival of undercut Douglas fir on drought prone sites but no significant difference in their growth performance. Root system modifications by various types of root cutting have been recommended for use in bare root nurseries world wide including the production of: radiata pine planting material in New Zealand (Rook, 1969; van Dorsser and Rook, 1972); seedlings of pines and Douglas fir in the United States (Tanaka *et al.* 1976; Edgren, 1981); a variety of conifer and hardwood species in British nurseries (Aldhous, 1972; Sharpe *et al.* 1988; Mason and McKay, 1989); and bare root seedlings in Finland (Huuri, 1978). Deans *et*

al. (1990) not only recommended the use of nursery treatments that promoted root fibrosity but also called for the inclusion of this characteristic in seedling grading.

Simpson (1988) reported that survival and growth of bare root conifer seedlings in British Columbia was poor and recommended undercutting and wrenching as one of the means of reducing shoot : root ratio, improving root growth capacity and, eventually, post planting survival and performance. However, nursery production of conifer species in British Columbia shifted abruptly during the 1980's from bare root nurseries to greenhouse culture of seedlings in containers. Currently, almost all seedlings produced in British Columbia are grown in polystyroblocs that are available in the assortment of sizes and spacing of rooting cavities. The confinement of root systems within a container modifies root system morphology, and deformation of root systems by containers have been of serious concern (see numerous citations in Sutton, 1980 and many articles in van Eerden and Kinghorn, 1978). Wenny *et al.* (1988) stated bluntly that containers deform seedling root systems which could inhibit tree growth after planting.

Van Eerden (1978) acknowledged the fact that root form of container-grown seedlings was determined by container design but suggested that this problem could be solved by improvements to container design. Chemical root pruning by coating the walls of containers with copper compounds (Saul, 1968; Burdett, 1978; Burdett and Martin, 1982) was considered to be a particularly promising innovation (Sutton, 1980). Wenny (1988) reported greater number of new roots in chemically pruned container-grown Douglas fir seedlings than in unpruned controls but the treatment did not result in increased height growth or survival of these seedlings up to the third year after planting. In another study, Wenny and Woollen (1989) and Wenny *et al.* (1988) found that chemically pruned seedlings of three conifer species produced more lateral roots than untreated controls during six months after planting without any adverse effects of the treatment on height and stem diameter growth. Burdett *et al.* (1983) reported lateral root development, height, and stem diameter growth improved by chemical pruning of lodgepole pine (*Pinus contorta*) compared to untreated seedlings. In British Columbia, pre-treated polystyroblocs for chemical root pruning are commercially available and are in use mostly for pine culture. However, on client's demand, other species are also grown in these polystyroblocs. Thus, the differences in the responsiveness of various species to different concentrations of the toxic compound reported by Burdett and Martin (1982) are not given much consideration.

Box pruning is a method in which seedling roots are pruned on five sides resulting in a box-like appearance of the root systems embedded in the growing medium (Chavasse, 1978; Burdett,

1981, Trewin and Hunter, 1986). Box-pruned seedlings grown in peat boards placed in reusable slotted (to facilitate cutting) plastic trays have been commercially produced in Finland. Recent trials with lodgepole pine seedlings in central British Columbia have shown better growth performance of box pruned seedlings than that of container-grown seedlings of the closest available size, whether chemically pruned or not (Krasowski, 1995¹⁴, unpublished, McGarvie, 1995¹⁵, unpublished). In these trials, box pruned seedlings grew equally or better in height and stem diameter than the other seedlings under study, had fewer stem curvatures, and were almost completely free of root deformations, especially of balling and twisting of roots and of the swelling at the root collar. Chemical root pruning reduced the occurrence of these deformations, compared to untreated seedlings, but their frequency was still disturbing, regardless whether the seedlings were grown in new or second-time used polystyroblocs.

The form, size, and physical condition of the tree seedling root system can have a strong influence on the survival and growth of seedlings transplanted to many field environments (Nambiar, 1980). Many studies postulate that the ability to grow new roots after planting could have a significant effect on seedling establishment and survival (Stone and Shubert, 1959; Bacon and Bachelard, 1978; Burdett, 1979). The purpose of this study was to compare the growth performance of containerized white spruce seedlings with and without chemically pruned roots with that of box pruned seedlings of similar sizes. While numerous morphological characteristics were examined and compared, particular attention was paid to the development of root systems within the post-planting period critical for early survival and to changes in hydraulic characteristics of these root systems during the first two months after planting.

¹⁴Krasowski, M.J. 1995. Comparison of root form, tree stability, and growth performance of three stock types of lodgepole pine. Third year progress report on the experimental project (EP 716.4), British Columbia Ministry of Forests, Research Branch files, 22p., unpublished.

¹⁵McGarvie, J. 1995. A comparison of growth performance, root form, and stem form of lodgepole pine produced by different root pruning treatments. University of Victoria Co-operative Program, Work Term Report, 30p., unpublished.

MATERIALS AND METHODS

Plant material and culture

A single seedlot of white spruce from the central interior of British Columbia (seed orchard full-sib, British Columbia Ministry of Forests registered seedlot # 6861) was used. Seeds were sown in March, 1996 into 1) PSB 415B polystyroblocks¹⁶ 2) PCT 415B³ polystyroblocks, and 3) Vapo¹⁷ peat boards. The first two are polystyrobblock containers of the same size: cavity diameter 35 mm, depth 149 mm, volume 93 ml, plant density 527/m². The PCT blocks were pretreated by the manufacturer with a copper-containing compound for chemical root pruning while the PSB blocks were not but have ribbed container walls to prevent root spiraling. The cavities were machine-filled with a mix of peat and vermiculite. The Vapo method used pressed and dried peat boards with holes drilled for seed placement. The boards were placed inside reusable plastic trays 80 mm deep with perforated bottoms for bottom air pruning of roots. The boards expanded when watered to the thickness of about 80 mm. The trays used in the experiment had slots in all 4 sides, 5 cm apart to enable mechanical root pruning. Thus, plant density in the Vapo trays was 400 plants/m². Apparently, other sizes of the trays are also available (Parviainen and Tervo 1989)

Seeds were single sown, germination was over 90% in all three types of growing units. Four units of each type were grown for the experiment. Seedlings remained in the same greenhouse throughout the nursery culture and were fertilized at the same time through the injection system coupled with overhead watering. Irrigation was occasionally adjusted as needed to prevent drought stress as drying of the media in polystyrobblocks was not the same as in the Vapo trays. The Vapo seedlings were manually pruned with an electric knife run lengthwise and crosswise as marked by the slots in the trays. This was done first when the seedlings were, on average, about 7-8 cm tall, as recommended by Parviainen and Tervo (1989). The pruning was repeated two more times, including the last time about a week before lifting to ensure that the seedlings could be separated and to allow time for healing before the seedlings were put into freezer

¹⁶Beaver Plastic Limited, Edmonton, Alberta, Canada. Mention of trade names does not constitute endorsement.

¹⁷Kekkilä OY, Vapo Group, Tuusula, Finland.

storage (-1° C) in October. Seedlings grown in different types of growing units are referred to as different types of planting stock or simply as stock types.

Planting on forest sites

Seedlings were removed from freezer storage, thawed, and planted in late May on two sites in the central interior of British Columbia. Both sites were within the elevation, latitude, and longitude range acceptable for the seed source, according to the British Columbia Ministry of Forests Seed Transfer Guidelines. Site 1 was located in the SBS mk1 zone (Madinger and Pojar, 1991). Five experimental blocks were located on a south-facing slope with rows of seedlings running up and down the slope. Each block contained three rows of about 20 (19-22) seedlings, each row planted with seedlings of one stock type. Stock type placement into rows in blocks was random. Site 2 was located in the SBS mk2 biogeoclimatic zone. Five experimental blocks were established on a flat part of the site. Seedlings were planted with shovels without mechanical site preparation. Both sites were well drained and had no obvious impediments to root growth. Vegetative competition was moderate on both sites. Seedling heights and ground-level stem diameters were measured after planting and again in the fall when their survival was also assessed.

Planting into soil-filled boxes

Four seedlings of each stock type of uniform and similar appearance were chosen from each polystyrobloc and Vapo tray and labeled. The seedlings were planted in mid-May, 1997 into 0.4 x 0.4 x 0.4 m wooden boxes (0.064 m³ capacity) filled with sandy nursery soil topped with 4 cm of organic mix (compost, ground bark, and peat). The boxes were buried in the soil for the entire growing season. Germinating weeds were manually removed. The seedlings were grown at ambient conditions with no supplemental fertilizing or irrigation. Boxes were placed in the soil in random order.

In October, the boxes were removed from the field and brought to an unheated greenhouse. Seedling height and stem diameter near the root collar were measured. Sturdy wire pins were pushed into the soil through holes drilled in the sides of the boxes - 5 cm below the top of the box, and 5 cm apart forming a crate for supporting the roots. Then, the boxes were unscrewed and taken apart leaving only two sides supporting the pins. The soil was gently removed and the

root systems were photographed, then carefully washed. Root systems were cut off the above ground portion and individually scanned using a desk - top Hewlett-Packard scanner. The root systems had to be subdivided by cutting off some or most of first - order laterals together with associated roots to minimize overlapping of roots during scanning. Scanned images were analyzed using the WinRhizo¹⁸ software. It was unnecessary to stain white roots to improve the contrast of images as adjusting the gray level of the images provided satisfactory resolution. After the measurement, the root systems were individually packaged and dried to a steady weight in a forced convection oven. Needle samples were taken from the previous and current year growth (mixed together) of each seedling and scanned using the WinNeedle⁵ software, then dried and weighed to determine the relationship between the dry weight and leaf surface area of the subsample. Total leaf surface areas were calculated individually for each seedling by multiplying the surface area per gram of subsampled leaves times the total dry weight (including the weight of the subsample) of the leaves. Dry weights of leaves and stems of individual seedlings were taken separately .

Planting into pots for measurements of root hydraulic conductivity

Six seedlings per each styroblock or a Vapo tray (further called stock type-treatment replicates) were sampled in early May from cold-stored boxes (a total of 24 seedlings per stock type). Seedlings of all three stock types were chosen to be of similar heights and stem diameters. Then, the seedlings were separated into three batches by randomly assigning one seedling from each stock type-treatment replicate into batches one and two, and four seedlings per treatment replicate into the third batch. Seedlings from batches one and three were immediately (May 5, 1997) planted into 4 L capacity pots filled with nursery soil. The pots were buried in the soil outdoors in rows by stock type but pots with the stock type-treatment replicates of batch three were buried in random order within the rows. Sampling for measurements of hydraulic conductivity was made across the rows. Since four to six seedlings were sampled daily during each sampling period, this ensured that all three stock-type treatments would be represented equally across each sampling time. Seedlings from batch two were kept in cold storage for another week, then potted and planted as the other batches. This was done to minimize the effect of the elapsing time. Batches one and two were sampled from beginning one week after planting The distinction between batches one and two was not maintained in the analyses and all these

¹⁸Regent Instruments, Quebec City, Quebec, Canada

seedlings were treated as samples the second week after planting (time 1). Sampling from the batch three commenced six weeks after planting (time 2).

Measurements of root hydraulic conductivity and root pressure

Pressure probes were purchased from Dr. E. Steudle, University of Bayreuth, Germany. A total of 24 seedlings (8 per stock type, 2 per stock type-treatment replicate) were tested at time 1. Using two pressure probes allowed processing four to six seedlings daily and the measurements were completed within 4 - 5 days in each week of time 1. Measurements at time 2 took 15 consecutive days to complete as 48 seedlings were tested (16 per stock type, 4 stock type-treatment replicate) and one pressure probe failed half-way through the measurements.

The general protocol for hydraulic conductivity measurements was similar to that described by Hallgren *et al.* (1994) but only hydrostatic pressure was used in pressure relaxation experiments. Briefly, pots removed from the soil were brought indoors, watered to dripping, and the entire above - ground seedling portions were covered with black plastic bags for a minimum of 16 hours in order to stop transpiration and prevent filling of the xylem with air when the stems were later severed. The stems were cut off and a pressure probe was sealed onto the remaining stump protruding approximately 25 mm above the soil level (Fig. 6.12). About 20 mm of the stump was placed into the pressure probe. Air bubbles were removed from the system by flushing the internal spaces of the probe with distilled water. About two hours were allowed for root pressure development and equilibration of the system. The probe was carefully checked for the presence of air bubbles which were removed, if necessary, and the time for equilibration was appropriately adjusted. Stabilized value of the root pressure was recorded. The function of the pressure probe and formulas required for the calculation of hydraulic conductivity were given by Zhu and Steudle (1991), Peterson and Steudle (1993a), Steudle *et al.* (1993), Steudle (1993). The elastic coefficient (β) was determined by rapidly moving the metal rod into a new position and immediately returning it back to the original position. This was done several times in both directions (applying negative or positive pressure) while carefully observing volume displacement (dV) in the pressure probe capillary and recording the corresponding pressure change (dP). The elastic coefficient was calculated as:

$$\beta = dP/dV \quad (1)$$

Pressure relaxations were next made by moving the metal rod to a new position but not returning it to the original position, so the displaced volume was maintained. The pressure change, which was exponential with time, was recorded on a chart recorder. The half time ($T_{1/2}$ = time required for relaxation of 1/2 of the applied pressure) was later determined from the recorded data and hydraulic conductivity (L_{p_s}) of the root system was calculated as:

$$L_{p_s} = \ln(2)/T_{1/2} A \beta \quad (2)$$

Water flux through a root system was calculated by multiplying L_{p_s} by root system surface area (A). Several (6 - 10) pressure relaxation measurements were made on each seedling and means of these measurements were used in statistical analyses.

After the completion of hydraulic measurements the root systems were removed from pots, washed, scanned and measured using the WinRhizo software. Individually tagged above-ground portions were oven - dried to a steady weight and so were the root systems once their images were taken. Dry weights were determined and total leaf surface areas were calculated from conversion values obtained by scanning leaf subsamples as described for the seedlings grown in large boxes.

Experimental designs and statistics

The SAS¹⁹ version 6.11 statistical package was used to analyze the data. The planting stock type was the treatment under study. A two-factor completely randomized block design was used for the planting on forest sites part of the study. The planting into the boxes portion used a completely randomized design with subsampling. The growth units (polystyroblocs and Vapo trays) were stock type-treatment replicates. The hydraulic conductivity portion of the study used a split - plot design in which the stock type was the main plot and time after planting was the split - plot factor, stock-type treatment replication was as previously described.

Most variables were analyzed using the General Linear Models procedure for both univariate and multivariate analyses of variance, the latter was used to analyze the distribution of root length and root surface area (the actual values and percentages of the whole root systems) in defined root diameter classes. The multivariate analyses were followed by univariate analyses of variance on individual variables. The Univariate procedure of the SAS was used to check the

¹⁹The SAS Institute, Carry, North Carolina, USA

normality of distribution of the residuals. In a few cases the data had to be transformed using the Ranking Procedure of the SAS. Least square means were obtained in the analyses and compared pairwise when necessary. Analysis of variance tables listing all error terms used for testing effects of particular sources can be found in Appendices 6.1 - 6.11.

RESULTS

Planting on forest sites

Although seedlings were randomly distributed to sites and blocks, a significant difference in height at planting was detected among the blocks and between the sites. Seedlings on site 1 were on average about 7 mm taller than those planted on site 2 (Table 6.1). While there was no overall difference in height among seedlings from the three treatments, the significant site and stock type interaction effect was caused by the Vapo seedlings on site 1 being on average 10 mm than seedlings of the other two stock types. On site 2, the PSB seedlings had the greatest mean height at planting but differences among the treatments were not significant. A significant stock type effect on stem - base diameter at planting was also detected and all three stock types differed significantly from each other (Table 6.1) in spite of only 0.27 mm range in mean stem diameters. No site or site and stock type interaction effects on stem base diameter were found.

No seedling losses occurred on either site over the first growing season. Seedlings on site 1 grew more than those on site 2 in height and in stem diameter (Table 6.1). The difference between mean heights and stem diameters on the two sites might have reflected the initial differences, particularly for the stem diameter as its increment on site 1 was almost significantly different from that on the site 2. However, there was no overall stock type effect on seedling height and height increment. The effect of the stock type on stem base diameter and its increment was very strong with the Vapo seedlings having the greatest increment and the greatest stem base-diameter after the first growing season (Table 6.1).

Growth of seedlings planted into the boxes

Although mean heights of the Vapo and PSB seedlings at planting were greater than that of the PCT seedlings (Table 6.2), these differences were not statistically significant. Mean stem base diameters of the three stock types were very similar at planting (Table 6.2) and no stock type effect was detected.

After one growing season the Vapo seedlings outperformed the other two stock types in virtually all measured characteristics (Table 6.2). Significant effects of stock type were detected for final

(end of the growing season) height and stem base diameter, height and stem diameter increments, foliage and stem dry weights, projected leaf area, total root surface area, the length of roots, and the number of root tips. The PCT and PSB seedlings were not different from each other in any of the above mentioned characteristics and it was only the Vapo stock type that was outstanding. There were no significant differences among the stock types in ratios of foliage : root system dry weight (Table 6.2), total above ground dry weight : root system dry weight (p. 0.29), and projected leaf surface area : root system surface area (Table 6.2), even though these ratios tended to be higher in the Vapo seedlings. When root and foliage surface area ratios were compared, the total rather than projected root surface area was used versus projected leaf area. This was thought to be more adequate than using the projected root surface area. Roots were presumed to absorb water equally through their surface around their entire root circumference while gaseous exchange in the leaves would be more one - sided due to the positioning of the stomata mostly on the abaxial side of a leaf.

Before planting, the Vapo seedlings had visibly smaller and differently configured root systems than did the PSB and PCT seedlings (Figure 6.1). After one growing season, the majority of roots occurred in the sandy soil ramifying through its entire volume and very few roots occurred in the organic soil (Figure 6.2). Root systems of the PSB seedlings (Figures 6.3-6.4) and PCT seedlings (Figures 6.5-6.6) tended to resemble expanded configuration of their nursery shapes with fewer roots spreading laterally than in the Vapo seedlings. The Vapo seedlings (Figures 6.7-6.8) had strikingly larger, wider, and more symmetrically spread root systems than the other two stock types. Configurations of root systems below the supporting pins collapsed when the soil was removed. Some root systems were supported with more than one layer of pins and it was visualized that the spreading of the roots in the lower portions of the boxes was much greater than that shown on Figures 6.3-6.8 in all three stock types but in the Vapo seedlings in particular. However, the pins were an obstacle to photography as they obscured the view of the roots so the number of pins was reduced. Nevertheless, the presented photographs demonstrate the difference between root systems of the container - grown and the mechanically root - pruned seedlings. The root systems in the three stock types were clearly heterorhizic with abundant long roots bearing some long branch roots and copious numbers of short, fine roots (Figure 6.9). The thicker long roots that appeared to be of the structural, anchoring type were the most prominent. These were almost always elongated first - order laterals. This occurred even in the Vapo seedlings in spite of the fact that most first order laterals were severed during pruning. Some second order laterals also elongated and thickened forming extensions of the stumps remaining after pruning of the first order laterals. Many very long roots of further orders were also present. Most of the short, fine roots were mycorrhizal.

Multivariate analysis of variance showed significant differences among the three stock types in the distribution of the root length and root surface area into three arbitrarily established root diameter classes. The three classes were 1) root diameters equal or smaller than 0.4 mm, 2) root diameters larger than 0.4 mm but equal or smaller than 0.8 mm, and 3) root diameters larger than 0.8 mm. There was no significant variation among the stock type-treatment replicates in these distributions. Separate, univariate analyses of variance of the root length and percent of root length in each root diameter class showed significant effects of the stock type on the actual length of roots in these classes but the stock type effect on the percent of the root system's length was present only in the greater than 0.8 mm root diameter class. The Vapo seedlings had greater length of their roots in all three diameter classes than did the other two stock types and had significantly greater percent of their root length in the class with the greatest diameters (Figure 6.10). The other two stock types did not differ significantly from each other in root length. The outcomes of the analyses of variance on the root surface area distribution in the three root diameter classes were similar to those for the length distribution except for the distribution of percentages of the surface area. The Vapo seedlings had a significantly lower percent of root surface area in the thinnest and more in the thickest root parts than had the other two stock types (Figure 6.11). When true values rather than percentages were compared, the Vapo seedlings also had greater surface area in every diameter class than the other two stock types (Figure 6.11). The PCT and PSB seedlings did not differ significantly from each other in this respect.

Changes in seedling morphology between the second week and 6-8 weeks after planting

When pots with seedlings were being successively removed from the soil outdoors during the second week after planting their roots had just began to elongate. Short white root tips were common in root systems of seedlings from all three treatments. In the PCT and PSB seedlings most of these white root tips were located near the bottom of the nursery plugs. Some white root tips were also present on the sides of the plugs, particularly in the lower two thirds of the plugs. In the Vapo seedlings the white root tips were protruding through every side of the root/peat cube. It was apparent that the elongating roots of the PCT and PSB seedlings were thicker than those of the Vapo. This was so because many of the existing thicker long roots resumed elongation in the PSB and PCT seedlings while thicker laterals of the Vapo had been pruned. Some progress in the process of root elongation occurred during the five days of sampling and the advancement of the elongation was greater in seedlings sampled last than in those sampled at

the beginning. However, the soil was still cold and night air temperatures were near or slightly below zero. There were no signs of bud swelling during sampling at the time 1.

At the time 2, buds had already broken and young shoots were extending. Projected leaf area of seedlings increased in all three stock types between the first and the second sampling time (Table 6.3) due to the elongation of new shoots and expansion of new foliage. The effect of time was thus significant but this occurred in all three seedling types and no time interaction with stock type was detected. The significant stock type effect was due to the Vapo seedlings having the greatest projected leaf area at the start. The effect of time on foliage dry weight was also significant as it increased in all three stock types between the sampling times. However, there was no significant effect of time on dry weight of stems. Dry weight of the root systems increased somewhat between the time 1 and time 2 in the PSB and PCT seedlings but not in the Vapo (Table 6.3) even though some white roots were visible in all seedling types at time 2. However, the changes to root dry weight were too small to result in a statistically significant difference over time. The significant stock type effect was related to the Vapo seedlings having substantially smaller root dry weights at the times 1 and 2 than those of seedlings in the two other stock types (Table 6.3).

There was no significant change in the length of roots in the Vapo seedlings over the time between the two sampling periods and only minimal, statistically insignificant increase to the root surface area (Table 6.3). The PCT seedlings increased root surface area by more than 25% and the PSB seedlings by over 40% between the two sampling times. Root length was also strikingly greater at the time 2 than at time 1 and so was the number of root tips in these two stock types (Table 6.3). All these changes were reflected in analytical results showing significant time and stock type effects on root length, root surface area and the number of root tips. The significant stock type interaction with elapsed time reflected different root growth behavior of the Vapo seedlings. These seedlings also had the greatest foliage weight to root weight and foliage surface area to root surface area ratios at both sampling times. Their total above to below ground dry weight ratio was greater at time 1 than in the other two stock types and increased even more at time 2 (Table 6.3).

Hydraulic conductivity and water flux through root systems

A schematic diagram of the experimental set - up for measuring root pressure and root hydraulic conductivity with a pressure probe is shown in Figure 6.12. The most important result of the

analysis of variance on root hydraulic conductivity was the detected significant interaction between stock type and time. This was caused by the significant decrease in hydraulic conductivity of the PCT seedlings at time 2, compared to the time 1, and by the significant increase in the hydraulic conductivity of the Vapo seedlings at the time 2, compared to the time 1 (Table 6.4). Hydraulic conductivity of the PSB stock type was about the same at both times (Table 6.4). Significant differences in hydraulic conductivity among the stock types occurred only at the time 2 when it was greater in the Vapo seedlings than in the other two stock types. However, this was the specific conductivity that apportioned the hydraulic conductivity of a root system equally to every unit of the root surface area. Water flux through root systems was also affected by the stock type and time interaction. At time 1, there were no significant differences (at $\alpha = 0.05$) in water flux through root systems among the three stock types. However, the least square means of the PCT and the Vapo stock types were at the border line of being significantly different ($p = 0.06$). At the time 2, the Vapo seedlings, in spite of having smaller root systems, had the greatest water flux through their roots. Statistically, it was not significantly different from those of the PCT and PSB seedlings. These results showed that the capacity for maximum water flux through root systems could change substantially during the first post-planting weeks. It did not reflect the size of seedling's root systems and their expansion after planting, including the changes in root length, root surface area, and the number of root tips.

In an attempt to relate between water flux and morphological characteristics of the root systems, the length and surface areas were partitioned into the same root diameter classes as it was described for seedlings grown in boxes. Multivariate analyses of variance showed that the distribution of both root length and root surface area and their percentages of the total root system differed significantly among the seedlings and between the sampling times. Univariate analyses of variance showed significant effects of stock type and elapsed time for both root length distribution and surface area distribution in each of the defined root diameter class. The distribution of root length in the three root diameter classes is presented on Figure 6.13 and the distribution of the surface area on Figure 6.14. Root length and root surface area increased between the two sampling times in all root diameter classes except for the thinnest - root diameter class of the Vapo seedlings where it declined with time. In all three stock types greater percentages of root length and root surface occurred in the two classes of thicker root diameters at time 2, compared to time 1. Root surface areas in each root diameter class were plotted against the measured flux for all seedlings pooled and for each stock type separately. Similar plots were made for root tip numbers and hydraulic conductivity. However, no pattern indicative of any consistent relationship between these variables could be detected. Correlation analyses and various regression analyses were tried but they only confirmed the poor relationship between the

distribution of root diameters, numbers of root tips, and water flux through root systems of white spruce seedlings.

Root pressure

The significant effect of stock type interaction with sampling time on root pressure was due to the difference between the root pressure of the Vapo seedlings at the times 1 and 2. This also caused the significant effects of the stock type and sampling time detected in the analysis of variance as the other two stock types contributed little to these results. The Vapo stock type had three times greater root pressure at time 1 than had each of the other stock types. Root pressure of Vapo seedlings declined significantly by the second sampling time (Table 6.4). At time 2, there was no longer any significant difference in root pressure among the three stock types. The PCT and PSB seedlings did not differ significantly from each other at any time.

DISCUSSION

This study examined the post - planting growth and morphological responses of white spruce seedlings to different treatments of their root systems during nursery growth. The three resulting stock types were compared in three different ways: 1) by measuring their height and diameter growth during the first growing season after planting on forest sites - the most widely used type of seedling performance assessment; 2) by the assessment of growth and a detailed examination of morphological characteristics of shoots and roots of the seedlings grown in the absence of competition by other vegetation and with little if any root system containment or other physical limitation to root growth; and, 3) by analyzing the growth of seedlings, particularly of their roots, and changes to root system hydraulic properties during the first weeks after planting. Collectively, these approaches provided a detailed evaluation of growth and morphological characteristics of the three types of planting stock at different times of the first post - planting season and under different growth conditions.

One of the most striking observations in this study was the lack of differences in growth and morphology, especially root system morphology, between the two container - grown stock types. The absence of significant differences in root length, root surface area, and the number of roots between these two stock types early after planting, during the second month after planting, and at the end of the growing season (in box - planted seedlings) demonstrated that chemical root pruning of white spruce grown in the commercially available polystyroblocs designed for that purpose was completely ineffective. Burdett and Martin (1982) showed that the effectiveness of chemical root pruning depended on the interaction among factors such as the species, the size of root cavity, the properties of the growing medium, and the concentration of the toxic compound. Clearly, these factors must be taken into account if chemical root pruning were to be used effectively across a range of species. While it would be possible to influence the release of copper ions from the container walls by regulating the properties of the growing medium, especially by regulating its pH, such manipulations could be risky. The pH could impact on the nutrient uptake by the roots (Jungk, 1991; Marschner, 1991), and on the microbial life in the rhizosphere (Smiley and Cook, 1973; Bowen and Rovira, 1991).

The Vapo seedlings grew generally better on the planted forest sites than did the other two stock types. The greater stem diameter growth was evident on both planted sites. It is dubious that the greater diameter increment in the Vapo seedlings was simply related to their greater size at

planting. There were no significant differences in the heights of the seedlings in the three stock types at planting and the differences in average stem base diameters, although statistically significant, were small. The Vapo seedlings also grew better in height on one site while there was no significant difference in height growth among the stock types on the second site.

In the soil - buried boxes, the Vapo seedlings grew more in height and stem diameter, had larger root systems with more individual roots, greater total root surface area, and root length than the two other stock types. This was clearly a superior performance. Numbiar (1980) wrote that the configuration of radiata pine root systems regenerated after transplanting closely resembled their initial configuration. In this study, container grown white spruce seedlings tended to have a cylindrical configuration of their root systems with many roots growing vertically downwards. They resembled an expanded shape of their nursery plug. The mechanically pruned seedlings had more widespread roots, with fibrous appearance and these root systems showed no signs of root containment during nursery growth. The sandy soil in the boxes offered little if any restriction to root growth and seedlings of all three stock types explored the soil to the bottom of the boxes.

The boxes were filled with infertile sand and the plants were not fertilized during the entire growing season. Yet, seedling growth was very good in all three stock types but outstanding in the Vapo. This could be attributed to the absence of vegetative competition. The plants had the entire soil volume in the box for their own exploitation. The contribution of the mineralization of the organic layer placed on the top of the sand to the nutrition of the plants in boxes was not known. It was very likely that leaching of soluble nutrients from the top layer into the sand occurred. Zutter *et al.* (1997) showed a positive relationship between the early growth of sweetgum (*Liquidambar styraciflua* L.) and loblolly pine (*P. taeda*) and the amount of the organic matter in the upper part of the soil profile. However, there did not appear to be any great concentration of the roots near the organic soil/sand interface and almost no root growth occurred in the organic layer in any stock type. However, the organic layer tended to dry very much during warm dry weather, similar to what happens on many forest sites (Sutton, 1991). Observations from this part of the study suggested that spruce roots would explore the soil to a considerable depth if there are no impediments to their vertical growth rather than proliferate just under the organic layer.

Numbiar (1980) remarked that the number of first order laterals in radiata pine did not appear to be amenable to nursery management practices. In the present study, roots were not classified relative to their developmental origin as the software used for the analysis of root images did not

provide such an option. However, roots systems were carefully observed during their preparation for scanning. These observations agree with Nambiar's (1980). The major structural roots of white spruce seedlings that provide the basic framework of the root system were the first order laterals. In the Vapo seedlings, the large first order laterals were pruned during nursery growth. After planting, the established major structural roots were either first order laterals that must have originated from elongated smaller roots or existing primordia. There were also second order laterals that extended from stumps left after pruning of the thicker first order laterals and formed major structural roots. Apart from the development of the framework of the large structural roots there did not appear to be any strong relationship between the developmental sequence and function of the roots, as was noted by Fitter (1991). Roots of any order could either elongate and support their own network of associated roots or remain short with or without mycorrhizae. Dichotomy of the short roots was uncommon in white spruce seedlings even if the short roots were mycorrhizal. After one growing season, the tap root was discernible only in its short basipetal portion as its prominence diminished through continuous forking and branching.

Root pressure of conifers has been reported (Rüdinger *et al.* 1994; Hallgren *et al.* 1994) as usually near zero (less than 0.02 MPa). This is very low, compared to the range of root pressure found in deciduous species which is between 0.05 - 0.5 MPa (Kramer, 1983). Steudle and Meshcheryakow (1996) reported root pressure of young oak seedlings to be in the range of 0.05 - 0.15 MPa. The root pressures found in white spruce seedlings in this study were surprisingly high, with means in the range between 0.09 - 0.3 MPa, values not previously reported for conifers. However, the potential relationship between root pressure and the growth stage of the plant has been rarely considered. Johnson-Flanagan and Owens (1986) reported respiratory changes of white spruce roots related to the changes in root morphology, the stage of shoot elongation, and to the environmental conditions. They concluded that the respiratory changes during the year were related to varying metabolic requirements. In this study, root pressure was measured at the time of the resumption of root growth shortly after planting and during the rapid phase of shoot elongation. Root pressure declined in the PCT seedlings and, especially, in the Vapo seedlings during shoot elongation, compared to the earlier measurement but it was still greater than expected from what had been reported in the literature. Root pressures measured in dormant seedlings in another part of this series of studies (Krasowski, M.J., unpublished data) were indeed negligible. Steudle and Meshcheryakov (1996) showed a decline of the steady-state root pressure in oak seedling with time. However, these authors remarked that results obtained during the first day of testing probably best resembled characteristics of roots in intact plants. Root pressures presented in this work on white spruce seedlings were obtained within 2 -4 hours from severing the top but when the root pressure was considered to be at the steady state.

What could be the reasons for such a high root pressure measured in the current study? One possibility could be the presence of large amounts of storage substances in the roots and their mobilization during growth resumption in the spring and during rapid shoot elongation. Rook (1969; 1971) found greater concentration of storage carbohydrates in wrenched than in uncut roots of radiata pine seedlings. Although soluble carbohydrates would be transported through the phloem rather than through the xylem, various metabolites produced in the roots during growth resumption could enter the xylem decreasing the water potential of its sap. Also, an increased concentration of solutes in the xylem parenchyma and in the phloem could generally intensify water influx into the stele. Root pressure could arise from osmotic potential of the xylem sap and/or from metabolic pumping of water into the xylem (Zholkevich, 1991). The metabolic constituent of the root pressure could also be significant during growth resumption and the period of shoot elongation. In their discussion of root pressure Steudle and Meshcheryakov (1996) compared the values obtained for oak with those reported for Norway spruce by Rüdinger *et al.* (1994), also measured with a pressure probe. Low root pressure of conifers was suggested to result from a low rate of active pumping of nutrients and by low root reflection coefficients (approximated by the maximum changes to root pressure at a given change in the osmotic pressure of the medium). However, it was shown by Steudle and Meshcheryakov (1996) that reflection coefficients of oak seedling roots were also low. Results presented by Hallgren *et al.* (1994) showed variation of root reflection coefficients calculated from measurements using different kinds of osmoticum. Results of Rüdinger *et al.* (1994) indicated that leak rates (solute permeability) were low in spruce. There has been no evidence of lower rates of active pumping of nutrients by conifer roots compared to deciduous species. Thus, why should conifer roots be different in these characteristics from roots of deciduous species? Exudation of xylem sap from decapitated conifer seedlings has been reported (Lopushinsky, 1980; Sands *et al.* 1982) which would support the existence of root pressure in conifers at least at certain times of the year.

Root pressure values recorded in the current study are closer to those reported for oak seedlings by Steudle and Meshcheryakov (1996) than to those reported by Rüdinger *et al.* (1994) and by Hallgren *et al.* (1994) for Norway spruce. However, the discrepancies could have been caused by differences in the application of the measurement technique and by different growth conditions that could have impacted on the rates of growth and shoot and root development. Hallgren *et al.* (1994) began their measurements 6 - 8 weeks after planting the two year old seedlings of *Picea abies*. This was the same time after planting as the time 2 of this study. Yet, they reported negligible root pressure of tested seedlings. Hallgren *et al.* (1994) kept the seedlings in a glasshouse where the temperature ranged between 18 - 28°C. This would have accelerated the

rates of growth, increased respiration, and possibly depleted the reservoir of root storage materials faster than in seedlings planted into ambient conditions. This, of course, would apply only if the breakdown of the storage materials really impacted on the root pressure. Also, Hallgren *et al.* (1994) pressurized the root systems for 12 hours prior to measurements and exuded the sap in order to remove air from the xylem. Thus, the original xylem sap was largely or completely removed and water from the soil in the pot was forced into the xylem. This could result in no osmotic potential gradient between the soil solution and the xylem sap and, consequently, no root pressure when the measurements began. If there is indeed a metabolic component of the root pressure it could also diminish several hours after decapitating the plant.

Hydraulic conductivity measured in this experiment was more in agreement with values obtained for Norway spruce by Hallgren *et al.* (1994) and by Rüdinger *et al.* (1994), than were the measurements of root pressure. The hydraulic conductivity measured in white spruce corresponded with the lower ranges of measurements obtained for Norway spruce. Hallgren *et al.* (1994) reported the hydraulic conductivity of Norway spruce seedlings to be in the range of $1.9 - 62 \times 10^{-8} \text{ m s}^{-1} \text{ MPa}^{-1}$ and considered these values to be in reasonable agreement with those reported by Sands *et al.* (1982) which were about $14 \times 10^{-8} \text{ m s}^{-1} \text{ MPa}^{-1}$. In white spruce seedlings tested in the currently reported study much smaller differences in hydraulic conductivity were found to be statistically significant. Therefore, where do these values become really different? It must be stressed that hydraulic conductivity is calculated per unit of root surface area, thus, it is a specific hydraulic conductivity that is the same for each and every part of the root system. Therefore, it does not consider differences in the resistance to flow among different parts of the root system. Sanderson (1982) showed a significant decline in water uptake some distance from the root tip of barley roots and attributed this observation to changes in the resistance to radial flow through the root caused by the maturation of root tissues, mainly to the developmental changes in the anatomy of the endodermis. Considering the fact that different regions of the root apparently differ in their resistance to radial flow, and that this could be related to the rates of root maturation, the hydraulic conductivity would be greatly influenced by the size of the root system. Therefore, it makes much more sense to look at the total flux through root systems than at the specific hydraulic conductivity calculated from the total root surface area.

It was shown in the present study that the size of the root system had little to do with water flux through that root system. The flux in the Vapo seedlings was equal or greater than that of the PCT and PSB seedlings that possessed larger root systems. Also, the rate of root system

expansion between the two measurement times did not result in increased water flux through these root systems, to the contrary, it rose in the Vapo seedlings that showed little root system expansion while it did not change in the PSB seedlings and even declined somewhat in the PCT seedlings in spite of their greater root system expansion during the period between the two measurement times. If the hydraulic properties of the root depended on the number of elongating roots then it could be expected that the significant increase in the number of root tips in the PCT and PSB seedlings between the two measuring times would also result in greater total hydraulic conductivity of these root systems. This could be anticipated as more young elongating roots would be expected to create larger surface area in regions of low resistance to flow. This, however, was not so. To the contrary, root systems that apparently grew less, initiated fewer root tips, did not increase significantly root length and root surface area in fact also significantly increased the maximum water flux they could support. Judging by the fact that the mean number of roots (indicated by the number of root tips) was smaller in Vapo seedlings sampled at time 2 than in those sampled at time 1, it could be suspected that, by chance, seedlings sampled at the second time had had substantially fewer lateral roots to start with than those sampled at time 1. This would not have been obvious during planting as the peat embedding the roots obscured their view. However, if root growth of the Vapo seedlings during these few weeks were substantial it would have shown in greater size of root systems sampled at the time 2.

The failure to find a consistent relationship between water flux through root systems and their surface area in different root diameter classes and the number of root tips suggests that there are other factors that impacted on hydraulic properties of the roots that were not considered here. Perhaps it would have been more meaningful to separate white, elongating roots from the rest of the root system and seek the relationship between measured water flux and the surface area of these roots. This remains to be examined.

Finally, it should be remarked that the initial root expansion after planting did not correspond to the differences in root and shoot biomass found at the end of the growing season among the three types of white spruce seedlings. Judging by the growth of seedlings in the boxes and on forest sites, the prediction of growth performance based on root growth during the first few weeks after planting would have been wrong. It appears that the rate of root growth in the container - grown seedlings varied greatly from that of the mechanically pruned seedlings during the growing season. The container - grown seedlings conformed to the growth pattern of containerized white spruce seedlings previously described by Johnson-Flanagan and Owens (1985) while the mechanically pruned seedlings did not. There is certain controversy about the necessity of vigorous root growth shortly after planting for the successful establishment and growth of

seedlings (see the debate in Simpson and Ritchie, 1997). The currently reported study does not support this notion. White, actively growing roots were seen a week after planting and 6 - 8 weeks later in all three tested stock types. However, the size of the root system and the lack of its significant expansion during the first post-planting weeks did not limit the root system's capability to conduct water and did not accurately predict the growth performance of these seedlings during the first growing season.

Table 6.1. Least square means and their standard errors (SE) for morphological variables height, stem-base diameter and their increments over one growing season in three types of planting stock (STK) planted on two sites in the central interior of British Columbia. The means are given by STK and S but only if these sources had a significant effect on the examined variables. Means with the same letter are not significantly different (at $\alpha = 0.05$) from other means within the group listed by the same source.

Variable	Separated by	Least square mean	SE
Height at planting (mm)	Site 1	124.2a	1.98
	Site 2	115.3b	2.00
Height after one season (mm)	Site 1	209.4a	2.49
	Site 2	185.8b	2.52
Height increment (cm)	Site 1	85.2a	2.57
	Site 2	70.5b	2.59
Stem base diameter at planting (mm)	STK:Vapo	2.48a	0.03
	STK:PCT	2.21c	0.03
	STK:PSB	2.39b	0.03
Stem base diameter after one season (mm)	Site 1	3.94a	0.07
	Site 2	3.65b	0.07
	STK:Vapo	4.23a	0.05
	STK:PCT	3.48c	0.06
	STK:PSB	3.72b	0.06
Stem base diameter increment (mm)	STK:Vapo	1.74a	0.06
	STK:PCT	1.28b	0.06
	STK:PSB	1.33b	0.06

Vapo - seedlings with mechanically pruned roots

PCT - styroblock - grown seedlings with chemically pruned roots

PSB - styroblock - grown stock, no root pruning

Table 6.2. Least square means of morphological characteristics of three different stocktypes of white spruce seedlings grown outdoors in soil-filled boxes for one season.

Variable	Stocktype means		
	PCT	PSB	Vapo
Height at planting (mm)	114.2a	123.2a	127.8a
Final height (mm)	234.2b	237.0b	312.1a
Height increment (mm)	120.0b	113.8b	184.3a
Stem diameter at planting (mm)	2.62a	2.77a	2.71a
Final stem diameter (mm)	5.54b	5.47b	6.99a
Stem diameter increment (mm)	2.92b	2.70b	4.28a
Foliage dry weight (g)	2.41b	2.32b	4.55a
Root dry weight (g)	2.79b	2.70b	4.75a
Stem dry weight (g)	2.11b	2.14b	4.24a
Total dry biomass (g)	7.31b	7.16b	13.54a
Foliage:root dry weight ratio	0.88a	0.89a	0.96a
Projected leaf area (cm ²)	120.8b	114.4b	200.6a
Root surface area (cm ²)	643b	645b	987a
Proj. leaf area: root surface area	0.20a	0.18a	0.21a
Total length of roots (cm)	4699b	4705b	6408a
Number of root tips	7543b	7124b	10999a

Note: means in the same rows followed by the same letter are not different from other means in the same row at $\alpha = 0.05$.

Table 6.3. Least square means and their standard errors (bracketed) of morphological characteristics of white spruce seedlings of three different stock types measured at two different times after planting.

Variable	Time 1			Time 2		
	PCT	PSB	Vapo	PCT	PSB	Vapo
Projected leaf area (cm ²)	46.6 (5.2)a	43.6(5.2)a*	59.1(5.2)a	57.4(4.3)a	62.0(4.3)a	65.2a(4.3)
Foliage dry weight (g)	1.00(0.11)a*	0.93(0.11)a*	1.22(0.11)a*	1.32(0.09)a*	1.35(0.09)a*	1.41(0.09)a
Root surface area cm ²	235(16)ab*	243(16)a	188(16)b	315(13)a*	347(13)a*	214(13)b
Root system dry weight (g)	0.95(0.05)a	0.95(0.05)a	0.69(0.05)b	1.04(0.04)a	1.09(0.04)a	0.68(0.04)b
Foliage area: root area ratio	0.20(0.02)a	0.18(0.02)a	0.32(0.02)b	0.18(0.02)a	0.18(0.02)a	0.32(0.02)b
Foliage:root dry weight ratio	1.05(0.09)b*	0.97(0.09)b*	1.77(0.08)a*	1.23(0.06)b*	1.25(0.06)b*	2.11(0.06)a*
Total above ground: below ground dry weight ratio	1.51 (0.12)b	1.33(0.12)b*	2.38(0.12)a*	1.68(0.08)b	1.69(0.08)*b	2.84(0.08)a*
Root length (cm)	1965(153)a*	2124(153)a*	1630(153)	2479(120)a*	2815(121)a*	1643(121)b
Number of root tips	3503(274)a*	3831(274)a*	3104(274)a*	5237(213)a*	5539(213)a*	2847(213)b*

Note: Means in columns with the same letters are not different from each other at $\alpha = 0.05$ within each time of the measurement. Asterisks indicate significant differences between means at Time 1 and Time 2 for the same stocktype at $\alpha = 0.05$.

Table 6.4. Least square means and their standard errors (bracketed) for hydraulic conductivity, total root system hydraulic conductivity, and root pressure of white spruce seedling's root systems for three types of planting stock measured at two different times after planting.

Variable	Time 1			Time 2		
	PCT	PSB	Vapo	PCT	PSB	Vapo
Specific hydraulic conductivity $\cdot 10^{-8} (\text{m} \cdot \text{s}^{-1} \cdot \text{MPa}^{-1})$	3.20 (0.48)a*	1.98 (0.48)a	2.29 (0.48)a	1.87 (0.35)b*	1.93 (0.35)b	3.88 (0.35)a*
Water flux rate $\cdot 10^{-12} (\text{m}^3 \cdot \text{s}^{-1} \cdot \text{MPa}^{-1})$	744 (107)a	489 (107)a	417 (107)a*	589 (76)a	665 (76)a	783 (76)a*
Root pressure MPa	0.11 (0.04)b	0.11 (0.04)b	0.30 (0.04)a*	0.11 (0.03)a	0.16 (0.03)a	0.09 (0.03)a*

Note: Means in columns with the same letters are not different from each other at $\alpha = 0.05$ within each time of the measurement. Asterisks indicate significant differences between means at Time 1 and Time 2 for the same stocktype at $\alpha = 0.05$. The $p > |T|$ value in the pairwise comparison of total hydraulic conductivity least square means of the PCT and Vapo at Time 1 was 0.06.

Figures 6.1-6.2. Root systems of white spruce seedlings grown in boxes filled with a nursery soil for one growing season.

Figure 6.1. Seedlings of the three stock types used in the study: a) seedling grown in a PSB 415B polystyrobblock, b) seedling grown in a PCT 415B polystyrobblock with container walls covered with a root pruning chemical, and, c) seedling grown in a Vapo peat board with mechanically pruned roots. Root systems were washed to remove adhering substrates.

Figure 6. 2. Roots of the Vapo seedling ramify through the entire volume of the soil in the box. Only a few roots (arrows) occurred at the interface (arrowhead) between the organic soil (OR) and the sand (S).

Figures 6.3 - 6.8. Root systems of box - grown seedlings of the three tested stock types of white spruce. Soil was gently removed but the roots were not washed. Some wire pins were removed before the photographs were taken but they were originally spaced 5 cm apart. This indicates the scale of the photographs and shows that photos on Figs. 6.7-6.8 are to a slightly more reduced scale than those in Figs. 6.3-6.6.

Figure 6.3. A side view of a PSB seedling root system showing some lateral roots (arrows) originated in the upper part of the nursery plug and more laterals growing out of its lower part.

Figure 6.4. A view from above on the root system of another seedling of the PSB type showing poor and asymmetrical lateral spread of the roots. Arrow shows the position of the cut stem.

Figure 6.5. a side view of a root system of a PCT seedling showing poor lateral spread of the roots and cylindrical shape of the root system resembling its nursery shape.

Figure 6.6. A top view of the same PCT-seedling root system as in Figure 6.5 showing poor extension of the laterals in the upper part of the root system (arrow).

Figure 6.7. A side view of a Vapo seedling root system showing good spreading of lateral roots in the upper part of the root system (arrows) and abundant roots in the lower part collapsed after the soil removal (arrowhead).

Figure 6.8. A top view of the same root system as in Figure 6.7. Note the soil adhering to the regions with abundant short laterals (arrows).

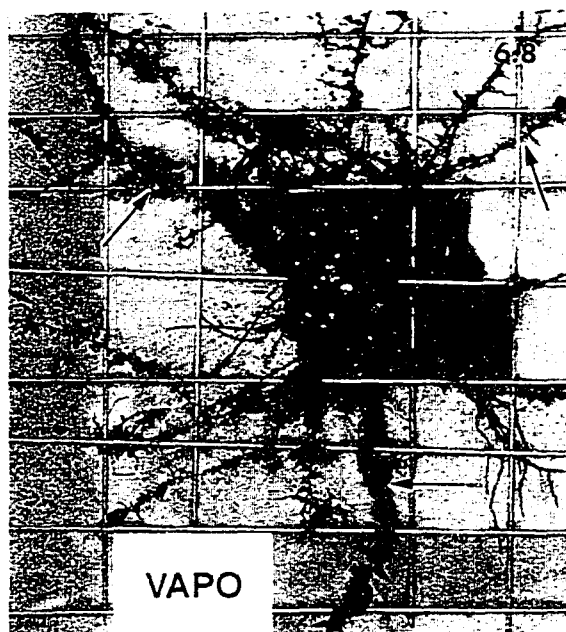
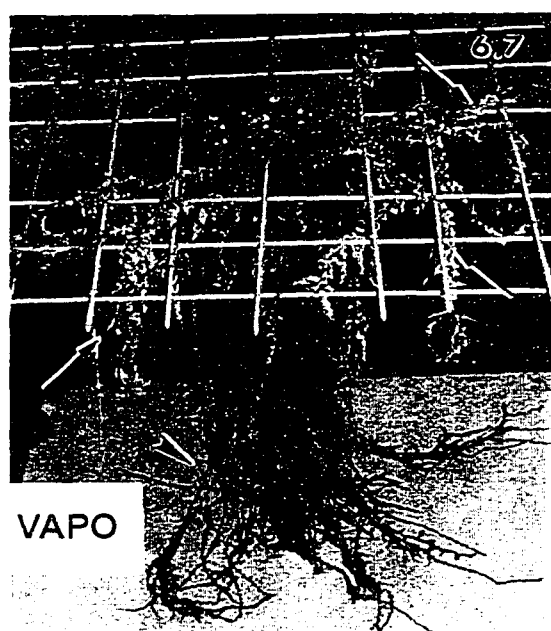
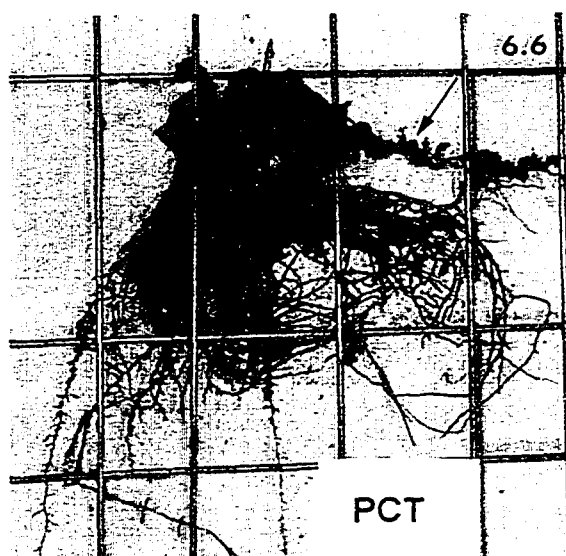
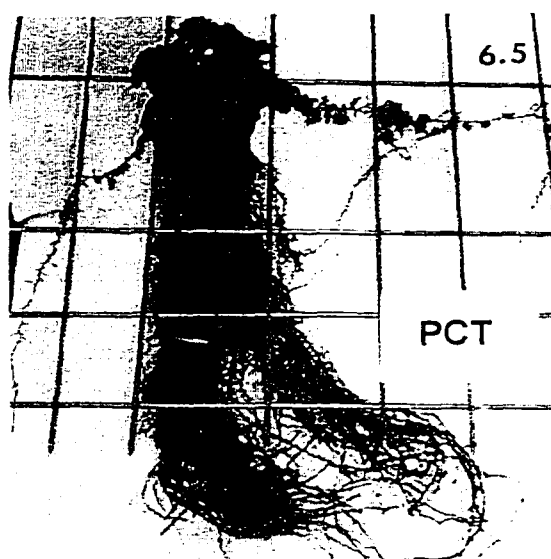
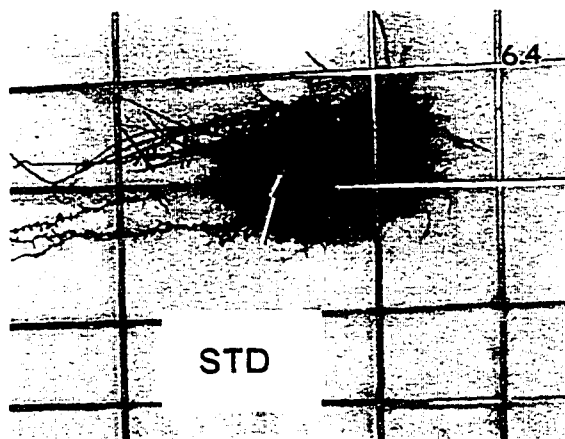


Figure 6.9 A scanned image of a Vapo seedling root system (framed insert) and an enlargement of the upper part of the image. One of the large laterals with associated roots was detached prior to scanning to minimize root overlapping and decrease the size of the image file. Note that the tap root was easily discernible at this stage but its forking near the tip would soon result in the loss of its characteristic appearance. The tap root was air pruned and the first order lateral elongated from the fork. Blunt tips of the larger first and second order laterals resulted from mechanical pruning. Most of the developing major structural laterals were first - order lateral roots. Some elongating second order laterals forming the major framework of the root system were also evident. Numerous white roots (empty arrowheads) appear gray on the enlarged image.

Figure 6.9

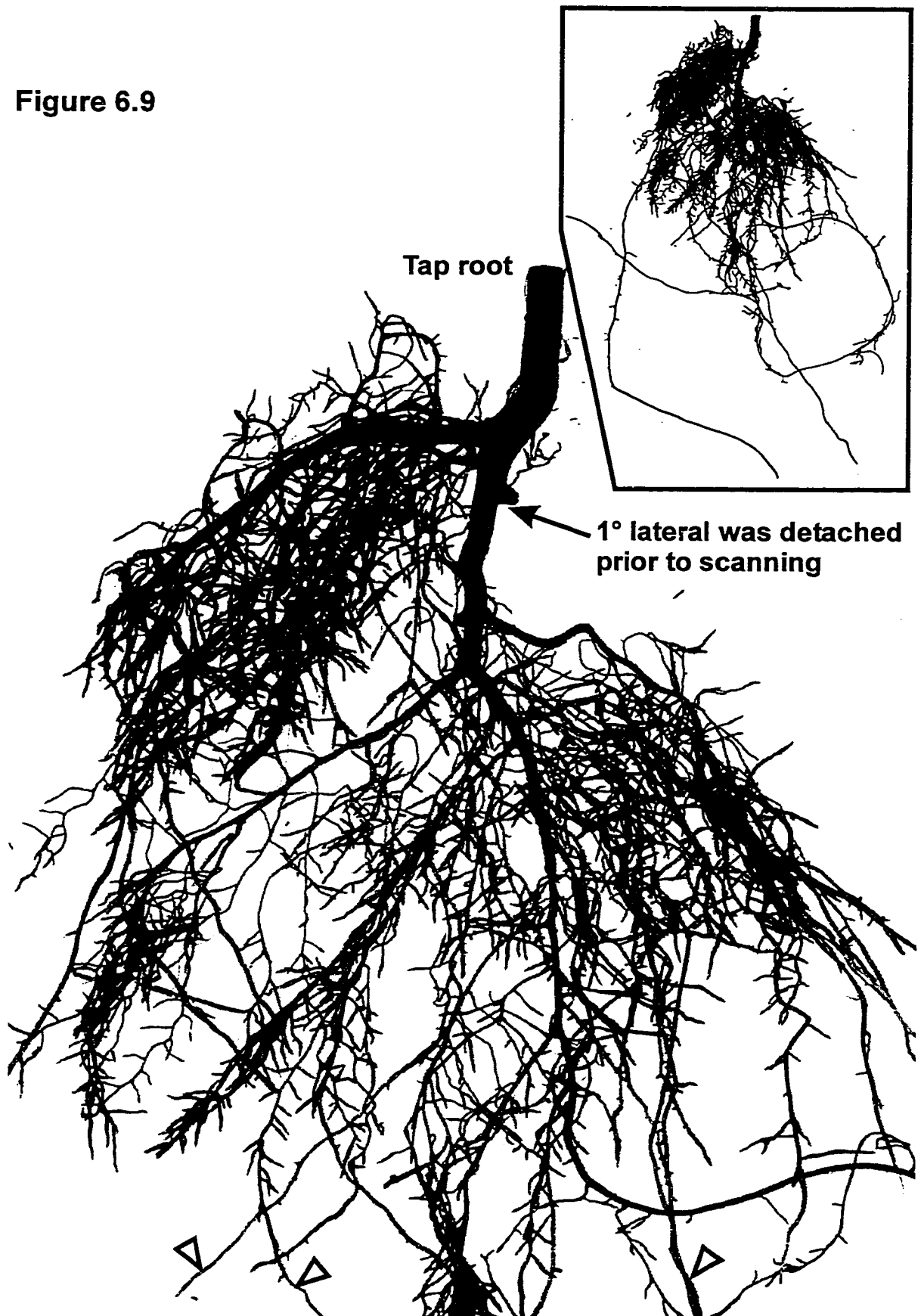


Figure 6.10-6.11. The average distribution of root length (Figure 6.10) and root surface area (Figure 6.11) in three root diameter classes (see legend). The actual least square means are plotted while the numbers indicate the percent of the class length or diameter in the whole root system. The y axis of Figure 6.10 is in cm, and in cm^2 in Figure 6.11.

Root diameter classes:

▨ ≤ 0.2 mm

▤ $0.4 \leq 0.8$ mm

■ > 0.8 mm

Figure 6.10

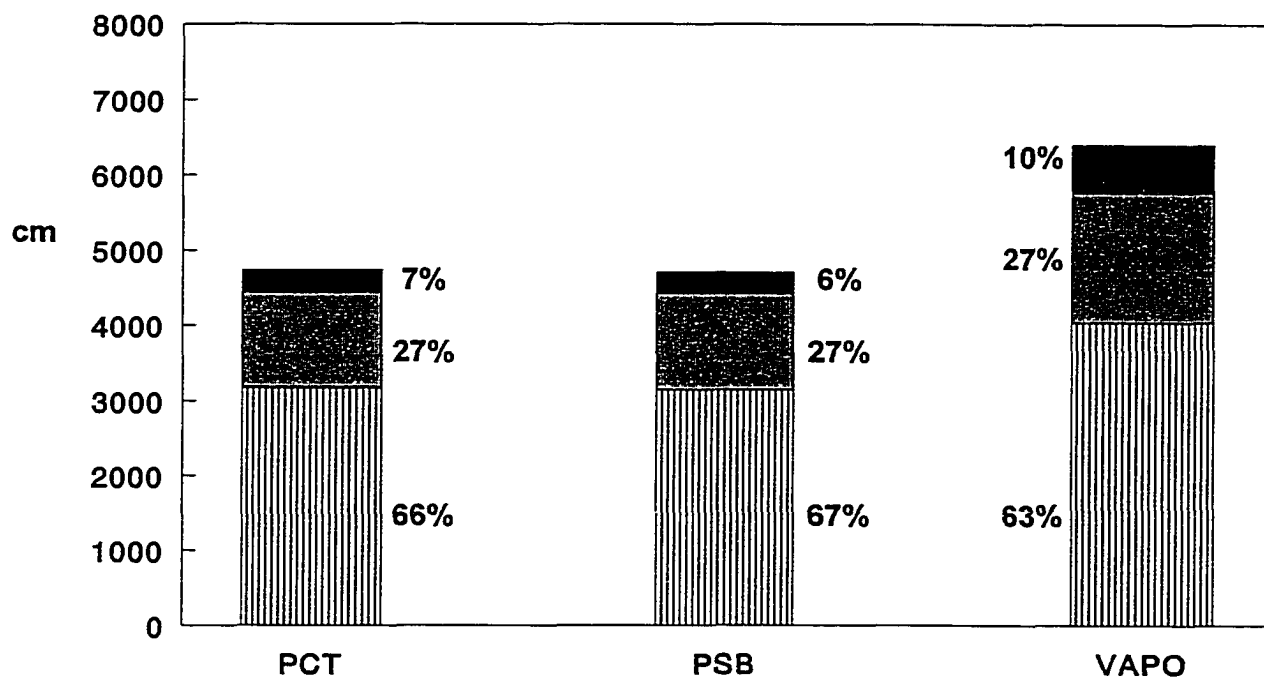


Figure 6.11

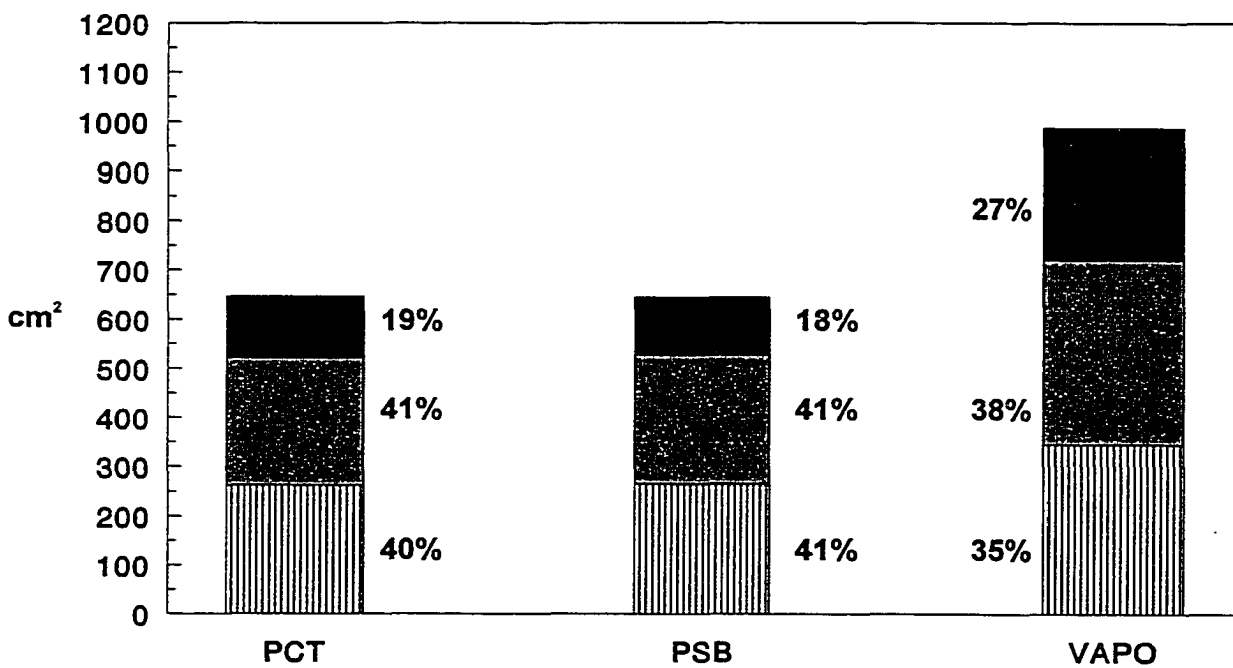


Figure 6.12. A schematic sketch of the pressure probe - root system set up for the measurement of root pressure and hydraulic conductivity. The entire space between the sealed stem above the root collar and the tip of the metal rod was filled with water on the root side and with silicon oil on the rod side. Moving the rod forward or backward displaced the volume and created positive or negative pressure in the system. Observing the distance by which the oil/water meniscus was displaced allowed to calculate the volume change while the pressure change was detected by the pressure transducer. The sketch was modified from that presented by Steudle and Meshcheryakov (1996).

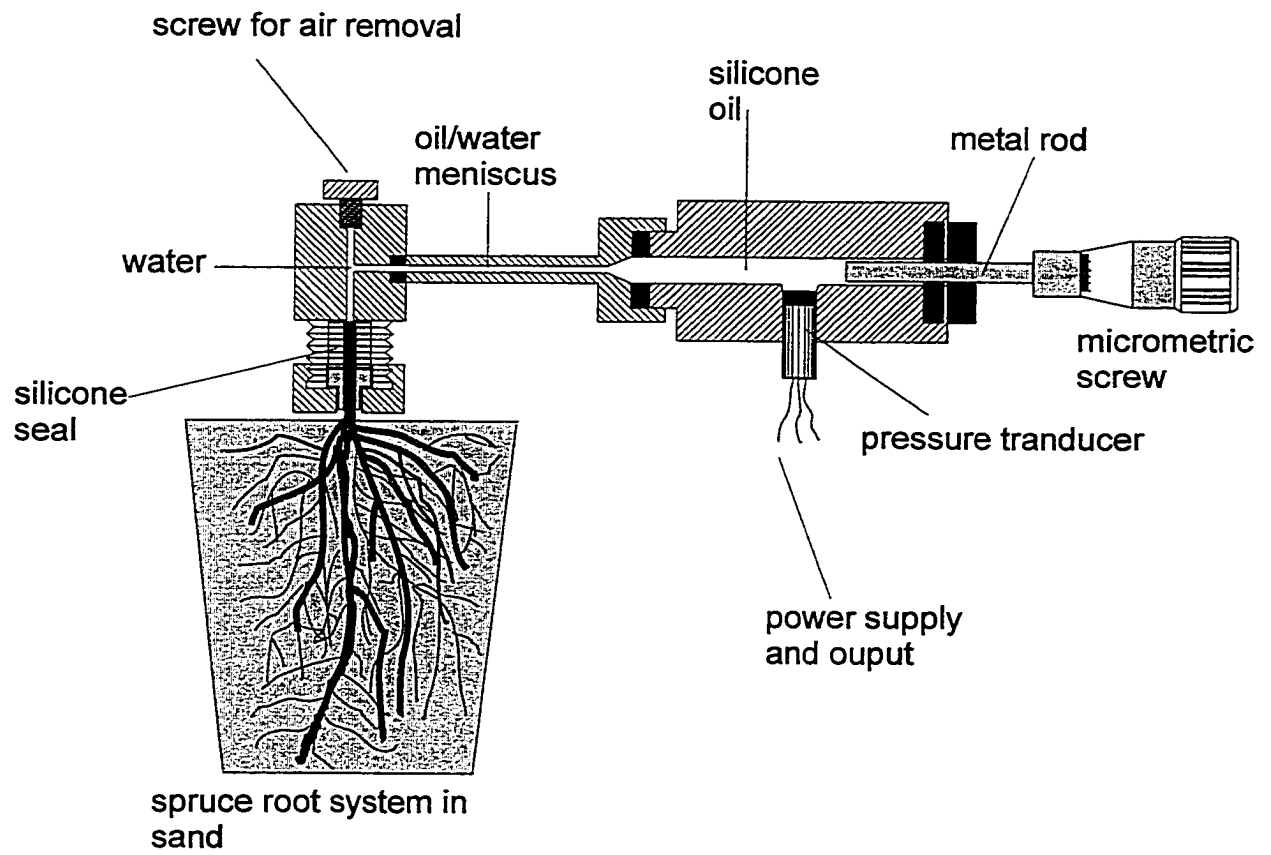
Figure 6.12

Figure 6.13 - 6.14. Distribution of root length (Figure 6.13) and root surface area (Figure 6.14) in the three defined root diameter classes (see the legend). The y - axis of the Figure 6.13 is in cm, and that of Figure 6.14 in cm^2 .

Root diameter classes:

▨ ≤0.2 mm

▤ 0.4 ≤0.8 mm

■ >0.8 mm

Figure 6.13

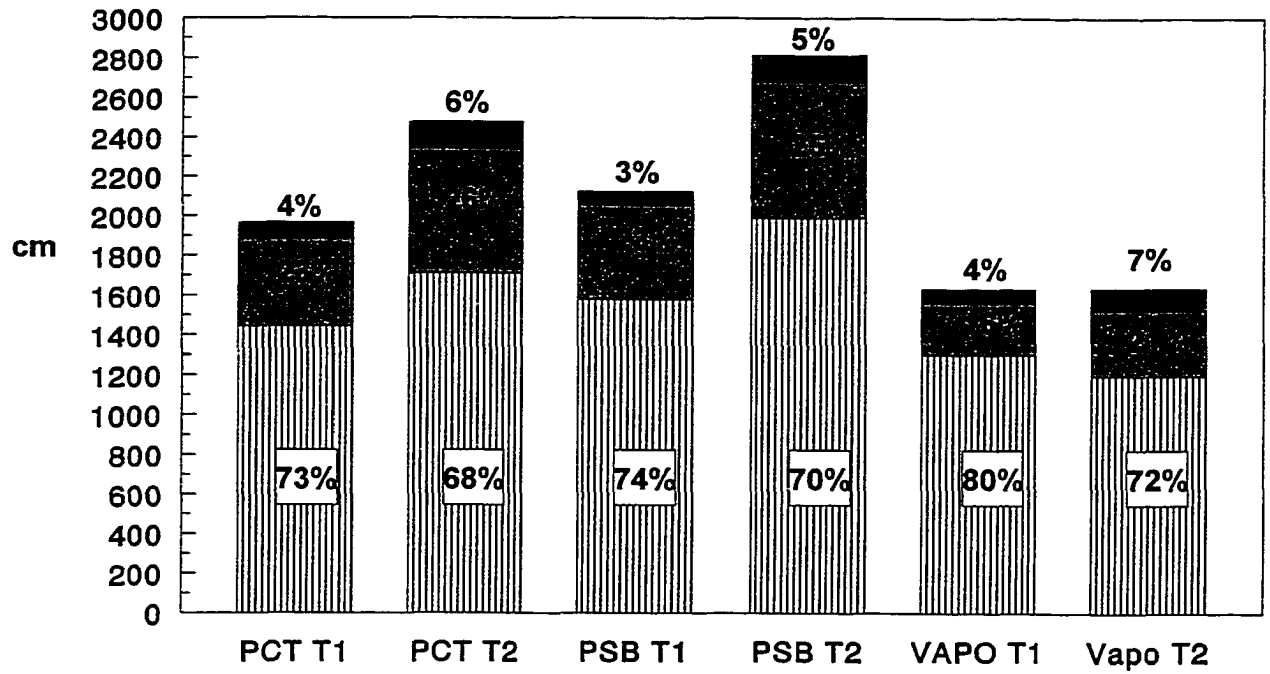
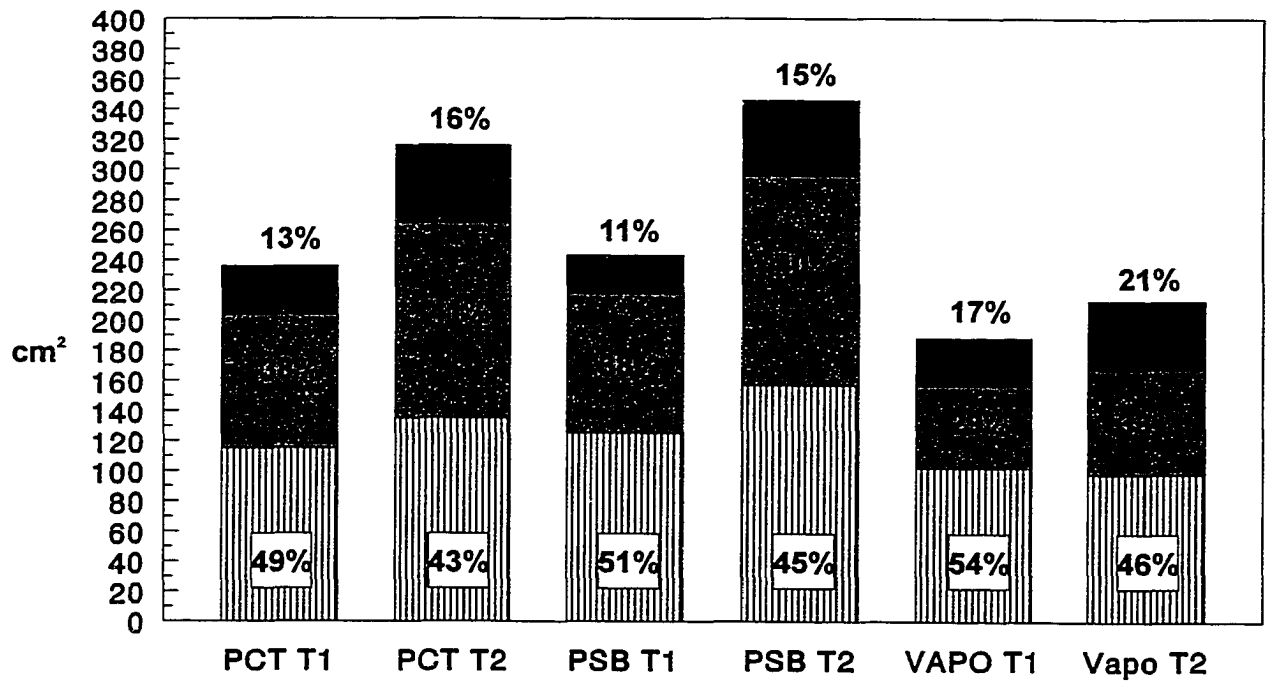


Figure 6.14



SUMMARY AND GENERAL CONCLUSIONS

The description of root growth and root system development patterns during the first post-germination season revealed some differences in comparison to previously reported results of investigations on the same subject made on roots of container-grown seedlings of white spruce during the second growing season. It appeared that the differences in root growth that lead to the dynamic changes in the development of root systems were related to the initial difference in the numbers of roots at the beginning of the growing season. Young germinants initially required a simultaneous growth of the entire root system, necessary for the gain of an adequate capacity for the uptake of water and nutrients as well as for the anchorage of the plant. Apparently, root systems of young germinants are required to surpass some minimal size needed for the fulfillment of these functions before switching to a more flexible mode of growth. The abundance of roots, especially in root systems with configurations altered by root confinement during nursery growth, as in container-grown seedlings, could be disadvantageous to the establishment of these seedlings after planting. Such seedlings, having numerous roots, could rely on a more conservative pattern of root system expansion than seedlings that are forced to regenerate their root systems after planting. This may explain a generally better performance of seedlings with roots pruned at the nursery, reported in the literature and observed in this study, in comparison to seedlings planted with large root systems, especially those disfigured at the nursery or at planting.

The portion of the study that investigated the effectiveness of different methods of root pruning on seedling performance and hydraulic conductivity after planting confirmed the superior growth of seedlings with effectively pruned roots, compared to those that were not pruned or were pruned ineffectively. This was demonstrated by the superior above and below-ground growth of mechanically pruned seedlings in comparison to the performance of container-grown seedlings, whether chemically pruned or not. However, this superiority was evident at the end of the first growing season and not immediately after planting. Initially, there was significantly less root elongation and initiation of new laterals in seedlings with mechanically pruned roots than in the unpruned or ineffectively pruned container-grown seedlings. This could make the mechanically pruned seedlings more prone to drought stress in the period immediately following the planting since hydraulic properties of their small root systems were not superior to those of the container-grown seedlings. This changed in 6-8 weeks after planting when the mechanically pruned seedlings developed a significantly greater capacity to conduct water through their roots than did

the container-grown seedlings. The fact that this occurred in spite of less root growth and less surface area in the former than in the latter seedlings indicated that root system expansion after planting did not guarantee an improved hydraulic capacity of the root system. This finding could explain some discrepancies between results of root growth capacity tests performed prior to planting and the post-planting performance of seedlings.

Differences in the capacity of root systems to conduct water are not easily explained. The developmental anatomy part of this study supports earlier reports regarding the relationship between the differentiation of primary root tissues and rates of root elongation. If suberization of the endodermis slows down the movement of water into the stele, it can be expected that root systems with numerous elongating roots will be more permeable to water than those with few roots or with roots elongating slowly. This is because fast-elongating roots with numerous young branches would have a greater proportion of segments free of the secondary-state endodermis. This could create less resistance to the flow of water to the xylem. This seemingly contradicts the results of the root pruning study. However, a likely explanation of this fact is that the initial fast expansion of the root systems of container-grown seedlings may be short lived and after the initial surge of root growth their root elongation rate declines. In mechanically pruned seedlings, the reverse may be true and after the initially slow growth, these seedlings may be entering a fast-expansion phase that makes their roots more permeable to water. The great sizes of root systems in mechanically pruned seedlings found at the end of the growing season support this notion and indicate that the period of rapid root expansion in these seedlings must have lasted longer than in the container-grown plants. Since water is the carrier of soil nutrients into the plant, high hydraulic capacity of root systems could impact on the nutrient uptake and this could be the reason explaining the excellent growth of the mechanically pruned seedlings. It should be noted, however, that the measured hydraulic conductivity of a root system is only indicative of its maximum capacity to meet a demand for rapid flow of water but it cannot be regarded as a measure of actual rates of flow. This qualifies the above discussion suggesting that the better growth of the mechanically pruned seedlings could also be the result of the avoidance of growth-hampering water stress that could be caused by insufficiently permeable roots during times of intense transpiration.

Results of the root permeability studies indicate that uncontrolled entry of solutes into the stele is more likely to occur long than in short roots. This could be again related to developmental differences between these root types demonstrated by the rapid development of Casparian bands in cells close to the apical meristem in elongating short roots, compared to long segments of elongating long roots in which Casparian bands have not yet developed. Although it was shown

that apical and subapical regions of the roots were poorly permeable to the apoplastic tracer, it was difficult to evaluate what proportion of the root length contained tissues mature enough to enable the movement of tracer molecules through the apoplast into the xylem. This difficulty was due to the variation in the differentiation of primary tissues, relative to the distance from the root apex, among individual roots that differed in diameter and, apparently, in the rate of growth. The presence of the apoplastic tracer in the xylem of proximal root portions suggested that the control of the movement of solutes through the root apoplast was not completely effective. While the entry of the apoplastic tracer into the stele could occur mainly in root portions distal to the first endodermal cells with Casparian bands, two more potential sites of such entry were identified: the sites of emergence of lateral roots from the parent root and the endodermal cells in which the plasma membrane pulled away from the Casparian band during early stages of deposition of suberin layer. Unfortunately, these suggestions were not verified in this study. Restrictions to the apoplastic and symplastic movement of molecules in the roots may not be indicative of the restriction to the passage of water through the same sites.

It was shown that the endodermis of white spruce roots was functional even at its secondary state as the unsuberized and viable passage cells provided the means of controlled solute transport between the stele and the cortex. Anatomical features of the endodermis in white spruce roots implied that the main mode of solute transport between the stele and the cortex was via the plasma membrane - the apoplast - plasma membrane mode, with minimal symplastic transport through plasmodesmata that were rarely present in the endodermis at any state of development. In such transport, the solutions had to pass twice through the plasma membranes. Thus, the means of selective control of solute passage were present in the roots. The presence of substantial root pressure in conifers was reported for the first time. There are no anatomical or physiological bases to believe that conifers could be incapable of developing root pressure. The ability to create root pressure during the early growing season could constitute an important survival mechanism enabling seedlings that lost foliage during winter to transport water to their buds in the absence or insufficiency of the transpirational pull.

Many anatomical studies of conifer roots have ignored mycorrhizae, concentrating only on the developmental anatomy of the roots. To my knowledge, there have been no publications on the development of the Hartig net relative to the development of primary tissues in roots of white spruce. The few studies of this kind performed on other conifers allow us to conclude that the coordination between the development of the primary tissues and the development of the Hartig net is a typical characteristic of the genus *Picea*. It is unknown whether the plant controls the development of the fungus or merely adjusts its own rate of development to that of the fungus.

Mycorrhizae were common and abundant in nursery-grown seedlings and morphotypes present at planting were also present a year after planting, although the proportion of root colonization by different morphotypes changed. Only one of the detected fungi appeared to have a preference for a specific soil type, although this could be different for natural-soil resident fungi than for fungi transferred from the nursery. Other morphotypes occurred in both investigated soil substrates and their abundance was apparently affected more by the condition of the roots than by the fertilizer. Most morphotypes occurred on healthy, living roots and only one occurred commonly on dying roots which indicated its possible sarcophagic character.

Seedlings with different types of root systems grew their roots differently in mineral than in organic soil substrates. Their root growth responses to the application of slow-release fertilizer also varied, depending to which soil substrate the fertilizer was added. This could be of practical significance as silviculturalists have the choice of the type of planting stock, planting method, and the option to apply fertilizers. Since there is currently a tendency for planting into undisturbed organic substrates, the application of slow-release fertilizer at planting could particularly benefit container-grown spruce seedlings whose root growth was greatly improved by the fertilizer. It was shown that elements such as nitrogen could affect root development by influencing cambial activity in roots and the size of produced tracheids. However, N did not significantly alter pit dimensions. The alterations to the size of xylem elements by the level of applied N could impact on hydraulic properties of roots. An additional portion of these studies, currently near completion, could clarify this matter.

This dissertation provides information on a broad range of subjects related to the growth and development of roots and root systems in white spruce seedlings. A substantial portion of this information is new and some contradicts other information presented to date. Much less is known about the growth regulation processes in roots than in shoots. Some discrepancies among published reports could be due to the impact from factors unknown at the time of investigation or not considered to be important at that time. Unfortunately, the progress in research on roots has been slow in spite of the increasing availability of sophisticated technology.

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Appendix 4.1 Results of analyses of variance performed on dependent variables root diameter (RD), section surface area occupied by the xylem (xylem area), and the proportion of xylem surface area: total section surface area (xylem proportion) in cross-sectioned samples of white spruce roots. The samples were collected from different distances above the tips of lateral roots of seedlings grown under low, medium or high levels of nitrogen (N) availability.

Variables:		RD			Xylem area*			Xylem proportion			Error term
Source	DF	MS	F	p>F	MS	F	p>F	MS	F	p>F	
N	2	6576662	15.6	0.0041	22806	14.3	0.0052	0.033	1.1	0.3974	MS T (N)
T (N)	6	419480	2.9	0.0157	1598	3.4	0.0059	0.031	3.7	0.0033	MS ER
R (NT)	36	185032	1.3	0.2016	714	1.5	0.0749	0.010	1.2	0.2619	MS ER
P	2	2105792	6.85	0.0103	23762	24.7	0.0001	0.343	21.3	0.0001	MS T (N)*P
N*P	4	555858	1.81	0.0077	1640	1.7	0.2135	0.020	1.3	0.3398	MS T (N)*P
T(N)*P	12	307234	2.1	0.0285	963	2.0	0.0352	0.016	1.9	0.0455	MS ER
ER	67	146372			475			0.008			

MS = mean square

N = nitrogen treatment

T - nitrogen level treatment replicate (tree)

R = root

P = sampling position on the root

brackets contain nesting factors

DF = degrees of freedom

ER = error

*Note: data were transformed using the ranking procedure of the SAS in order to normalize their distribution.

Appendix 4.2 Results of analyses of variance performed on dependent variables root diameter (RD), section surface are occupied by the xylem (xylem area) and the proportion of xylem surface area: total section surface area (xylem proportion) in cross sectioned roots of white spruce seedlings grown under different nitrogen levels. Sampling position was replaced in the analytical model with an arbitrarily assigned category of xylem development.

Variables:		RD			Xylem area*			Xylem proportion			Error term
Source	DF	MS	F	p>F	MS	F	p>F	MS	F	p>F	
N	2	1087879	5.7	0.0415	3874	5.1	0.0508	0.0009	0.1	0.9019	MS T (N)
T (N)	6	191930	1.4	0.2329	759	3.3	0.0067	0.0089	2.3	0.0474	MS ER
R (NT)	36	166077	1.2	0.2588	264	1.2	0.3014	0.0027	0.71	0.8676	MS ER
X	3	1861179	11.9	0.0003	17569	54.1	0.0001	0.2318	42.2	0.0001	MS T (N)*P
N*X	6	265226	1.7	0.1899	495	1.5	0.2374	0.0048	0.9	0.5341	MS T (N)*P
T(N)*X	15	156337	1.1	0.3480	324	1.4	0.1640	0.0055	1.4	0.1688	MS ER
ER	61	137771			227			0.0039			

MS = mean square

N = nitrogen treatment

T = nitrogen level treatment replicate (tree)

R = root

X = category of xylem developmental advancement

brackets contain nesting factors

DF = degrees of freedom

ER = error

*Note: data were transformed using the ranking procedure of the SAS to normalize their distribution.

Appendix 4.3a-b. Results of analyses of variance performed on dependent variables tracheid cross sectional area (Tracheid area) and the proportion of cell-wall cross sectional area: tracheid cross sectional area (wall proportion) in roots of white spruce seedlings grown under different levels of nitrogen availability. Transformed data are marked with (*).

Appendix 4.3a Sampling position was entered into the analytical model.

Source	DF	Tracheid area*			Wall proportion			Error term
		MS	F	p>F	MS	F	p>F	
N	2	1406431394	55.7	0.0001	0.0708	0.6	0.58	MS T (N)
T(N)	6	25261394	8.2	0.0001	0.1220	15.2	0.0001	MS ER
R(NT)	35	50725457	16.5	0.0001	0.2364	29.5	0.0001	MS ER
P	2	614066839	12.8	0.001	2.1897	14.6	0.0006	MS T(N)*P
N*P	4	105353356	2.2	0.13	0.8991	6.0	0.007	MS T(N)*P
T(N)*P	12	47941634	34.3	0.0001	0.1499	18.6	0.0001	MS ER
R(NT)*P	60	43817079	14.3	0.0001	0.2192	27.3	0.0001	MS ER
Rep (N T R P)	169	15525491	5.1	0.0001	0.0318	3.9	0.0001	MS ER
Er	7164	3070789			0.0080			

Rep = replicated subsampled areas containing measured tracheids, other abbreviations are the same as in Appendix 4.1.

Appendix 4.3b The xylem development class was entered into the analytical model.

Source	DF	Tracheid area*			Wall proportion			Error term
		MS	F	p>F	MS	F	p>F	
N	2	1003154466	57.7	0.0001	0.1592	0.8	0.50	MS T (N)
T(N)	6	17389844	5.2	0.0001	0.2054	23.3	0.0001	MS ER
R(NT)	35	61843603	18.5	0.0001	0.2716	30.8	0.0001	MS ER
X	3	86050606	1.4	0.3	0.9188	2.9	0.50	MS T(N)*X
N*X	6	73216602	1.2	0.3	0.5943	1.9	0.15	MS T(N)*X
T(N)*X	15	60511557	18.1	0.0001	0.3131	35.5	0.0001	MS ER
R(NT)*X	24	66475938	19.9	0.0001	0.2459	27.9	0.0001	MS ER
Rep	125	18521158	5.5	0.0001	0.0406	4.6	0.0001	MS ER
Error	7038	3341848			0.0088			

Rep = replicated subsampled areas containing measured tracheids, all other abbreviations are the same as in Table 4.2.

Appendix 4.4a-b. Results of analyses of variance performed on dependent variables cross sectional cell wall area (wall) and lumen cross sectional area (lumen) of tracheids from white spruce roots grown under different levels of nitrogen availability. Transformed data are marked with (*).

Appendix 4.4a Sampling position was entered into the analytical model.

Source	DF	Wall			Lumen*			Error term
		MS	F	p>F	MS	F	p>F	
N	2	949888	62.3	0.0001	844074951	35.2	0.0005	MS T (N)
T(N)	6	15241	8.7	0.0001	23969500	8.1	0.0001	MS ER
R(NT)	35	16808	9.6	0.0001	71141586	23.4	0.0001	MS ER
P	2	353055	14.5	0.0006	656229739	11.5	0.0016	MS T(N)*P
N*P	4	32630	1.3	0.31	176917788	3.1	0.06	MS T(N)*P
T(N)*P	12	24281	13.9	0.0001	56826707	19.2	0.0001	MS ER
R(NT)*P	60	19830	11.3	0.0001	59856014	20.2	0.0001	MS ER
Rep	169	8463	4.8	0.0001	14627631	4.9	0.0001	MS ER
Error	7164	1751			2965872			

Rep = replicated subsampled areas containing measured tracheids, all other abbreviations are the same as in Appendix 3.1.

Appendix 4.4b The xylem development class was entered into the analytical model.

Source	DF	Wall			Lumen*			Error term
		MS	F	p>F	MS	F	p>F	
N	2	548314	22.5	0.0016	690452697	38.5	0.0004	MS T (N)
T(N)	6	24370	12.8	0.0001	179370041	5.5	0.0001	MS ER
R(NT)	35	21707	11.4	0.0001	86611723	26.6	0.0001	MS ER
X	3	39267	1.5	0.26	1738463320	2.1	0.15	MS T(N)*X
N*X	6	2637	0.1	0.99	141362930	1.7	0.19	MS T(N)*X
T(N)*X	15	26325	13.8	0.0001	83812270	25.8	0.0001	MS ER
R(NT)*X	24	35415	18.6	0.0001	83726559	25.8	0.0001	MS ER
Rep	125	9292	4.9	0.0001	18080290	5.6	0.0001	MS ER
Error	7038	1902			3250183			

Rep = replicated subsampled areas containing measured tracheids.

All other abbreviations are the same as in Appendix 4.2.

Appendix 4.5a-b. Results of analyses of variance performed on cell wall thickness and lumen radius of tracheids from white spruce roots grown under different levels of nitrogen availability.

Appendix 4.5a Sampling position was entered into the analytical model.

Source	DF	Wall thickness			Lumen radius			Error term
		MS	F	p>F	MS	F	p>F	
N	2	71.2	20.5	0.0021	805.4	38.8	0.0004	MS T (N)
T(N)	6	3.48	14.6	0.0001	20.8	8.5	0.0001	MS ER
R(NT)	35	3.63	15.2	0.0001	50.3	20.6	0.0001	MS ER
P	2	41.6	41.6	0.0022	669.2	19.0	0.0002	MS T(N)*P
N*P	4	15.7	4.0	0.0270	218.0	5.9	0.0072	MS T(N)*P
T(N)*P	12	3.9	16.3	0.0001	36.8	15.5	0.0001	MS ER
R(NT)*P	60	3.9	16.3	0.0001	52.6	21.6	0.0001	MS ER
Rep	169	0.9	4.0	0.0001	12.9	5.3	0.0001	MS ER
Error	7164	0.2			2.4			

Rep = replicated subsampled areas containing measured tracheids, all other abbreviations are the same as in Appendix 3.1.

Appendix 4.5b The xylem development class was entered into the analytical model.

Source	DF	Wall thickness			Lumen radius			Error term
		MS	F	p>F	MS	F	p>F	
N	2	36.8	5.0	0.0531	565.5	38.2	0.0004	MS T (N)
T(N)	6	7.4	29.0	0.0001	14.8	5.4	0.0001	MS ER
R(NT)	35	3.5	13.8	0.0001	62.9	22.8	0.0001	MS ER
X	3	13.3	2.1	0.1421	122.7	1.8	0.1867	MS T(N)*X
N*X	6	5.6	0.9	0.5286	103.6	1.5	0.2331	MS T(N)*X
T(N)*X	15	6.3	24.8	0.0001	67.4	24.5	0.0001	MS ER
R(NT)*X	24	4.0	15.6	0.0001	79.5	28.9	0.0001	MS ER
Rep	125	1.0	3.8	0.0001	16.9	6.1	0.0001	MS ER
Error	7038	1902			2.7			

Rep = replicated subsampled areas containing measured tracheids.

All other abbreviations are the same as in Appendix 3.2.

Appendix 4.6 Results of analyses of variance performed on dependent variables cross sectional tracheid area (Tracheid area) and the proportion of cell wall cross sectional area (Wall proportion): tracheid cross sectional area in tracheids from white spruce roots grown under different levels of nitrogen availability. The type of xylem (primary or secondary) and the sampling position were entered into the analytical model.

Source	DF	Tracheid area*			Wall proportion			Error term
		MS	F	p>F	MS	F	p>F	
N	2	958643919	33.9	0.0005	62678122	5.0	0.05	MS T (N)
T(N)	6	282281170	10.1	0.0001	12495603	6.7	0.0001	MS ER
R(NT)	35	41469397	14.9	0.0001	65447700	35.2	0.0001	MS ER
P	2	279587183	8.0	0.006	89439234	1.9	0.20	MS T(N)*P
N*P	4	65157699	1.9	0.18	150327889	3.1	0.05	MS T(N)*P
T(N)*P	12	34717846	23.4	0.0001	47901701	25.7	0.0001	MS ER
R(NT)*P	60	40815818	12.4	0.0001	68920872	37.0	0.0001	MS ER
Xyl	1	788087230	125.8	0.0001	3968305084	991.7	0.0001	T(N)*Xyl
N*Xyl	2	51380145	8.2	0.02	8293046	2.1	0.21	MS ER
T(N)*Xyl	6	62654191	2.2	0.04	40015116	2.1	0.04	MS ER
R(N)*Xyl	25	113141119	4.1	0.0001	7356084	3.9	0.0001	MS ER
P*Xyl	2	9741354	1.7	0.26	6407418	1.1	0.40	T(N)*P*Xyl
N*P*Xyl	2	84124415	30.2	0.0001	19602240	3.3	0.11	MS ER
T(N)*P*Xyl	6	5834190	14.4	0.005	5930408	3.2	0.004	T(N)*P*Xyl
R(NT)*P*Xyl	9	3228605	1.2	0.31	6076245	3.3	0.0006	MS ER
Rep(N T R P Xyl)	220	13780965	4.9	0.0001	8142197	4.4	0.0001	MS ER
Error	7059	19679437464			1860034			

Xyl = xylem type (primary or secondary)

Rep = replicated subsampled areas containing measured tracheids.

All other abbreviations are the same as in Appendix 4.1

*Note: Data were transformed using the ranking procedure of the SAS to normalize their distribution.

Appendix 4.7 Results of analyses of variance performed on dependent variables cell wall cross sectional area and lumen cross sectional area of tracheids from white spruce roots grown under different levels of nitrogen availability. The xylem type (primary or secondary) and the sampling position were entered into the analytical model.

Source	DF	Wall			Lumen*			Error term
		MS	F	p>F	MS	F	p>F	
N	2	719242	88.5	0.0001	609244947	26.2	0.001	MS T (N)
T(N)	6	8125	5.1	0.0001	22429020	9.0	0.0001	MS ER
R(NT)	35	17372	10.9	0.0001	53142780	21.2	0.0001	MS ER
P	2	221016	12.4	0.0011	237939202	6.0	0.01	MS T(N)*P
N*P	4	105378	6.0	0.007	898883014	2.3	0.12	MS T(N)*P
T(N)*P	12	17628	11.1	0.0001	39297028	15.7	0.0001	MS ER
R(NT)*P	60	17217	10.8	0.0001	52508869	20.9	0.0001	MS ER
Xyl	1	12034	9.4	0.02	18479668232	549.1	0.0001	T(N)*Xyl
N*Xyl	2	124178	96.8	0.0001	30472940	9.0	0.01	MS ER
T(N)*Xyl	6	1282	0.8	0.57	3365584	1.3	0.23	MS ER
R(N)*Xyl	25	7643	4.8	0.0001	7151163	2.9	0.0001	MS ER
P*Xyl	2	24109	6.46	0.03	10042431	4.0	0.02	T(N)*P*Xyl
N*P*Xyl	2	175918	47.1	0.0002	68935852	12.4	0.007	MS ER
T(N)*P*Xyl	6	3730	2.3	0.03	5534979	2.2	0.04	T(N)*P*Xyl
R(NT)*P*Xyl	9	2713	1.7	0.08	2130798	0.8	0.57	MS ER
Rep(N T R P Xyl)	220	7157	4.5	0.0001	12306274	4.9	0.0001	MS ER
Error	7059	1593			2504015			

Xyl = xylem type (primary or secondary)

Rep = replicated subsampled areas containing measured tracheids.

All other abbreviations are the same as in Appendix 4.2

*Note: Data were transformed using the ranking procedure of the SAS to normalize their distribution.

Appendix 4.8 a-b Results of the analyses of variance performed on the variable tracheid length measured on macerated root segments of white spruce seedlings grown under different levels of nitrogen supply.

Appendix 4.8a The analysis using sampling position on the lateral root in the analytical model.

Source	DF	MS	F	p>F	Error term
N	2	79501894	2.9	0.13	MS T(N)
T(N)	6	27713862	25.6	0.0001	MS ER
R(NT)	36	6732078	6.2	0.0001	MS ER
P	2	82348449	10.4	0.0024	MS T(N)*P
N*P	4	20469746	2.6	0.09	MS T(N)*P
T(N)*P	12	7939294	7.3	0.0001	MS ER
R(NT)*P	70	6798403	6.3	0.0001	MS ER
ER	3927	1080257			

All abbreviations are the same as in Appendix 4.1

Appendix 4.8b The analysis using an arbitrarily established classification of the xylem developmental advancement instead of the sampling position.

Source	DF	MS	F	p>F	Error term
N	2	114862285	11.2	0.0096	MS T(N)
T(N)	6	131131	9.3	0.0001	MS ER
R(NT)	36	7803	5.5	0.0001	MS ER
X	3	22345	1.7	0.26	MS T(N)*P
N*X	6	49492	3.3	0.03	MS T(N)*P
T(N)*X	15	14983	10.6	0.0001	MS ER
R(NT)*X	34	7050	5.0	0.0001	MS ER
ER	3957	1135400			

Note: Data in tables a and b were transformed using the ranking procedure of the SAS to normalize their distribution. Abbreviations are the same as in Appendix 4.2

Appendix 4.9 a-b Results of the analyses of variance performed on the variable tracheid width measured on macerated root segments of white spruce seedlings grown under different levels of nitrogen supply.

Table 4.10a The analysis using sampling position along the root length in the analytical model.

Source	DF	MS	F	p>F	Error term
N	2	2245001334	31.7	0.0006	MS T(N)
T(N)	6	70920775	25.7	0.0001	MS ER
R(NN)	36	21450515	7.8	0.0001	MS ER
P	2	204171673	7.2	0.0089	MS T(N)*P
N*P	4	60137729	2.1	0.14	MS T(N)*P
T(N)*P	12	28477976	10.3	0.0001	MS ER
PR(NT)	71	21999498	8.0	0.0001	MS ER
ER	6727	2761685			

Abbreviations are the same as in Appendix 4.1.

Appendix 4.9b The analysis using an arbitrarily established classification of the xylem developmental advancement instead of the sampling position.

Source	DF	MS	F	p>F	Error term
N	2	1656797214	26.5	0.0011	MS T(N)
T(N)	6	62602143	21.4	0.0001	MS ER
R(NT)	36	25634846	8.8	0.0001	MS ER
X	3	89805329	4.0	0.02	MS T(N)*P
N*X	6	51279817	2.3	0.09	MS T(N)*P
T(N)*X	15	22298181	7.6	0.0001	MS ER
R(NT)*X	34	14235775	4.9	0.0001	MS ER
ER	6758	2927750			

Note: Data in tables a and b were transformed using the ranking procedure of the SAS to normalize their distribution. Abbreviations are the same as in Appendix 4.2.

Appendix 4.10 Results of analyses of variance performed on dependent variables pit border width (pit border), pit aperture width (pit aperture), and the ratio of pit aperture: pit border widths (ratio) measured on tracheids from roots of white spruce grown under different levels of nitrogen availability.

Source	DF	Pit border			Pit aperture*			Ratio*			Error term
		MS	F	p>F	MS	F	p>F	MS	F	p>F	
N	2	844	2.7	0.14	101397056	3.0	0.12	33889287	1.2	0.35	MS T (N)
T(N)	6	311	107.3	0.0001	33634044	88.8	0.0001	27328898	62.8	0.0001	MS ER
R(NT)	18	29	10.2	0.0001	4515927	11.9	0.0001	5535127	12.7	0.0001	MS ER
P	1	126	0.8	0.41	25990067	5.1	0.06	66565477	7.9	0.03	MS T(N)*P
N*P	2	107	0.7	0.55	964868	0.2	0.83	12783061	1.5	0.29	MS T(N)*P
T(N)*P	6	160	55.4	0.0001	5089176	13.4	0.0001	8465745	19.4	0.0001	MS ER
R(NT)*P	18	88	30.6	0.0001	2490569	6.6	0.0001	2743714	6.3	0.0001	MS ER
TRA	549	18	6.2	0.0001	2343493	6.2	0.0001	2215745	5.1	0.0001	MS ER
Error	2696	3			378558			435272			

TRA - tracheid.

All other abbreviations are the same as in Appendix 4.1

*Note: Data were transformed using the ranking procedure of the SAS to normalize their distribution.

Appendix 5.1a-b Results of multivariate analysis of variance on root length distribution into three root diameter classes:

Appendix 5.1a: Root length distribution analysis:

Source	Wilk's Lambda	Num DF	Den DF	F	p>F
STK	0.80	3	24	2.0	0.1435
Fert	0.24	6	48	8.4	0.0001
STK*Fert	0.62	6	48	2.1	0.0682
Comp	0.33	3	24	16.1	0.0001
Comp*Fert	0.11	6	48	16.2	0.0001
Comp*STK	0.68	3	24	3.7	0.0254
Comp*STK*Fert	0.84	6	48	0.7	0.6210

Appendix 5.1b: Percent of root length distributions:

Source	Wilk's Lambda	Num DF	Den DF	F	p>F
STK	0.80	3	24	2.0	0.1377
Fert	0.43	6	48	4.1	0.0021
STK*Fert	0.76	6	48	1.2	0.3309
Comp	0.37	3	24	13.5	0.0001
Comp*Fert	0.15	6	48	12.4	0.0001
Comp*STK	0.66	3	24	4.0	0.0188
Comp*STK*Fert	0.97	6	48	0.1	0.9945

STK = planting stock, Fert = fertilizer treatment, Comp = rooting medium compartment, Num - numerator, Den = denominator, DF = degrees of freedom.

Error terms used for testing the sources were as explained in the materials and methods section of Chapter 5 and is shown in Table 5.2.

Appendix 5.2a-b Results of multivariate analysis of variance on root surface area distribution into three root diameter classes.

Appendix 5.2a:

Source	Wilk's Lambda	Num DF	Den DF	F	p>F
STK	0.84	3	24	1.4	0.2532
Fert	0.24	6	48	8.3	0.0001
STK*Fert	0.62	6	48	2.1	0.0684
Comp	0.31	3	24	17.5	0.0001
Comp*Fert	0.10	6	48	16.9	0.0001
Comp*STK	0.75	3	24	2.6	0.0767
Comp*STK*Fert	0.86	6	48	0.4	0.8401

Appendix 5.2b:

Source	Wilk's Lambda	Num DF	Den DF	F	p>F
STK	0.80	3	24	1.9	0.1565
Fert	0.40	6	48	4.6	0.0009
STK*Fert	0.76	6	48	1.2	0.3398
Comp	0.51	3	24	7.6	0.0010
Comp*Fert	0.20	6	48	10.0	0.0001
Comp*STK	0.81	3	24	1.9	0.1547
Comp*STK*Fert	0.83	6	48	0.8	0.5706

Note: Abbreviations are the same as in Appendix 5.1.

Error terms used for testing the sources were as explained in the Materials and Methods section of Chapter 5.

Appendix 5.3 Results of multivariate (MANOVA) composition analysis on the percent of root colonization of white spruce in the divided root experiment by different mycorrhizal fungi and percent of non-colonized roots.

Source	Wilks Lambda	Num	Den	F	P
Intercept	0.030	5	22	143.3	0.0001
STK	0.391	5	22	6.8	0.0005
Fert	0.637	10	44	1.1	0.3761
STK*Fert	0.624	10	44	1.2	0.3380
Comp	0.583	5	22	3.1	0.0274
STK*Comp	0.695	5	22	1.9	0.1309
Fert*Comp	0.276	10	44	3.9	0.0007
Fert*STK*Comp	0.668	10	44	0.9	0.4722

STK = planting stock, Fert = fertilizer treatment, Comp = divided rooting medium compartment

Note: Ratios of percent of root tips colonized by each of the five detected fungi species to the percent of non-colonized root tips were log transformed to normalize data distribution.

Appendix 6.1. Results of analyses of variance on height at planting (HT1), height after one growing season (HT2) and the 1997 height increment (HTINC) of white spruce seedlings of three different stocktypes planted on two sites in central British Columbia.

Variable	Source	DF	MS	F	p>F	Error term
HT1	S	1	9645	10.0	0.0132	MS B (S)
	B(S)	8	961	2.2	0.0283	MS Error
	STK	2	3335	2.5	0.2860	MS S*STK
	S*STK	2	1336	3.0	0.0497	MS B(S)*STK
	B(S)*STK	16	724	1.6	0.0557	MS Error
	Error	457	442			
HT2	S	1	67452	44.4	0.0002	MS B(S)
	B(S)	8	1520	1.4	0.1915	MS Error
	STK	2	8873	0.8	0.5579	MS S*STK
	S*STK	2	11197	5.2	0.0176	MS B(S)*STK
	B(S)*STK	16	2131	2.0	0.0136	MS Error
	Error	457	1081			
HTINC*	S	1	733938	14.6	0.0051	MS B(S)
	B(S)	8	50316	3.1	0.0020	MS Error
	STK	2	23790	0.2	0.8594	MS S*STK
	S*STK	2	145400	3.0	0.0760	MS B(S)*STK
	B(S)*STK	16	47824	3.0	0.0001	MS Error
	Error	457	16176			

* Data were transformed by the ranking procedure of the SAS to normalize their distribution.

S = Site; B = block; STK = planting stock; nesting factors are in brackets.

Df = degrees of freedom; MS = mean square

Appendix 6.2. Results of analyses of variance on stem-base diameter at planting (DIA1) and after one growing season (DIA2) and the 1997 diameter increment (DIAINC) of white spruce seedlings of three different stocktypes planted on two sites in the central interior of British Columbia.

Variable	Source	DF	MS	F	p>F	Error term
DIA1	S	1	0.006	0.0	0.9539	B (S)
	B(S)	8	1.68	10.7	0.0001	MS Error
	STK	2	3.06	132.1	0.0075	MS S*STK
	S*STK	2	0.02	0.1	0.9278	MS B(S)*STK
	B(S)*STK	16	0.31	2.0	0.0144	MS B(S)*STK
	Error	4576	0.16			
DIA2	S	1	11.77	10.2	0.0127	MS B(S)
	B(S)	8	1.15	2.2	0.236	MS Error
	STK	2	22.95	97.3	0.0102	MS S*STK
	S*STK	2	0.24	0.3	0.7647	MS B(S)*STK
	B(S)*STK	16	0.86	1.7	0.0473	MS Error
	Error	457	0.51			
DIAINC	S	1	11.02	3.3	0.1069	B (S)
	B(S)	8	3.34	5.7	0.0001	MS Error
	STK	2	10.48	30.1	0.0321	MS S*STK
	S*STK	2	0.35	0.5	0.6208	MS B(S)*STK
	B(S)*STK	16	0.71	1.2	0.2493	MS Error
	Error	457	0.58			

S = Site; B = block; STK = planting stock; nesting factors are in brackets.
Df = degrees of freedom; MS = mean square

Appendix 6.3. Results of analyses of variance on morphological characteristics of white spruce seedlings of three different stocktypes grown for one growing season in 76 L boxes buried in the soil outdoors.

Variable	Source	DF	MS	F	p>F
Final height	STK	2	31269	19.8	0.0005
	Rep (STK)	9	1580	0.6	0.7833
	Error	36	2605		
Height increment	STK	2	24386	32.6	0.0001
	Rep (STK)	9	748	0.4	0.9382
	Error	36	1979		
Final stem base diameter	STK	2	11.90	17.8	0.0007
	Rep (STK)	9	0.67	1.1	0.3651
	Error	36	0.59		
Stem diameter increment	STK	2	11.72	19.2	0.0006
	Rep (STK)	9	0.61	1.1	0.4025
	Error	36	0.57		
Foliage dry weight	STK	2	25.5	28.5	0.0001
	Rep (STK)	9	0.9	1.5	0.1785
	Error	36	0.6		
Root system dry weight	STK	2	21.4	44.9	0.0001
	Rep (STK)	9	0.5	0.7	0.7339
	Error	36	0.7		
Stem tissue dry weight	STK	2	23.9	26.5	0.0002
	Rep (STK)	9	0.9	1.2	0.3035
	Error	36	0.7		
Projected leaf area	STK	2	36930	23.2	0.0003
	Rep (STK)	9	1592	1.3	0.2629
	Error	36	1210		
Root surface area	STK	2	627808	38.2	0.0001
	Rep (STK)	9	16437	0.3	0.9539
	Error	36	47883		
Total length of roots	STK	2	15516977	15.5	0.0012
	Rep (STK)	9	10022335	0.4	0.9017
	Error	36	2258217		
Number of root tips	STK	2	7235443	18.8	0.0006
	Rep (STK)	9	3845956	0.4	0.9063
	Error	36	8819095		

DF = degrees of freedom; MS = mean square; STK = stocktype; Rep = replicate

Note: Stocktype effects were tested by the MS Rep (STK) as the error term denominator while the rep (STK) was tested by the MS error.

Appendix 6.4. Results of multivariate analyses of variance on the distribution of root length and root surface area into three root diameter classes: 1) ≤ 0.4 mm, 2) $0.4 \leq 0.8$ mm, and 3) >0.8 mm in root systems of three different stocktypes of white spruce seedlings grown outdoors in 76 < boxes filled with soil.

Variable	Source	Wilk's Lambda	Num DF	Den DF	F	p>F
Root length	STK	0.057	6	14	7.4	0.0010
	Rep (STK)	0.651	27	100	0.6	0.9436
Percent of root length	STK	0.112	4	16	8.0	0.0010
	Rep (STK)	0.681	18	70	0.8	0.6657
Root system surface area	STK	0.457	6	14	8.6	0.0005
	Rep (STK)	0.661	27	100	0.6	0.9555
Percent of root system surface area	STK	0.090	4	16	9.3	0.0004
	Rep (STK)	0.676	18	70	0.8	0.6459

Note: Effects of stock type were tested using Rep (STK) as an error term.

Effects of stocktype were tested using MS Rep (STK) as an error term. Rep (STK) effects were tested with the matrix error term. The F statistics for Wilk's Lambda are exact.

S = Site; B = block; STK = planting stock; nesting factors are in brackets.

DF = degrees of freedom; MS = mean square; Num = numerator; Den = denominator

Appendix 6.5. Results of univariate analyses of variance on the distribution of root length and percent of root length into three root diameter classes in white spruce seedlings of three stocktypes grown outdoors in soil-buried boxes.

Variable	Source	DF	MS	F	p>F
Length in the ≤ 0.4 mm class	STK	2	4280337	7.0	0.0146
	Rep (STK)	9	611146	0.6	0.8225
	Error	36	1097206		
Length in the $0.4 \leq 0.8$ mm class	STK	2	1133433	16.0	0.0011
	Rep (STK)	9	70962	0.4	0.9416
	Error	36	191417		
Length in the > 0.8 mm class	STK	2	649142	72.4	0.0001
	Rep (STK)	9	8970	0.4	0.9488
	Error	36	25269		
Percent of length in the ≤ 0.4 mm class	STK	2	60.4	2.5	0.1347
	Rep (STK)	9	23.9	1.0	0.4794
	Error	36	24.6		
Percent of length in the $0.4 \leq 0.8$ mm class	STK	2	1.2	0.1	0.9248
	Rep (STK)	9	15.8	1.2	0.3263
	Error	36	13.2		
Percent of length in the > 0.8 mm class	STK	2	75.4	27.6	0.0001
	Rep (STK)	9	2.7	0.5	0.8351
	Error	36	5.1		

Note: Effects of stocktype were tested by the MS Rep (STK) as an error term, effects of Rep (STK) by the MS error.

S = Site; B = block; STK = planting stock; nesting factors are in brackets.
Df = degrees of freedom; MS = mean square

Appendix 6.6. Results of analyses of variance on the distribution of root surface area and percent of root surface into three root diameter classes in white spruce seedlings of three different stocktypes grown for one growing season outdoors in soil-buried boxes.

Variable	Source	DF	MS	F	p>F
Surface area in the ≤0.4 mm class	STK	2	35997	10.1	0.0050
	Rep (STK)	9	3569	0.5	0.8783
	Error	36	7434		
Surface area in the 0.4 ≤ 0.8 mm class	STK	2	69257	21.0	0.0004
	Rep (STK)	9	3292	0.4	0.9335
	Error	36	8501		
Surface area in the >0.8 mm class	STK	2	115374	82.9	0.0001
	Rep (STK)	9	1392	0.3	0.9701
	Error	36	4648		
Percent of surface area in the ≤ 0.4 mm class	STK	2	161.0	8.2	0.0095
	Rep (STK)	9	19.7	0.8	0.6176
	Error	36	24.6		
Percent of surface area in the 0.4 ≤ 0.8 mm class	STK	2	42.3	2.6	0.1251
	Rep (STK)	9	16.0	1.3	0.2475
	Error	36	11.9		
Percent of surface area in the > 0.8 mm class	STK	2	365.1	43.7	0.0001
	Rep (STK)	9	8.3	0.4	0.9322
	Error	36	21.4		

S = Site; B = block; STK = planting stock; nesting factors are in brackets.
Df = degrees of freedom; MS = mean square

Appendix 6.7. Results of analyses of variance on leaf and root system characteristics of white spruce seedlings of three different planting stocktypes used in measurements of root system hydraulic conductivity after planting and after approximately two months of post-planting growth.

Variable	Source	DF	MS	F	p>F	Error term
Projected leaf area	STK	12	680	4.1	0.0549	MS Rep (STK)
	Rep (STK)	9	167	1.2	0.2866	MS Error
	T	1	2214	5.2	0.0484	MS Rep (STK)*T
	STK*T	2	206	0.5	0.6313	MS Rep (STK)*T
	Rep (STK)*T	9	425	3.2	0.0042	MS Error
	Error	48	133			
Foliage dry weight	STK	2	0.20	2.5	0.1398	MS Rep (STK)
	Rep (STK)	9	0.08	1.2	0.2071	MS Error
	T	1	1.61	9.6	0.0126	MS Rep (STK)*T
	STK*T	2	0.07	0.4	0.6771	MS Rep (STK)*T
	Rep (STK)*T	9	0.17	2.5	0.0190	MS Error
	Error	48	0.07			
Root system dry weight	STK	2	0.75	41.4	0.0001	MS Rep (STK)
	Rep (STK)	9	0.02	0.7	0.6686	MS Error
	T	1	0.09	2.8	0.1282	MS Rep (STK)*T
	STK*T	2	0.03	0.8	0.4592	MS Rep (STK)*T
	Rep (STK)*T	9	0.03	1.3	0.2372	MS Error
	Error	48	0.02			
Root surface area	STK	2	53358	36.4	0.0001	MS Rep (STK)
	Rep (STK)	9	1466	0.7	0.7279	MS Error
	T	1	78213	39.1	0.0001	MS Rep (STK)*T
	STK*T	2	8857	4.4	0.0459	MS Rep (STK)*T
	Rep (STK)*T	9	2002	0.9	0.5155	MS Error
	Error	48	2174			
Root length	STK	2	3924751	18.9	0.0006	MS Rep (STK)
	Rep (STK)	9	207261	1.3	0.2770	MS Error
	T	1	2608198	24.1	0.0008	MS Rep (STK)*T
	STK*T	2	674924	6.2	0.0201	MS Rep (STK)*T
	Rep (STK)*T	9	108404	4.1	0.0220	MS Error
	Error	48	163074			
Number of root tips	STK	2	17598903	28.1	0.0001	MS Rep (STK)
	Rep (STK)	9	626664	1.1	0.4052	MS Error
	T	1	17131321	41.2	0.0001	MS Rep (STK)*T
	STK*T	2	6968978	16.8	0.0009	MS Rep (STK)*T
	Rep (STK)*T	9	415395	0.7	0.7004	MS Error
	Error	48	4307920			

STK = planting stock; Rep = replicate T = time after planting; DF = degrees of freedom; MS = mean square

Appendix 6.8. Results of analyses of variance on hydraulic properties of white spruce root systems in seedlings of three planting stock types measured at two different times after planting.

Variable	Source	DF	MS	F	p>F	Error term
Hydraulic conductivity	STK	2	6.74	5.3	0.0299	MS Rep (STK)
	Rep (STK)	9	1.27	.73	0.6806	MS Error
	T	1	0.08	.004	0.8432	MS Rep (STK)*T
	STK*T	2	11.38	5.7	0.0253	MS Rep (STK)*T
	Rep(STK)*T	9	2.00	1.1	0.3477	MS Error
	Error	544	1.74			
Total conductivity of root systems	STK	2	46261	1.0	0.3978	MS Rep (STK)
	Rep (STK)	9	45225	0.4	0.9085	MS Error
	T	1	265361	2.6	0.0908	MS Rep (STK)*T
	STK*T	2	369319	3.6	0.0348	MS Rep (STK)*T
	Rep(STK)*T	9	74018	0.7	0.6930	MS Error
	Error	544	103615			
Root pressure	STK	2	745	9.2	0.0067	MS Rep (STK)
	Rep (STK)	9	81	0.2	0.9949	MS Error
	T	1	1936	6.4	0.0321	MS Rep (STK)*T
	STK*T	2	2083	6.9	0.0152	MS Rep (STK)*T
	Rep(STK)*T	9	302	0.7	0.7180	MS Error
	Error	48	440			

STK = Stocktype; Rep = replicate, DF = degrees of freedom, MS = mean square T = time after planting

* Data transformed by the ranking procedure of the SAS to normalize its distribution.

Note: Hydraulic conductivity and total hydraulic conductivity were calculated from 6-10 measurements per tree.

Appendix 6.9. Results of multivariate analyses of variance on the distribution of root length and root system surface area into three root diameter classes in root systems of white spruce used in measurements of root system's hydraulic conductivity at two different times after planting.

Variable	Source	Wilk's Lambde	Num DF	Den DF	F	p>F
Root length	STK	0.037	6	14	9.8	0.0002
	T	0.140	3	7	14.3	0.0023
	STK*T	0.384	6	14	1.4	0.2706
Percent of root length	STK	0.080	4	16	10.2	0.0003
	T	0.209	2	8	15.1	0.0019
	STK*T	0.730	4	16	0.7	0.6158
Root system surface area	STK	0.032	6	14	10.4	0.0001
	T	0.126	3	7	16.2	0.0016
	STK*T	0.402	6	14	1.3	0.3012
Percent of root surface area	STK	0.019	6	14	14.3	0.0001
	T	0.005	3	7	416.0	0.0001
	STK*T	0.070	6	14	6.5	0.0019

Note: Error terms used: Rep (STK) for testing STK effects, Rep (STK)*T for testing the effects of T, and the effects of STK(T)*T.

Rep = replicate, STK = stocktype, T = time after planting, DF = degrees of freedom, Num = numerator, Den = denominator. The F statistics for Wilk's Lambda are exact.

Appendix 6.10. Results of univariate analyses of variance on the distribution of root length into three root diameter classes in white spruce seedlings used in root system hydraulic conductivity measurements at two different times after planting.

Variable	Source	DF	MS	F	p>F
Length in the ≤ 0.4 mm class	STK	2	1549873	10.1	0.0050
	Rep (STK)	9	153500	1.3	0.2777
	T	1	57605	9.9	0.0119
	STK*T	2	378915	6.5	0.0179
	Rep(STK)*T	9	58308	0.5	0.0793
	Error	48	120906		
Length in the 0.4 ≤ 0.8 mm class	STK	2	519321	61.7	0.0001
	Rep (STK)	9	841	1.1	0.3466
	T	1	412453	43.1	0.0001
	STK*T	2	31130	3.2	0.0864
	Rep (STK)*T	9	9567	1.3	0.2570
	Error	48	7305		
Length in the >0.8 mm class	STK	2	1957	2.2	0.1683
	Rep (STK)	9	895	0.8	0.5959
	T	1	45422	39.6	0.0001
	STK*T	2	977	0.8	0.4583
	Rep(STK)*T	9	1147	1.1	0.4103
	Error	48	1084		

Note: Effects of stocktype were tested with the MS Rep (STK) with MS error.

S = Site; B = block; STK = planting stock; nesting factors are in brackets.

Df = degrees of freedom; MS = mean square

Appendix 6.11. Results of univariate analyses of variance on the distribution of root system surface area into three root diameter classes in white spruce seedlings used in root system hydraulic conductivity measurements at two different times after planting.

Variable	Source	DF	MS	F	p>F
Surface area in the ≤0.4 mm class	STK	2	9270	14.0	0.0017
	Rep (STK)	9	660	0.9	0.5521
	T	1	4247	11.6	0.0078
	STK*T	2	1810	4.9	0.0356
	Rep(STK)*T	9	366	0.5	0.8765
	Error	48	753		
Surface area in the 0.4 ≥ 0.8 mm class	STK	2	18386	71.6	0.0001
	Rep (STK)	9	257	0.8	0.5798
	T	1	18466	53.1	0.0001
	STK*T	2	1501	4.3	0.0485
	Rep(STK)*T	9	348	1.1	0.3522
	Error	48	304		
Surface area in the > 0.8 mm class	STK	2	89	0.6	0.5567
	Rep (STK)	9	142	0.8	0.5978
	T	1	6180	31.0	0.0003
	STK*T	2	186	0.9	0.4277
	Rep(STK)*T	9	199	1.1	0.3458
	Error	48	173		

Note: Effects of stocktype were tested with the MS Rep (STK) with MS error.

S = Site; B = block; STK = planting stock; nesting factors are in brackets.

Df = degrees of freedom; MS = mean square