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ANATOMICAL AND METABOLIC RESPONSE OF ROOTS OF *Picea mariana* (Mill) BSP, *Picea glauca* (Moench) Voss, *Pinus banksiana* Lamb., *Pinus contorta* Dougl. var. latifolia Engelm., AND *Larix laricina* (DuRoi) K. Koch TO ANOXIA AND COLD TEMPERATURE.

BY

TIMOTHY S. S. CONLIN

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Ph.D. in Forest Science.

DEPARTMENT OF FOREST SCIENCE,
UNIVERSITY OF ALBERTA,
EDMONTON, ALBERTA
FALL, 1993

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ISBN 0-315-88300-6

Canada

University of Alberta
Edmonton

Canada T6G 2H1

Department of Forest Science
Faculty of Agriculture and Forestry

855 General Services Building
Telephone (403) 492-4413

October 7, 1993

Faculty of Graduate Studies
University of Alberta
Edmonton, Alberta

Dear Sir or Madame,

Timothy Conlin is just finished his Ph.D. thesis in Forest Science "Anatomical and metabolic response of roots.....". Two of the chapters of this work were published prior to completion of the entire thesis. Most of the work in these papers was done by Timothy and my role in the work was the usual commitment of advise, assistance and editorial comments normally provided by a supervisor during the development of a thesis. I have no objections in having these chapters included in his Ph.D. thesis.

I hope that his thesis is accepted.

Sincerely,

Victor Lieffers
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Timothy Conlin
3119 Glasgow Ave.,
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V8X 1L8

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The undersigned certify that they have read and recommended to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled "Anatomical and metabolic response of roots of Picea mariana (Mill) BSP, Picea glauca (Moench) Voss, Pinus banksiana Lamb., Pinus contorta Dougl. var. latifolia Englm. and Larix laricina (Du Roi) K. Koch to anoxia and cold temperature" submitted by Timothy S. S. Conlin in partial fulfillment of the requirements for the degree of Ph.D. in Forest Science.

Dr. V. Lieffers (Supervisor)

Dr. J. J. Zwiazek (Committee Chair)

Dr. G. Taylor

Dr. F.C. Yeh

Dr. R. Guy (External)

ABSTRACT

Rates of carbon dioxide efflux were measured from roots of Picea mariana, Picea glauca, Larix laricina, Pinus contorta and Pinus banksiana exposed to oxia (OX), root anoxia (RA) and total plant anoxia (TPA). Ratios of mean TPA to OX efflux rates at 5°C suggested that P. mariana had a higher TPA CO_2 increase than the other species. This elevated ratio was further demonstrated in a follow-up experiment, which also showed that L. laricina had the highest OX rates. These data supported the hypotheses that, at low temperatures, roots of P. mariana are capable of high rates of fermentation and roots of L. laricina are capable of high rates of respiration. Further, L. laricina showed rhizospheric oxidation, which is evidence of internal O_2 diffusion, while P. mariana did not. This indicated the potential for respiration sustained by internal gas diffusion in L. laricina, but not for P. mariana. Both pine species also showed evidence for respiration supported through internal O_2 diffusion, although their oxic CO_2 rates were low in comparison to L. laricina. Picea glauca showed no evidence of O_2 diffusion from roots. Examination of root anatomy suggested limited internal O_2 diffusion in these species.

A combination of low temperature and anoxia in solution culture caused a significant decrease in percent translocation of ^{14}C to Pinus contorta roots. Increasing the solution temperature eliminated this reduction in translocation. Anoxia, but not low temperature, decreased translocation in Picea mariana roots. Anoxia did not reduce translocation in Pinus banksiana, Picea glauca, or Larix laricina. Therefore, with the exception of Picea mariana, the apparent internal diffusion of O_2 within the root does not appear to be necessary for

translocation during periods of solution anoxia. Cold soil temperatures in combination with anoxia may be equally important in influencing translocation of carbohydrate to roots of conifers.

Regardless of physiological differences, growth of roots of P. mariana and L. laricina did not occur during periods of high water table in the field. This indicates that internal O_2 diffusion does not support root growth in these species.

ACKNOWLEDGEMENT

Funding for this work was provided through Natural Sciences and Engineering Research Council of Canada Forestry Transition and Operating grants.

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CHAPTER ONE

INTRODUCTION

Future management of the boreal forest in harvested areas will depend upon the long term performance of species planted in logged-over sites. This performance will be difficult to foresee without baseline information on conifer root ecophysiology in response to soil flooding stress. This information is necessary because large areas of the boreal forest are subjected to soil waterlogging, as demonstrated by extensive tracts of peatland and poor drainage characteristics found in post-glacial boreal terrain. In addition, the hydrology of forest areas can change dramatically following harvest. Planted trees could be subjected to periods of flooding stress at an early age or to gradual waterlogging as the site is modified through biological and physical processes. In both instances mortality of trees due to waterlogging could lead to considerable loss of aesthetic, natural and economic resources.

The most obvious and immediate cause of flooding stress to plants is the impedance of O_2 flow to roots. This is a result of the lower diffusion rates of O_2 in water. These low diffusion rates are further decreased by the normal complement of soil fauna and flora which quickly use up available O_2 in flooded soil and then begin using other substances such as NO_3, SO_4, and $Fe_2(OH)_3$ as electron acceptors during respiration (Ponnamperuma, 1984). The result is O_2 starvation and discontinuation of respiration in root tissues. This will result in root death unless the plant finds alternate ways to support energy conversion, such as development of aerenchyma which promotes O_2 flow through roots to respiring tissue, or through energy metabolism which does not require O_2 as an electron receptor (see Chapter Two - Literature Review).

Research suggested that improved survival of roots of species associated with boreal peatlands, Picea mariana (Mill) BSP. (black spruce) and Larix laricina (DuRoi) K. Koch (tamarack), occurred under high water table conditions when seedlings are subjected to low soil temperatures (8.0 to 10.5 °C)(Lieffers and Rothwell, 1986). The control treatment consisted of seedlings grown at higher soil temperatures of 17 to 19°C with high water tables. Lieffers and Rothwell suggested that this increased survival may have been the result of decreased soil respiration and increased solubility of O_2 in soil water, making more O_2 available for root respiration. However, survival of roots of these seedlings may have been the result of anatomical and metabolic adaptations to flooding (Coutts and Armstrong, 1976), the existence of which should not be discounted until investigated.

The objective of the work presented in this thesis was to provide baseline information on metabolic and anatomical responses to flooding stress in Picea mariana, Picea glauca, Larix laricina, Pinus banksiana and Pinus contorta. This type of approach has been used on several occasions for commercial and non-commercial plant species (Armstrong, 1969; Garcia-Novo and Crawford, 1973; Hook and Brown, 1973; Joshi et al., 1973; Crawford, 1976; Smirnoff and Crawford, 1983; Tripepi and Mitchell, 1984; Justin and Armstrong, 1987; Kimmerer and MacDonald, 1987). I chose to measure flooding stress response by examining changes to root anatomy following exposure of conifers to waterlogged soil, changes in CO_2 efflux

rates from roots exposed to oxic or anoxic conditions, and ^{14}C translocation patterns to roots exposed to oxic or anoxic conditions. I incorporated cold temperatures into the experiments dealing with CO_2 efflux and ^{14}C translocation because it is an important feature of boreal peatland soils and plays a fundamental role in metabolism.

In addition to laboratory experiments, I also conducted a small field experiment. The purpose of this experiment was to determine seasonal timing of root growth of P. mariana and L. laricina on a peatland. Root growth under flooded conditions is either correlated with a internal supply of O_2 to the root (Coutts and Armstrong, 1976) or increasing soil temperatures in drained soils (Tryon and Chapin, 1983). Information on the timing of root growth, when correlated with information on water table and soil temperature, would allow me to refine my conclusions derived from observations of anatomy and metabolism of conifers.

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CHAPTER TWO

LITERATURE REVIEW

Plant roots survive soil flooding through the use of several adaptive features (Drew, 1983). This section reviews these features in order to give the reader a better understanding of what is involved in survival of roots under anoxic conditions. I also address the influence of cold upon root metabolism as this stress may be important to the survival of roots experiencing anoxia in boreal peatlands.

Aerenchyma and Oxygen Movement in Plant Roots

It has become widely accepted that oxygen moves from the atmosphere to root tissues via leaves and stems. Evidence supporting this view comes from studies using oxygen isotopes to trace oxygen movement through the plant (Evans and Ebert, 1960; Barber et al., 1962; Jensen et al., 1967), from studies using polarographic techniques to measure oxygen diffusing from root surfaces (Greenwood 1967a; 1967b; Armstrong, 1975; Ando et al., 1983) and from use of redox dyes used to localize oxygen diffusion from root surfaces (Philipson and Coutts, 1978; Conlin and Crowder, 1989). Indirect evidence also comes from observations of intercellular air spaces (Ando et al., 1983) and aerenchymatous lacunae found in root tissues of herbaceous plants (Sifton, 1945; Smirnoff and Crawford, 1983; Justin and Armstrong, 1987). These air spaces are thought to facilitate oxygen transport throughout the root in plants subjected to waterlogging.

Supply of oxygen to the root is necessary for root elongation (Geisler, 1965; Huck, 1970; John et al., 1974; Atwell et al., 1985), energy dependent trans-membrane transport activity (Bravo-F and Uribe, 1981; deBoer and Prins, 1984; Morris and Dacey, 1984), nutrient uptake (Morris and Dacey, 1984; Drew and Saker, 1986), and prevents anaerobic respiration from replacing aerobic respiration (Mendelssohn et al., 1981; Monk and Brandle, 1982). A constant supply of oxygen to the roots also sustains an oxidized rhizosphere, which is thought to reduce damage done to roots by reduced toxins formed in flooded soils (Hook, 1984; Hendry and Brocklebank, 1985; Koncalova, 1990).

Two physical forces promote O_2 movement into root tissues. The first is movement of O_2 down through the root in response to a diffusion gradient. The diffusion gradient is the result of O_2 consumption by respiring root tissues (Luxmoore et al., 1970). An anoxic soil rhizosphere also promotes diffusion of O_2 from root tissues (Armstrong, 1979), and this may contribute to the overall diffusion forces encouraging O_2 movement down the root.

The second driving force consists of mass flow of gases, including O_2, from the leaves and shoot to roots and rhizomes. Mass flow of O_2 can develop through one of two processes. The first and most thoroughly examined process is convective flow of gasses induced by positive gas pressures. This was first demonstrated in Nuphar luteum by Dacey (1980), and has since been shown to occur in several species of water lily, Phragmites australis (Armstrong and Armstrong, 1990) and in Alnus glutinosa Gaertn. (Grosse and Schroder, 1985; Schroder, 1989). Another form of mass flow is generated via negative pressure gradients created

through solublization of CO_2 in soil water after it has diffused from root tissues. This type of convective gas flow has been demonstrated in Oryza sativa (Raskin and Kende, 1985).

The effectiveness of O_2 diffusion in maintaining adequate oxygen levels within the root is subject to some speculation. For example, diffusion of O_2 to respiring root tissues can be disrupted by very low rhizospheric redox levels which increase the rate of O_2 loss from the root and deprive the root of O_2. This sort of process has been shown to occur in Spartina alterniflora Loisel (Mendelssohn et al., 1981; Morris and Dacey, 1984) and in Pisum sativum (Armstrong and Healy, 1984) under laboratory conditions. In wetland graminoids like S. alterniflora, the presence of multicellular, tightly packed and highly lignified exodermal tissues is thought to mitigate O_2 loss by slowing the diffusion rate from root to rhizosphere (Clark and Harris, 1981; Conlin and Crowder, 1989; Koncalova, 1990). Oxygen is only allowed to diffuse from the root apices of these graminoids where exodermal tissue has not developed or where lateral roots penetrate the exodermis. However, even with this type of root anatomy, it is obvious that reliance upon O_2 diffusion to sustain root growth and survival is a precarious strategy for plants experiencing soil flooding.

Tree roots are also thought to have tissue air spaces which facilitate movement of oxygen into root tissues (Armstrong, 1968; Armstrong and Read, 1972; Hook and Brown, 1973; Coutts and Philipson, 1978; Kawase and Whitmoyer, 1980). Lysigenous aerenchyma is often present within the cortex of Salix spp. (Kawase and Whitmoyer, 1980) and Armstrong and Read (1972) demonstrated diffusive O_2 transport to root tips in seedlings of several species of conifers. Pines had higher rates of O_2 diffusion than spruces and it was suggested by the authors that this was due to a larger amount of intercellular air space within the primary root cortex of pines. Armstrong and Read (1972) suggested that this greater amount of cortical intercellular air space and high root O_2 diffusion rates were "...indicative of a plant well adapted to the anaerobiosis of wet soil ... at least in the early seedling stage."

Coutts and Philipson (1978) also investigated tolerance to flooding by Pinus contorta and Picea sitchensis through examination of root anatomy and use of redox dyes to localize oxygen diffusion. Their study showed that roots of P. contorta developed cavities within the stele of primary roots exposed to flooded soil. These cavities were not present in roots from drained soils. The steler cavities, along with cortical intercellular spaces, were structures which were identified as being involved in enhanced O_2 transport into pine roots. In further studies of woody roots, Philipson and Coutts (1980) showed that O_2 transport in P. sitchensis took place via the bark (no other tissues were identified) and in P. contorta via embolised tracheids .

Root Growth and Morphology in Response to Soil Flooding

The architecture of root systems often reflects the physical and biotic stresses which plants are exposed to. In tropical mangroves lateral roots extending from the main root axis tend to avoid contact with flooded substrate, except where secondary laterals of primary structure enter the wet soil (Gill and Tomlinson, 1977; McKee et al.,

1988). Taproots and sinkers in mangrove tend to assume "obconical" shapes, with secondary thickening occurring in the upper root portions at the soil surface. Below the surface of flooded soils these roots tend to retain their primary anatomy.

McQuilken (1953) observed that root systems of pitch pine showed strong tap roots which extended into the saturated zone of flooded soils. Below the saturated zone the taproot became a mass of smaller roots. These "shaving brush" patterns are also found in P. contorta and Picea sitchensis (Coutts and Armstrong, 1976). This suggests that secondary growth in roots of conifers, like mangrove, is inhibited when roots grow into flooded, anoxic soil.

Keeley (1979) showed that swamp populations of one-year-old Nyssa sylvatica replaced their root system with succulent roots within one month of flooding. These roots showed increased fermentative capacity above that of the original roots, but were replaced within the year by non-succulent roots which showed a reduced capacity for fermentation, and an increased capacity for oxygen transport.

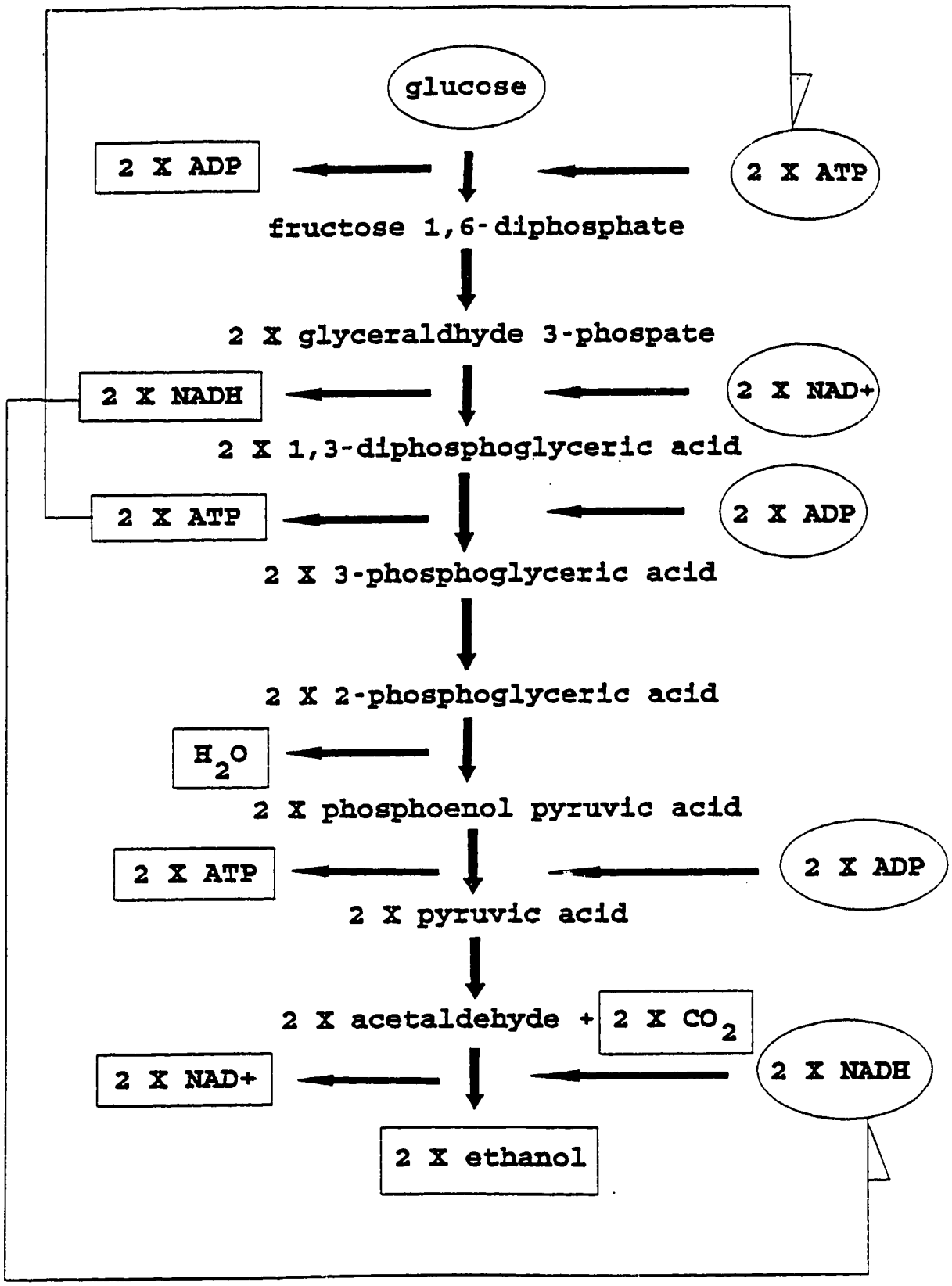
Flooding tolerance may also be related to the timing of root growth in flooded soils. In most temperate tree species, root growth occurs in bi-seasonal surges before and after bud flush (Lyer and Hoffman, 1972; Langlois et al., 1983). Warm soil temperatures are usually required for root growth (Lyer and Hoffman, 1972; Deans, 1979), while conditions of low soil redox potential brought about by flooding often slow growth of roots of tree species adapted to wetland conditions (Pezeshki, 1991). Peatland black spruce and tamarack bud flush usually occurs at the beginning of the season when soils are cold (Rothwell and Silins, 1990) and water table levels are high (Lieffers and Rothwell, 1987). Cold, flooded soil conditions are not likely to encourage root growth prior to bud flush in these species, and root growth phenology may be governed by either a drop in the water table or by increasing soil temperatures with the progression of the growing season.

Fermentation and Flooding Tolerance in Plants

Under anaerobic conditions, living tissue metabolizes glucose without oxygen. This metabolism is termed glycolysis (Embden-Meyerhof pathway) and is the primary step in respiration. It consists of phosphorylation of ADP through the partial breakdown of glucose, resulting in the production of lactate as a terminal product. If the terminal step of lactate production is replaced with the production of ethanol, this form of glycolysis is termed ethanolic fermentation (Figure 2.1). Ethanolic fermentation is the predominant form of anaerobic energy metabolism found in plants (Drew, 1983; Smith and ap Rees, 1979; Smith et al., 1984; 1986). However, it is possible to find both lactate, malate, and ethanolic forms of glycolysis in plant tissues (Turner, 1960; Drew, 1983; Roberts et al., 1985; 1989).

There is controversy over the value of ethanolic fermentation as a means of ensuring survival of plant tissue under anaerobic conditions. Several authors (Fulton and Erickson, 1964; Crawford and Tyler, 1969; Crawford, 1976; Crawford and Baines, 1977; Pradet and Bomsel, 1978; Crawford, 1984; Moore, 1982; Smits et al., 1990) asserted that ethanol accumulation in tissues is harmful to the plant and that flooding

Figure 2.1. Abbreviated schematic of fermentation representing the metabolism of glucose into ethanol and CO_2. Circled items represent input of compounds into pathway while items in squares represent output of compounds. Items with no borders are compounds intermediate between glucose and ethanol. The arrows bordering the schematic and connecting box's and circle's of NADH and ATP are to illustrate that there is a net production of only two ATP per glucose molecule in fermentation, despite the fact that other reducing energy is produced in the form of NADH and extra ATP. The extra ATP and NADH is used in the fermentative pathway to metabolize glucose. Alcohol dehydrogenase catalyses the production of ethanol from acetaldehyde.


glucose
2 X ADP
2 X ATP
fructose 1,6-diphosphate
2 X glyceraldhyde 3-phospate
2 X NADH
2 X NAD+
2 X 1,3-diphosphoglyceric acid
2 X ATP
2 X ADP
2 X 3-phosphoglyceric acid
2 X 2-phosphoglyceric acid
H2O
2 X phosphoenol pyruvic acid
2 X ATP
2 X ADP
2 X pyruvic acid
2 X acetaldehyde + 2 X CO2
2 X NAD+
2 X NADH
2 X ethanol

survival and tolerance of anoxia is mediated through glycolytic processes which produce alternate end products such as malate, glycerol and shikimate. This theory is often referred to as the "metabolic theory" of flooding tolerance (Davies, 1980; Drew, 1983). However, a number of authors agree that there is little evidence for a glycolytic process other than those which produce ethanol or lactate (Drew, 1983; Smith and ap Rees, 1979; Smith et al., 1984; Roberts et al., 1985). Others, such as Jackson et al. (1982) have shown that exogenous supply of ethanol to pea plants produced no injury and the authors stated that "... ethanol plays only a minor role in flooding injury to roots and shoots." (Jackson et al., 1982).

Reservations have also been expressed over the belief that ethanol accumulates in plant tissues (Davies, 1980). Experimental evidence shows that large amounts of ethanol are carried via the transpiration stream from anoxic roots to shoots of P. contorta and Populus deltoides where it is oxidized (Crawford and Finegan, 1989; MacDonald et al., 1989). Ethanol has also been observed in xylem exudate of flooded Lycopersicum esculentum (Fulton and Erikson, 1964).

A growing body of literature indicates a correlation between survival of anoxia and increases in both alcohol dehydrogenase (ADH) activity and ethanol levels of tissues after imposition of anoxia stress. A variety of species including Oryza sativa, (John and Greenway, 1976), Carex riparia, Poa trivialis, Utrica dioica, Ranunculus repens, Filipendula ulmaria (Smith et. al., 1986), Fraxinus pennsylvanica (Good and Patrick, 1987), Avicennia germinans (McKee and Mendelssohn, 1987), Scirpus acutus, S. validus, Scolochloa festucacea, Phragmites australis (McKee et al., 1989), Betula nigra L. and B. pendula Roth (Tripepi and Mitchell, 1984) showed a rise in ADH following flooding stress. Several other examples of species showing anoxia tolerance linked to ADH activity are given in reviews by Drew (1983) and Harry and Kimmerer (1991).

In contrast, several other studies which set out to show correlations between plant tissue anoxia and increased ADH activity showed no ADH increase. However, several of these studies do not take into consideration factors which prevent anoxia from occurring within tissues. For example, Fagerstedt (1984) compared ADH levels between Carex rostrata and four cultivars of Hordeum vulgare L. grown in drained soilconditions and then subjected to flooding of up to 15 days duration. Carex rostrata showed no increase in ADH activity compared to non-flooded controls, while H. vulgare showed increased ADH activity with flooding. However, Fagerstedt did not examine root and shoot anatomy C. rostrata, which is known to produce extensive, well developed root, shoot and leaf aerenchyma under aerobic as well as anaerobic conditions (Conlin, 1986). Hordeum vulgare produces root aerenchyma only after it is exposed to flooding (Bryant, 1934) and there is no indication that it produces leaf aerenchyma. Thus, C. rostrata under Fagerstedt's experimental conditions may have been better able to supply its roots with oxygen, allowing this species to sustain respiration without having to switch to fermentation due to lack of oxygen (therefore, no rise in ADH or ethanol levels). H. vulgare roots, on the other hand, with no aerenchyma, would have been forced to ferment, causing a rise in ADH activity.

Similar problems have arisen in other experiments. The research conducted by McKee et al. (1989) showed low ADH levels in roots of Typha

latifolia while at the same time demonstrating high levels of root air space cross-sectional area (i.e., roots had large amounts of aerenchyma). Another good example is Hook and Brown's (1973) work which showed low level accumulation of ethanol in roots of Nyssa aquatica along with rhizospheric O_2 diffusion.

It should also be recognized that a rise in tissue ADH and ethanol or the presence of ADH or ethanol in plant tissues does not necessarily indicate response to anoxia stress. Nor does a large increase in ADH or ethanol levels indicate that one plant variety or species is more tolerant to anoxia than another. There is evidence to indicate some fermentation activity occurs in plant tissues even in the presence of high concentrations of oxygen. Apples and other fruits produce ethanol under aerobic conditions without needing the stimulus of anoxia (Boersig et al., 1988). Ricard et al. (1986) state that Oryza sativa embryos have high constitutive ADH activity that appears to be inhibited under aerobic conditions. Ethanol was shown to be present in the trunks and roots of P. contorta and Picea sitchensis under all conditions (Crawford and Baines, 1977). One paper by Crawford (1976) appears to show more ethanol in roots of P. contorta, Alnus incana, L. laricina, P. mariana, Picea sitchensis, Larix europea and Pinus sylvestris exposed to ambient O_2 concentrations than roots under anoxia.

It has also been shown that ADH and ethanol levels in tissues increase in response to stress other than anoxia. An example of a rise in ADH was shown to occur in roots and shoots of Zea mays and Oryza sativa exposed to low temperature for 24 hours. During this period, ADH showed an eight-fold increase activity over that shown by control plants (Christie et al., 1991).

In another study, maize lines with ADH activity higher than 20 nmol NADH/min/mg protein showed no improvement in energy metabolism of root tips under anaerobic conditions (measured as length of survival time of root tip under hypoxia), although there was a level at which ADH activity correlated with root tip intolerance to anaerobiosis (about 10 nmol NADH/min/mg) (Roberts et al., 1989). Roberts et al. (1989) also made the point that pretreatment of root tips with mild hypoxia increased their survival time under severe hypoxia, but this was not due to any increase in ADH activities.

Although ethanol is a product of ethanolic fermentation, and ADH has been used as a marker of this activity, there have been few studies examining alternate roles of ADH in plants. This is despite the fact that several plant species are capable of producing more than one isozyme of ADH (Harry and Kimmerer, 1991). One interesting paper by Ricard et al. (1986) showed that a large proportion of the ADH formed in Oryza sativa in response to anoxia was a different isozyme from that of the constitutive ADH present before anoxia. Ricard et al.'s (1986) paper brings to mind several questions. For example, why would there be a need for a different isozyme of ADH when there is a large amount of constitutive ADH in place in this species? And why does this new ADH increase with time following anoxia? It seems to me that energy production is essential to life and it does not make sense that a plant like Oryza sativa would experience O_2 deprivation and then increase the amount of ADH necessary for anoxic energy production. What does the plant do for energy in the meantime?

Possible answers to these questions could be realized if it is understood that ADH has reversible properties (Tajima and LaRue, 1982). When ADH is considered a component of metabolism responsible for oxidation of ethanol to acetaldehyde (Eisses et al., 1985), then the rise in ADH levels following imposition of anoxia in plant tissue could be seen as a response to increased ethanol, not anoxia. Thus, in the case of Ricard et al.'s (1986) work, the increase of one form of ADH following anoxia could be seen as an attempt by the plant to oxidize ethanol created by the constitutive ADH. Other examples which could be used as circumstantial evidence supporting this view are the studies of kinetic properties of Zea mays ADH-2 isozymes, which increase in activity following anoxia. Both isozyme variants show a high K_m for the production of acetaldehyde from ethanol (Davies, 1980).

Production of carbon dioxide has also been used as a measure of fermentation activity in plants (Taylor, 1941; Turner, 1951; 1960; Bourne and Ranson, 1965; Effer and Ranson, 1967; Crawford, 1976; John and Greenway, 1976; Tripepi and Mitchell, 1984; Borsig et al., 1988). Use of CO_2 production can be advantageous in fermentation studies since it is possible to avoid pitfalls associated with correlating ADH or ethanol with flooding tolerance. For example, Borsig et al. (1988) showed that use of ethanol to define the Pasteur effect curve is not prudent since ethanol is not a good indicator of onset of fermentation. Additionally, Turner (1960) showed that in many cases the amount of ethanol recovered from fermenting tissues cannot account for their observed rates of CO_2 efflux, indicating the fermentation of substrate other than carbohydrates.

In addition, CO_2 efflux rates give a measure of fermentation and respiration which are comparable to one another. This is because respiration of a single glucose molecule will result in the production of six CO_2 molecules, while fermentation will result in the production of only two CO_2 molecules from a glucose molecule (Turner, 1951). Thus, comparison of CO_2 efflux rates from fermentation under anaerobic conditions to respiration at 21% O_2 can give us an idea of the size of the glycolytic component of the respiratory pathway in plants (Turner, 1960). Plant species which produce ratios of 0.33 should have a 1:1 ratio of glycolytic components to TCAC components.

A ratio greater than 0.33 seems to be far more common than not (Turner, 1951; 1960). It appears that Fagopyrum esculentum is one of the few plant species to show a fermentative CO_2 efflux which is one-third that of its respiratory efflux (Leach, 1936 ; Turner, 1960; Effer and Ranson, 1967). Turner (1960) indicated that there is little reason to believe that CO_2 is produced by a metabolism other than ethanolic fermentation, although a recent paper by Vanlerberghe et al. (1989) provides evidence for the existence of mitochondrial fermentation in algae based upon the investigation of CO_2 efflux rates under anoxia. Other possible hypothetical metabolic pathways do not produce CO_2, or require the fixation of CO_2 to function, or, as in the case of the possible functioning of the pentose phosphate pathway under anoxia, represent untested hypotheses (Turner, 1960; Davies, 1980).

Fermentation and Respiratory Substrate

Starch is broken down into soluble sugars prior to its use in glycolysis and the TCA cycle, and the energy stored in these sugars is used to drive the synthesis of ATP. The ability of a plant tissue to maintain fermentation or respiration can be limited by the availability of soluble sugars. The availability of soluble sugars are often limited by several factors, including cold temperature and anoxia (Crawford and Huxter, 1977; Barclay and Crawford, 1983; Delucia, 1986).

Forces governing the flow of sugars to roots from shoots include the increasing viscosity of flowing sap with cooling temperatures and the decreasing strength of respiratory sinks which promote flow of sugars (Geiger and Sovonick, 1975). Interruption of translocation of photoassimilated ^{14}C to anoxic organs and through anoxic stems and petioles has been demonstrated by several authors (Sij and Swanson, 1973; Geiger and Sovonick, 1975; Fensome et al, 1984). Decreased transport of ^{14}C to roots after exposure to anoxia has also been reported and it has been hypothesized that this is a response to lack of internal O_2 transport to roots (Nuritdinov and Vartapetyan, 1980; Schumacher and Smucker, 1985). It has also been shown by Webb and Armstrong (1983) and Waters et al. (1991) that growth and viability of roots are prolonged under anoxia when an exogenous supply of glucose are added to the culture medium. These authors concluded that anoxia reduced the flow of carbohydrate through the phloem and that added glucose partially replaced a shortfall in translocated sugar.

Aside from the forces influencing sugar translocation in phloem sap, there is also evidence indicating that respiration and fermentation within root tissue is affected by the availability of sugars in situ, the presence of which is influenced by anoxia or cold. For example, Crawford and Huxter (1977), showed that the availability of soluble carbohydrates for respiration and growth of corn and pea root tips was governed by root temperature. At 2°C, corn root segments ceased growth as soluble carbohydrate levels decreased, while pea root tips maintained carbohydrate levels and continued growing over 24 hours. A supply of exogenous glucose resulted in resumption of growth in excised corn root tips at 2°C. The decrease in soluble carbohydrate levels with a concomitant halt in root growth in corn were not due to exhaustion of available sugars through respiration, since it was shown that at higher temperature levels, both corn and pea root tips maintained their growth rates. Therefore, some other factor must have been responsible for decrease in soluble carbohydrates in corn at 2°C. This other factor may have been the adaptive nature of pea root invertase, which showed a lowering of its K_m value after cold pretreatment, while corn invertase did not (Crawford and Huxter, 1977).

Cold temperatures have also been shown to affect the form of carbohydrate available to conifer roots. Delucia (1986) showed that a 5 day, 0.7°C chilling of roots of Picea engelmannii seedlings (shoots were maintained at a external temperature of 20°C) resulted in breakdown of starch to glucose. Chilling treatments also resulted in the decreased starch content of needles and stems. Roots kept at 10 and 20°C did not show conversion of starch to glucose. Roots of conifers grown in cold soils, i.e. boreal peatlands, would be better able to sustain

fermentation or respiration if carbohydrates were available in their soluble form.

Another experiment which measured the adenylate energy charge (ATP+0.5ADP/ATP+ADP+AMP) of fermenting corn root tips under anoxia at 25°C showed that addition of 0.2 M glucose resulted in an increase in energy charge from 0.2 to 0.6 (Saglio et al., 1980). The energy charge of roots in air was 0.9.

Finally, it has been shown by Barclay and Crawford (1983) that rhizomes of three emergent plant species, Scirpus maritimus L., Phalaris arundinacea L. and Glyceria maxima (Hartm.) Holmberg showed different levels of tolerance to total anoxia. Scirpus maritimus apparently showed active shoot extension from rhizomes over a 7 day period of anoxia; P. arundinacea did not show shoot elongation while under the same treatment conditions, but resumed shoot growth after re-exposure to ambient levels of oxygen. Rhizomes of G. maxima, died during exposure to anoxia over 7 days. After 4 days of anaerobic conditions, it was discovered that S. maritimus and P. arundinacea showed no statistically significant differences between non-structural carbohydrate levels (starch and fructosans) in the treated rhizomes and the control rhizomes. Scirpus maritimus did not show any difference between the treatment and the control in tissue sugar level (glucose, fructose, sucrose and raffinose), while raffinose levels in P. arundinacea decreased and fructose levels increased fourfold over the control. In contrast, G. maxima showed a decrease in sucrose and raffinose and total non-structural carbohydrate levels by the fourth day of anoxia.

Barclay and Crawford's (1983) work indicates that the survival of rhizomes of wetland plants is related to the maintenance of adequate levels of soluble carbohydrates. These carbohydrates are likely required for fermentation and production of ATP. Glyceria maxima was the only plant species in their experiment whose rhizomes did not survive 7 days of anoxia and also showed statistically significant decreases in rhizome sucrose and total non-structural carbohydrate. Therefore it would seem that this species' inability to survive anoxia was correlated with a drop in starch and sucrose levels. Starch and sucrose levels did not change in those species which survived anoxia. Webb and Armstrong (1983) reinforced this view by showing that a exogenous supply of sucrose increased viability of excised root tips of Oryza sativa exposed to anoxia.

Temperature also seems to play a role in making soluble carbohydrates available for fermentation. Pea root invertase is one enzyme which seems to be affected by a drop in temperature to 2°C, causing a reduction in soluble carbohydrate available for respiration and growth (Crawford and Huxter, 1977). On the other hand, a drop to 0.7°C causes the build-up of soluble carbohydrate in the roots of Englemann spruce (Delucia, 1986), while growth of roots of Picea glauca at 4°C increased soluble carbohydrate levels by approximately 50% over that seen at higher growth temperatures (Weger and Guy, 1991); these carbohydrates could be available to supply fermentation in roots which experience anoxia under cool soil conditions.

Finally, work with corn adenylate energy charge (Saglio et al., 1980) shows that glucose is important in increasing energy output from fermentation. Although this study did not show relative levels of ADH in

severed root tips, the fact that AEC increased upon addition of glucose indicates that the level of ADH alone does not guarantee increased survival of anoxia by roots. Survival of anoxia is therefore probably a combination of increased fermentative capacity and, equally important, the ability to maintain a increased supply of respiratory substrate, e.g. soluble carbohydrate, to fermentation.

Summary

This literature review illustrates the physiological complexity of flooding tolerance in plants and the pitfalls inherent in trying to correlate survival of flooding stress with a single feature. Several examples were given where studies were conducted without taking into consideration the effect of anatomy on O_2 diffusion and its sustenance of root respiration. Examples were also given of the uncertainty of the role of ADH in the response of plants to anoxia. The validity of ethanol as a true measure of anoxia response was also brought into question. Finally, examples were given in which it was shown how fermentation and tolerance to anoxia could be influenced by carbohydrate metabolism in underground plant organs. These examples have led me to believe that a range of attributes should be examined to provide information on what I believe is a whole plant response to flooding stress. This is a belief shared by other scientists investigating flooding stress in plants (Hook and Brown, 1973; Drew, 1983).

The attributes that I believed should be studied include anatomical responses to flooding as well as metabolic response which are not confounded by use of uncertain parameters such as ADH and ethanol. This leaves the option of using CO_2 as a measure of the metabolic response of roots to anoxia. In addition, I believe it is essential to obtain information on how carbohydrate supply to respiring or fermenting roots is influenced by anoxia stress. I feel that this could be accomplished through the use of ^{14}C to trace translocation of carbohydrate in plants whose roots were subjected to anoxia.

Finally, it is difficult to extrapolate laboratory results to the field under natural conditions. Evidence of internal O_2 diffusion within roots of conifers has been demonstrated under laboratory conditions and is thought to be necessary for root growth. I believe that the collection of data on root growth through a growing season and comparing it with information on soil waterlogging at the same site was one method of indirectly corroborating this hypothesis. Lack of growth during soil flooding could mean insufficient internal O_2 diffusion. In the following chapters I present the results of work looking at metabolic and anatomical adaptations to anoxia stress as well as anaerobic translocation activity and seasonal root growth in five species of conifer.

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CHAPTER THREE

RESPIRATION AND FERMENTATION IN ROOTS OF CONIFERS AT LOW TEMPERATURES*

Preamble to Chapter Three

Early in the development of my thesis proposal, I hypothesized that cold peatland soils may aid Picea mariana and Larix laricina in surviving soil anoxia by inducing root dormancy. A preliminary experiment measuring respiration rates did not support this hypothesis. This led me to hypothesize that respiratory and fermentative metabolism of the roots of these species were adapted to colder peatland soils. It also led me to the realization that Pinus contorta, which has been reported as being flooding tolerant (Crawford and Baines, 1977), may be metabolically tolerant of soil anoxia at warmer soil temperatures but may not be able to cope with colder peatland soils. This hypothesis was reinforced by my observations that P. contorta does very well in flooded soils in greenhouse experiments where the ambient soil temperature is 18°C or higher. The following chapter is a manuscript based on the results of an experiment which measured respiration and fermentation of five species of conifers throughout a range of temperatures.

Introduction

Results of laboratory and greenhouse experiments suggest that pines are better adapted to flooded substrate than are spruces (Crawford and Baines, 1977; Levan and Riha, 1986). Armstrong and Read (1972) and Philipson and Coutts (1978; 1980) reported that O_2 transport in Pinus contorta Dougl. and Pinus sylvestris L. roots extended down a greater root length than in Picea sitchensis Carr. and Picea abies Karst roots. Coutts and Philipson (1978) also report that flooded P. contorta roots develop cavities in the stele which are thought to facilitate air flow to submerged roots. The implications of these studies are that pine roots avoid ethanolic fermentation through respiration which is sustained via efficient O_2 transport to the roots. On the other hand, Drew (1983), Smith et al. (1984), Tripepi and Mitchell (1984), Smith et al. (1986), Good and Patrick (1987) all reported that flooding tolerance is dependent upon fermentative glycolysis in plant roots. This is because anoxia will occur in root tissues and has to be compensated for, even with efficient root aeration (Mendelssohn et al., 1981).

The literature on O_2 diffusion in roots suggests that peatland soils should be ideal for species like P. contorta. Peatland soils are cold and should encourage lower rates of root and soil respiration which in turn should facilitate O_2 diffusion down roots, and as a consequence, sustain root respiration over fermentation (Armstrong and Read, 1972; Philipson and Coutts, 1978; Ando et al., 1983). However, Picea mariana (Mill) BSP. and Larix laricina (DuRoi) K. Koch., not P. contorta or Pinus banksiana

* A version of this manuscript has been published by the 1993 Canadian Journal of Forest Research, 23:767-771.

Lamb. (Corns and Annas, 1986), are the dominant conifer tree species found on Canadian boreal peatlands (Van Cleve et al., 1983; Lieffers and Rothwell, 1987).

Cold soil temperatures are an important factor influencing the composition of vegetation associations of boreal forest peatlands (Van Cleve et al., 1983). However, Crawford and Baines (1977) and Levan and Riha (1986) did not consider the influence of low soil temperatures on survival of flooded conifers. I hypothesized, therefore, that P. mariana and L. laricina might be metabolically adapted to cold, flooded soils while P. contorta and P. banksiana might be better able to cope with warm, flooded soils.

This paper reports on experiments designed to test this hypothesis by measuring the rates of root fermentation (CO_2 efflux from roots under anoxic conditions) and root respiration (CO_2 efflux under oxic conditions) from roots of five conifer species at 5°C (the approximate median temperature during peak flooding in peatland soils (Rothwell and Silins, 1990)). I also report on the ability of these species to transport O_2 to roots at lower temperatures using the redox dye method (Philipson and Coutts, 1978).

Materials and Methods

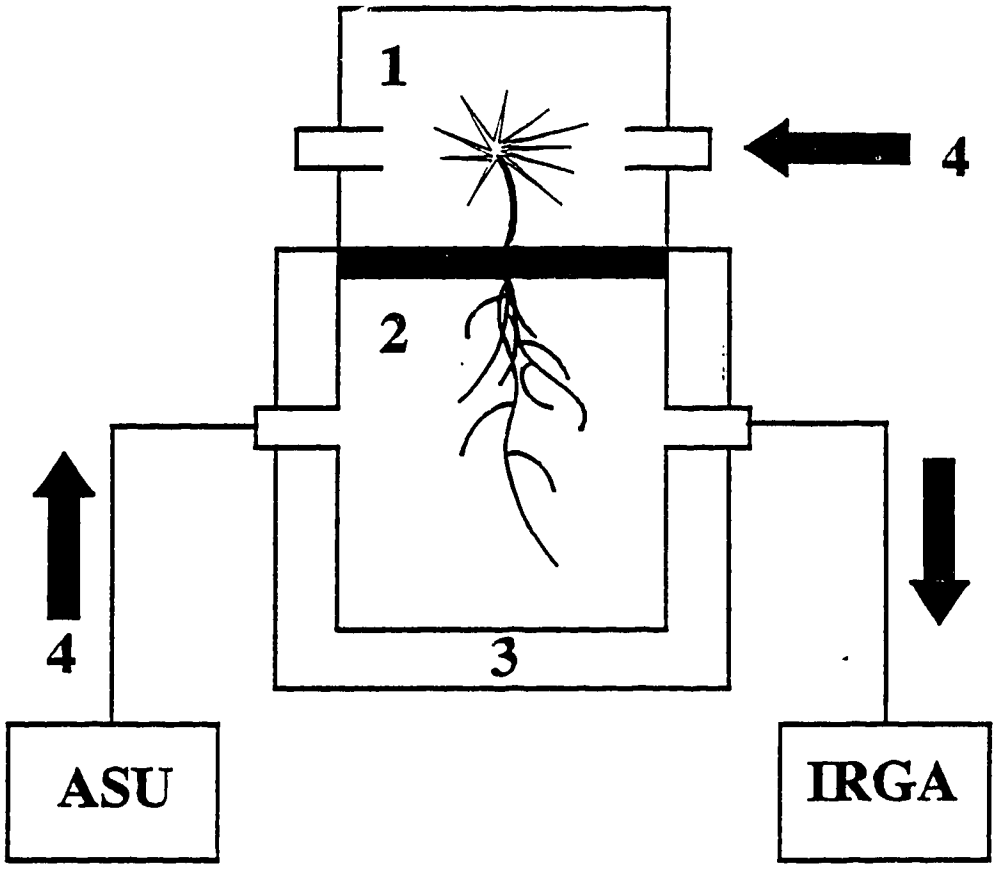
Plant Culture

Seeds of Pinus banksiana and Picea glauca (Moench) Voss were obtained from stands in the Calling Lake region of Alberta, while Picea mariana, and Larix laricina were harvested from the Saulteaux field site (Lieffers and Rothwell, 1987). Pinus contorta seed was obtained from seed lot DG 64-8-6-83PI. Seeds were germinated in an autoclaved 2:2:1 sand-terralite-peat mixture in flats. After emergence of cotyledons, seedlings were transplanted to a hydroponic system consisting of 5-litre buckets with opaque plastic lids. Seedlings were held in place by a foam plug through a hole in the bucket lid. The hydroponics nutrient solution was a modified New Jersey solution composed of: 2.0×10^{-3} M KH_2PO_4, 0.5×10^{-3} M K_2SO_4, 2.0×10^{-3} M $Ca(NO_3)_2$, 1.0×10^{-3} M $MgSO_4$, 1.0×10^{-3} M $(NH_4)_2SO_4$, equimolar amounts of 4.5×10^{-6} M $FeCl_3$ and NaEDTA, 2.3×10^{-6} M $MnCl_2$, 4.6×10^{-6} M H_3BO_3, 0.8×10^{-6} M $ZnSO_4$, 0.2×10^{-6} M $CuSO_4$, and 6.2×10^{-8} M $HMoO_4$. The pH of the nutrient solution was adjusted using NaOH or HCl to 5.5 for the Pinus spp. and 6.5 for all other species. Pinus species were found to grow better at pH 5.5 than at a pH of 6.5. All nutrient solutions were continuously aerated and replaced approximately every two days. The germination and growth of seedlings took place in a greenhouse at a median temperature of 22.5°C with an 18 hour photoperiod.

Experimental Apparatus

A robust seedling of approximately 12 weeks age was selected at random from one of the buckets. Its stem was embedded in terrostat putty and mounted in a split rubber cork, which was then wrapped in plumbers teflon tape. The cork with its plant was mounted in an airtight plexiglass chamber (Figure 3.1). Carbon dioxide flux was measured from the roots using an Analytical Design Corp. (ADC) portable Infra-red Gas Analyzer. Gases were fed into the cuvette using an ADC air supply unit set at a flow

Figure 3.1. Schematic of apparatus used to measure CO_2 flux from roots. 1, upper chamber provided oxic or anoxic atmosphere around shoot; 2, lower chamber provided oxic or anoxic conditions around roots; 3, water jacket regulated temperature around roots; 4, stream of nitrogen or 21% oxygen. Air supply unit (ASU) regulated flow of nitrogen or 21% oxygen gas. An infrared gas analyzer (IRGA)was used to measure the efflux of CO_2 from root tissues.


1
4
2
4
3
ASU
IRGA

rate of 10 mL sec^{-1}. Temperature was controlled by a water jacket around the cuvette connected to a Haake F3-C digital Refrigerated Bath and Circulator. Interior temperature of the cuvette was recorded using a thermocouple.

Experiment One

Carbon dioxide effluxes from roots of individual trees were measured at three temperatures (5°C, 15°C and 25°C) while flushing roots and shoots with N_2 gas (total plant anoxia treatment; TPA) or a prepared mix of 21% O_2 + 79% N_2 gas (oxic treatment; OX) or while flushing the roots with N_2 gas and leaving the shoot exposed to ambient oxygen levels (root anoxia treatment; RA). The gases used were free of CO_2 and were bubbled through distilled water maintained at the cuvette temperature. This prevented roots from being desiccated by the flow of dry air through the cuvette. Moisture was scrubbed from the cuvette gas stream using anhydrous calcium sulphate before measurement by the IRGA. The cuvette was tested for leakage periodically during the experiment by conducting a run without a plant in place; in all tests the IRGA registered zero levels of CO_2 gas, indicating no leakage of atmospheric gases into the experimental apparatus. Temperature levels represented the range between peat soil temperatures (Lieffers, 1988; Rothwell and Silins, 1990) and upland soil temperatures or greenhouse soil conditions (Levan and Riha, 1986). The order of the temperature levels was randomized for each treatment. Plants were allowed a period of 20 to 30 minutes to acclimate prior to measurement of CO_2 flux when treatment series were stepped down in temperature level after a steady baseline of CO_2 efflux was achieved at 25°C (e.g. 10 minutes to allow the equipment to ramp down to 15°C followed by a 10 minute stabilization period).

Each treatments was replicated three times for each species using a separate plant for each replicate. Root dry weights were measured for each plant and CO_2 efflux in nmoles CO_2 g^{-1}root dry w. min^{-1} were estimated at each temperature level.

Multiway analysis of variance was not attempted because independence was not maintained between temperature levels (each plant was tested at all temperatures). However, one-way analysis of variance was used to test for significant differences between species at each temperature level. Planned comparisons (α = 0.05) were used to compare means if significant differences were detected between species. One-way analysis of variance was also used to detect significant differences between TPA, OX, and RA for each species. Detection of significant differences was followed by planned comparison of means (α = 0.05) between TPA and OX.

Carbon dioxide efflux values from TPA treatments were divided by OX CO_2 efflux values to determine TPA/OX efflux ratio differences between species at each temperature level. My expectations were that peatland species should show ratios well above the expected 0.33 value found in flooding intolerant species (Turner, 1951), especially at low temperatures.

Experiment Two

A second, more focused experiment using the same experimental apparatus was designed to further investigate the TPA and OX values and their

ratios for the five species at 5°C. This was done in order to test the significance of the TPA:OX ratios of the five species, which could not be done using the small sample sizes in experiment one. Six to twelve trees per species were incubated under both TPA and OX conditions and TPA, OX and TPA to OX ratio data sets were analyzed using analysis of variance. Planned comparisons of means were used to test for differences in TPA to OX ratios between black spruce and all other species or jack pine and all other species. Tamarack responses to TPA and OX treatments were compared to all other species.

Oxygen transport

Oxygen transport to the roots of conifers has been qualitatively measured using reduced indigo-carmine dye, which remains colorless until it is exposed to oxygen diffusing from roots (Philipson and Coutts, 1978). A modified version of this method was used in which sodium dithionite was used to reduce agar gel containing indigo-carmine dye. This gel was cooled and poured into plexiglass boxes containing roots which were left attached to the plant (Conlin and Crowder, 1989). The presence or absence of diffusing O_2 from roots of each species was noted and the length of O_2 diffusion halos around each root was measured after 20 hours incubation in growth chambers at 5, 15 and 25°C.

Results

Experiment One

All the species exhibited an increase in CO_2 efflux with increased temperature for TPA, OX and RA (Figure 3.2). This was the expected temperature response of CO_2 efflux from glycolysis or respiration. With the exception of the pines under OX at 15°C, which gave significantly different CO_2 effluxes from that of the spruces and L. laricina, analysis of variance detected no differences between species for each treatment at all temperature levels. The only TPA and OX means which were statistically different from one another were found in the pines at 15°C and in P. contorta at 25°C.

All species showed a TPA to OX CO_2 efflux ratio greater than one third at all the temperatures (Table 3.1). Picea mariana showed a very high TPA to OX CO_2 efflux ratio at 5°C which decreased with increased temperature. Pinus banksiana also showed a high TPA to OX ratio at 5°C. Pinus contorta, on the other hand had a very low ratio at 5°C. The highest ratio for L. laricina occurred at 15°C. Picea glauca ratios rose as temperature increased.

Experiment Two

A planned comparison of means showed that P. mariana had a significantly higher TPA to OX ratio than all the other species (t prob. = 0.001) (Table 3.2). Furthermore, a planned comparison of L. laricina with all other species showed that this species had significantly higher levels of TPA and OX CO_2 production (t prob. = 0.001). Unlike the results in experiment one, P. banksiana exhibited a low TPA to OX ratio and its

Figure 3.2. Mean CO_2 flux from conifer roots under TPA (total plant anoxia), OX (aerobic treatment) or RA (root anoxia) conditions at 5, 15 and 25°C. Each bar represents the mean and standard error of three samples. Pj, P. banksiana; Pl, P. contorta; Sb, P. mariana; Sw, P. glauca; Ll, L. laricina.

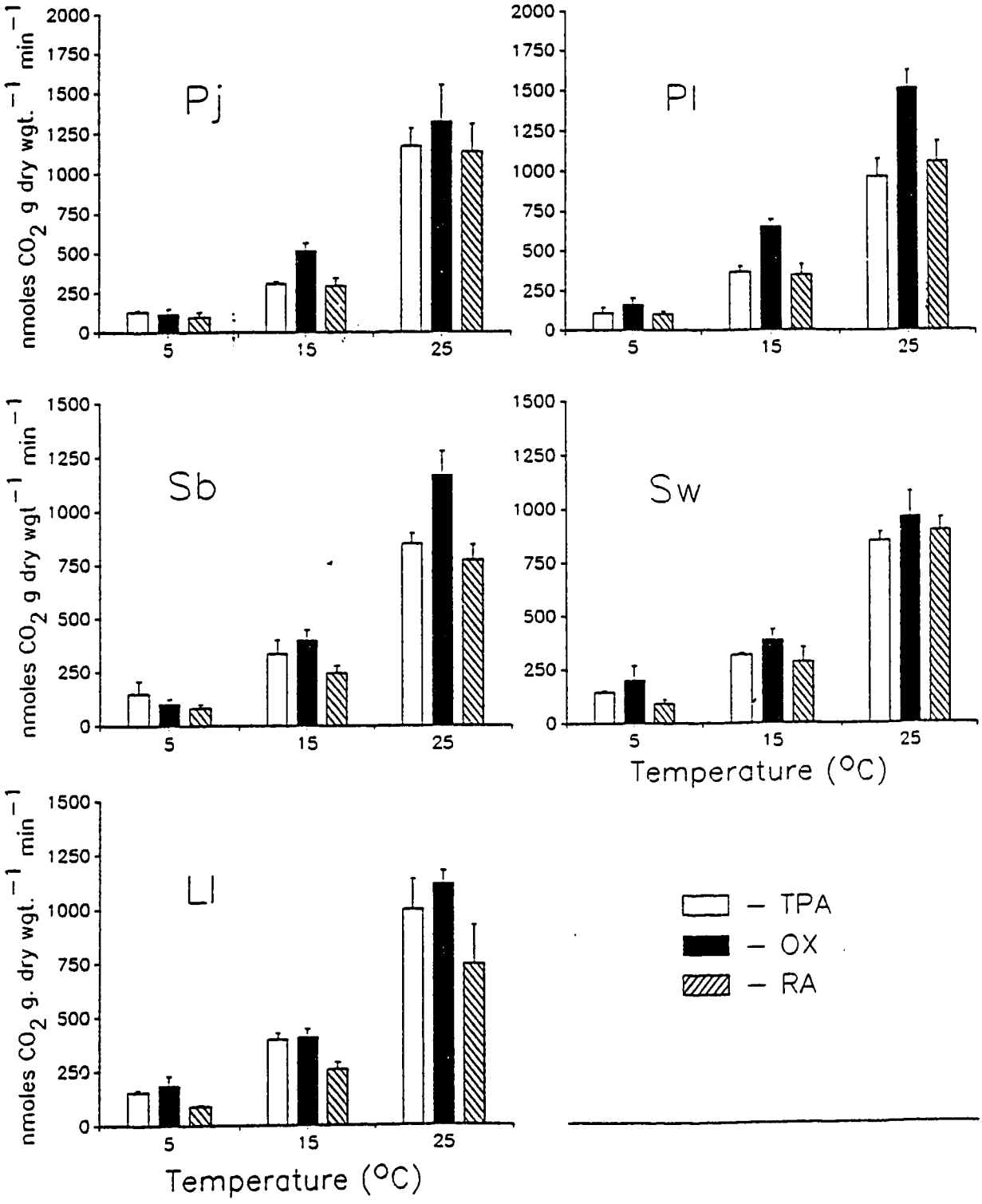

Pj
Pl
Sb
Sw
Ll
nmoles CO_2 g dry wgt.$^{-1}$ min^{-1}
nmoles CO_2 g. dry wgt.$^{-1}$ min^{-1}
0
250
500
750
1000
1250
1500
1750
2000
5
15
25
Temperature (°C)
– TPA
– OX
– RA

Table 3.1. Ratio of mean TPA CO_2 efflux to mean OX CO_2 efflux for Pl, P. contorta; Pj, P. banksiana; Sw, P. glauca; Sb, P. mariana; Ll, L. laricina,. Each mean derived from three replicates. Experimental design does not allow calculation of standard error for values in this table.

	Species				
Temperature	Pl	Pj	Sw	Sb	Ll
5°C	0.67	1.09	0.71	1.45	0.83
15°C	0.56	0.60	0.82	0.84	0.97
25°C	0.64	0.88	0.88	0.73	0.89

Table 3.2. Mean efflux rates (nmoles CO_2 g^{-1} dry w. min^{-1}) generated by conifer roots at 5°C under TPA and OX conditions and average ratio of TPA to OX flux for each species (s.e.). Values marked with asterisk indicate species which are significantly different from all other species using a planned comparison of means (t = 0.001). sample size given in brackets under species. Pl, P. contorta; Pj, P. banksiana; Sw, P. glauca; Sb, P. mariana; Ll, L. laricina.

Species (n)	TPA	OX	TPA/OX
Pl(12)	128.9(8.5)	194.0(12.8)	0.67(0.03)
Pj(12)	95.8(6.2)	135.4(5.8)	0.71(0.04)
Sw(6)	78.7(25.3)	116.9(25.8)	0.61(0.1)
Sb(10)	124.2(14.9)	143.9(16.6)	0.87(0.05)*
Ll(6)	173.6(13.4)*	251.8(14.5)*	0.69(0.04)

capacity to produce CO_2 was no greater than that of P. contorta. A comparison of P. banksiana's mean TPA to OX CO_2 efflux ratio with all other species except P. mariana showed no significant differences between these species (t prob. = 0.315).

Oxygen transport

The spruces showed no sign of O_2 diffusion from root surfaces at any of the temperatures measured. Larix laricina and the pines showed O_2 diffusion at all temperatures, but not all roots showed oxidation. All oxidized dye patterns extended along the roots from root bases, but stopped well short of encompassing the full length of the root (Table 3.3). No apical oxidation patterns were observed. Pinus banksiana was the only species which showed any increase in mean length of the oxidation halo with lower temperature. Oxidation was very slow and could not be observed until the end of 20 hours.

Discussion

Ratios of TPA to OX CO_2 efflux serve to illustrate the fermentation performance of each species at each of the three temperature levels. This is because alcoholic fermentation along with the production of CO_2 seems to be the predominate
metabolic response to anoxia stress in trees (Smith et al.,1984; Tripepi and Mitchell, 1984). Picea mariana had a high TPA to OX CO_2 efflux ratio at the lowest temperature tested in this experiment. This feature, which was confirmed in our second experiment, may be indicative of metabolic adaptations which include increased rates of fermentation in response to flooding in cold soils. Similar adaptations occur in other flooding tolerant species. For example, Tripepi and Mitchell's (1984) comparison of CO_2 effluxes from excised root tips of flood-tolerant Betula nigra and flood-intolerant B. pendula indicated that river birch had a higher CO_2 production rate per unit fresh weight and a higher hypoxic to oxic ratio (0.6) than European birch (0.3) after one day of hypoxia. In another instance, data obtained by John and Greenway (1976) showed that a ratio of 0.30 could be derived from anaerobic-adapted Oryza sativa root apices while non-adapted apices showed a ratio of 0.05. A ratio of 0.05 was also derived from Smith and ap Rees (1979) measurements of flooding intolerant Pisum sativum root apices. Experiments with intact O. sativa and Triticum aestivum seedlings growing on enriched carbohydrate substrate showed that O. sativa's fermentative to respiratory CO_2 output ratio was 1.5 while T. aestivum's ratio was 0.58 (Taylor, 1942).

Larix laricina had the highest TPA and OX CO_2 efflux of the five species tested at 5°C. These higher rates indicate that this species may be able to survive and exploit cold soils by virtue of having higher respiration rates. Higher root respiration rates have been observed under cold soil conditions within species native to cold soils when compared to species native to warm soils (Sowell and Spomer, 1986).

Pinus contorta, P. banksiana and L. laricina showed evidence of O_2 transport to roots over the 5°C to 25°C temperature range. Philipson and Coutts (1978) showed that roots of P. contorta have greater overall

Table 3.3. Mean length (s.e.) in centimetres of oxidized dye along root and mean root length of immersed roots of P. contorta, P. banksiana and L. laricina at 5, 15 and 25°C. Oxidized dye zone length is measured from the base of the roots. Sample size is based upon those roots which actually showed oxidation within a sample of three plants per temperature level.

	Pinus contorta			Pinus banksiana			Larix laricina		
Temp.	n	Dye	Root	n	Dye	Root	n	Dye	Root
5°C	8	6.8(1.0)	20.8(1.4)	5	9.3(0.7)	28.8(3.7)	4	4.2(0.2)	16.1(1.8)
15°C	2	8.5(1.1)	19.9(5.6)	5	5.4(0.9)	21.5(0.8)	0	-	-
25°C	3	5.2(0.3)	34.8(8.4)	3	6.9(0.3)	25.3(1.0)	5	6.3(0.1)	34.9(4.1)

lengths of O_2 diffusion patterns than Picea sitchensis. Armstrong and Read (1972) demonstrated that P. contorta had greater O_2 diffusion rates from roots than P. sitchensis. This would suggest that O_2 is transported more efficiently and along greater distances in P. contorta. The significant differences between TPA and OX CO_2 efflux rates in the pines at 15°C and P. contorta at 25°C (Fig. 3.2) also indicate that these species have greater respiration than fermentation rates at warmer temperatures, and, therefore, probably depend upon O_2 diffusion to sustain respiration rather than allow root tissues to ferment. Internal O_2 diffusion could also support the high root respiration rates seen in L. laricina at low soil temperatures.

Philipson and Coutts (1978) reported that experiments carried out at 4°C with non-flood adapted P. contorta roots caused the dye halo around roots to extend to a mean length of 8.7 cms (which was also the mean length of their roots) over 16 hours. Experiments with single, longer root lengths showed dye halos extending as much as 36 cm on a 37 cm root. The halo's around roots of my P. contorta at 5°C were not nearly as long and covered only a small section of the overall root length. However, Philipson and Coutts (1978) measured oxidation halos of severed roots extracted from soil, while we measured halos of hydroponically grown roots still attached to the plant. It is possible that severing of the roots could have led to alterations in O_2 consumption by root tissue, or, that removal of roots from soil may have caused the inadvertent ripping away of laterals and outer root tissues. Both actions may have altered respiratory consumption of O_2 and changed O_2 diffusion patterns from roots. Cut ends may have also promoted a more efficient diffusion of O_2 into roots.

Although it is possible that high rates of fermentation may be a characteristic of trees adapted to anoxia stress, it should be pointed out that fermentation requires greater amounts of carbohydrate in order to produce the same energy charge as respiration. Barclay and Crawford (1983) showed that plant survival of flooded soils depended upon carbohydrate reserves within the root. Those plants which succumbed to flooding stress were not able to maintain carbohydrate reserves throughout anoxia. Therefore, if increased fermentation is an adaptation to flooding, it must be accompanied by adequate carbohydrate supply to the fermenting tissues.

Summary

Roots of Pinus contorta, Pinus banksiana, Picea glauca, Picea mariana, and Larix laricina all showed increasing oxic and anoxic CO_2 efflux rates with increasing temperature. This indicated that CO_2 originated from respiration or fermentative metabolism. Division of mean anoxic CO_2 efflux rates by oxic CO_2 efflux rates gave ratios greater than the expected ratio of 0.33. The anoxic to oxic ratio of P. mariana at 5°C was much higher than all other species, indicating a correlation with flooding tolerance as demonstrated by other flood tolerant plant species. Pinus banksiana also showed a high anoxic to oxic ratio.

The results of a second, more rigorous experiment designed to examine CO_2 efflux response of all species at 5°C could not repeat P. banksiana's performance, although P. mariana again showed the highest ratio. The second experiment also showed that roots of L. laricina had the highest overall anoxic and oxic CO_2 efflux rates.

A test using a redox dye in a reduced agar medium revealed O_2 diffusing from root surfaces of P. contorta, P. banksiana and L. laricina, demonstrating internal O_2 transport. However, this oxidation also demonstrated that internal O_2 transport may have been restricted to basal regions of roots, in which case it would only be beneficial to support of respiration in these portions of the root.

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CHAPTER FOUR

COMPARISON OF ROOT ANATOMY OF CONIFERS GROWN IN FLOODED AND NON-FLOODED PEAT.

Preamble to Chapter Four.

Coutts and Philipson (1978b) showed that primary roots of Pinus contorta grown in flooded soils contained steler air-space cavities. Roots that were grown in drained soils did not show these cavities. They suggested that this species ability to extend its roots deep into flooded soils was due to the facilitation of oxygen flow to roots via these cavities. Armstrong and Read (1972) also showed that roots of pine seedlings, including P. contorta, gave off a greater diffusive O_2 efflux than spruce seedlings. They attributed this larger flux rate to greater cortical air space in roots of the Pinus species.

On my arrival at Edmonton I was given roots from an experiment where Pinus contorta and Pinus banksiana were grown under flooded and drained conditions. After examining fresh hand-cut sections from these roots, I found cavities in the stele of roots of pines grown under both flooded and drained treatments. I also saw intercellular cortical spaces in roots of Picea glauca, Picea mariana, Larix laricina P. contorta and P. banksiana from flooded and drained treatments.

I felt that a good deal of anatomical detail was lost with hand-cut sections, which resulted in my being unable to distinguish aerenchyma from torn or damaged tissue. As a result I decided to repeat the experiment, but this time I embedded root tissues in paraffin and used a microtome and histological stains to prepare thin sections. I also used a pycnometer to determine if there was any change in percent air space between roots of the same species grown either under flooded or drained treatments. Pinus contorta was used as a control species since it had already been reported as showing steler air spaces in response to flooding. If steler spaces showed up in P. contorta under flooded conditions, I could be sure that this species had responded to soil flooding and that conditions favoured a flooding response in the other four species.

The results showed no anatomical response to flooding.

I then repeated the experiment and again tried to find anatomical differences between flooded and drained treatments. Again, I couldn't find any differences.

After looking at the root anatomy from three experiments over a period of two years, it finally occurred to me that perhaps there wasn't an anatomical response to flooding by conifers and that perhaps Coutts and Philipson were wrong about steler cavities in roots of Pinus contorta. The following chapter describes the results of the second and third experiments examining root anatomy in conifers.

Introduction

Roots of herbaceous and woody angiosperms often respond to soil flooding by creation of aerenchyma in cortical tissues (Kawase and Whitmoyer, 1980; Jackson et al., 1985; Justin and Armstrong, 1987). Aerenchyma allows for the transport of O_2 throughout roots; this ensures that roots do not become anaerobic and allows them to respire normally (Armstrong, 1979; Drew, 1983). However, changes in root anatomy of gymnosperms as a response to soil flooding is not as clearly documented and it seems that there are many opinions on how conifer root anatomy facilitates O_2 transport. For example, Coutts and Philipson (1978b) have that roots of Pinus contorta produced continuous steler spaces in response to flooding. These spaces were in the pericycle adjacent to phloem tissue. Coutts and Philipson suggested that these cavities facilitated oxygen transport into and through P. contorta roots and speculated that this was the reason this species had greater root penetration into flooded soil than Picea sitchensis, which had no steler spaces. Similar cavities were seen to occur in roots of Pinus taeda (McKevlin et al., 1987) along with formation of lysigenous cavities in root primary cortex. However, in another study, Yamamoto et al. (1987) showed that xylem tissue from stems of flooded Pinus halepensis seedlings contained extensive intercellular spaces. They reasoned that these were the conduits for O_2 transport in P. halepensis roots. Also, Philipson and Coutts (1980) reported that pressurized air flow occurred through embolised root tracheids in P. contorta to a distance of 45 cm, while flow was limited to under 10 cm in Picea sitchensis. In addition, Armstrong and Read (1972) suggested that the greater rates of O_2 transport seen in pine seedlings when compared to spruce seedlings were due to abundant cortical intercellular spaces. These spaces were not seen in spruces.

Of the conifer tree species found in the boreal forests of Alberta, Canada, only two are commonly found on peatlands. These are Picea mariana and Larix laricina (Corns and Annas, 1986). Pinus contorta and P. banksiana, which are also distributed over the same region, are usually found on well drained soils. Some provenances of P. contorta has been shown to do well in wet soils in the United Kingdom (Crawford and Baines, 1977). Pinus banksiana, when compared in a trial to Pinus resinosa, performed poorly in flooded soils (Tang and Kozlowski, 1983). Pinus resinosa is intolerant of flooded soils in the field and root penetration of the water table under flooded conditions was comparable to Pinus strobus L. and P. mariana (Levan and Riha, 1985). In addition, Levan and Riha (1985) found no sign of increase in root porosity, a measure of aerenchyma tissue present in roots, in Pinus strobus, P. resinosa, P. mariana or Picea glauca in response to flooding.

There are no published reports on the quantification of porosity changes which take place in roots of P. contorta, P. banksiana, and L. laricina when these species are grown in flooded soils. In addition, no work has been done on changes in root anatomy, if any, which occur in response to flooding in P. mariana, P. glauca, L. laricina, and P. banksiana. Large differences between anatomical, porosity and rooting depth attributes in a species may be indicative of adaptive features responsible for root survival in poorly drained soils (Justin and Armstrong, 1987). This chapter reports upon the effect of flooding stress

upon these features in these species.

Materials and Methods

Experimental Design

Seeds of P. mariana, P. glauca, P. contorta, P. banksiana and L. laricina were germinated in a 2:2:1 sand-terralite-peat mixture contained in seed flats with plastic lids. Seedlings were transplanted to peat-filled tubes 17 days after planting for P. banksiana, 18 days after planting for P. contorta and P. glauca, 19 days after planting for P. mariana, and 20 days after planting for L. laricina. Tubes were vertically oriented 4.3 cm i.d. PVC pipe 25 cm in length. The bottom of each pipe was embedded in a 1.5 cm sand layer across the bottom of a 25.5 cm deep x 51 x 61 cm water-proofed wooden box. The rest of the pipe was packed with peat to within 1 cm of the top end of the tube. Tubes were kept in place by packing the spaces between the tubes with peat. One extra tube was allowed to remain empty as a well to monitor water table levels in the boxes. Ten tubes in each box were randomly planted with one conifer seedling from each species for a total of 50 tubes per box.

One box was flooded to the peat surface eight days after seedlings were transplanted. The other box was periodically watered via its PVC tube well. Each species were grown in the boxes for the following lengths of time; P. banksiana, 119 days; L. laricina, 121 days; P. contorta, 125 days; P. mariana, 140 days; P. glauca, 169 days. Germination and growth throughout the experiment took place in a greenhouse with 16 hours of supplemental lighting from high pressure sodium lamps. Median temperature during the course of the experiment was 22.5°C.

This experiment was repeated at a later date with the following differences: all conifer species were transplanted into tubes 16 days after germinating and all samples were harvested 168 days later. Sample harvest was restricted to 2 plants per species per treatment.

Root penetration of flooded soil

In the first experiment six tubes were lifted from each box for each species and the peat core containing the roots gently pushed out intact. The rooting depths of the plants were easily distinguished because the roots tended to grow to a certain depth and no further in both treatments. Roots which reached their maximum depth tended to grow horizontally around the inner surface of the tubes. Rooting depth was therefore measured as the distance to which the roots grew.

Root porosity measurements

Three of the six plants per species from each box used in estimation of rooting depth were randomly selected for porosity estimation using the technique described by Van Noordwijk and Brouwer (1988). This method consisted of cutting roots into 0.5 cm lengths, recording their fresh weight, placing these lengths in a pycnometer, filling the pycnometer with water and then recording the weight of the tissue both before and after vaccum infiltration of the pycnometer. The difference in weight gave an

estimation of specific gravity which was expressed as a percent.

Steler porosity was also determined from thin sections taken at the 3.0 and 4.0 cm length (see "Methods - Root anatomy"). These measurements of porosity were determined by photographing thin sections, measuring the area of the steler cavities and area of the stele using a grid micrometer, and expressing the area of the cavities as a percentage of the total stele area.

Root anatomy

The tap roots of the remaining three plants from the first experiment were sectioned into 1.0 cm lengths. These lengths were taken from the apical four cm and the basal centimeter of each plant. The sections were killed and fixed using formalin-acetic acid-alcohol or FAA (ethanol content 50%), washed and dehydrated using a alcohol series graded upward in 10% increments and embedded in Paraplast^{R} Plus. Radial longitudinal and transverse sections were cut with a American Optical Spencer 820 rotary microtome.

Roots from the second experiment were killed and fixed using 2% gluteraldehyde (O'Brien and McCully, 1981) and embedded in glycol methacrylate (GMA) using Kushida's (1977) embedding protocol. GMA embedded sections were cut using a Reichert Om U2 ultramicrotome.

Handcut sections of live root tissue were also taken at various root lengths and photographed. Toludine blue was used as a background stain for embedded and handcut sections (O'Brien and McCully, 1981). Periodic acid-Schiff stain was used to stain for carbohydrate (Feder and O'Brien, 1968) and Sudan black was used for detection of lipids and endodermis (O'Brien and McCully, 1981).

The distance from the quiescent centre at which cortical cell, protoxylem initial cell, and pericycle cell elongation, and endodermal formation occurred were measured in root apices from both treatments. When possible, distance from the quiescent centre to protoxylem and metaxylem development were measured. However, this was a difficult measurement to obtain since development seemed to occur in the join where the root was severed into 1.0 cm lengths. The number of cortical cell layers were also counted in each root apice.

Results and Discussion

Root penetration of flooded soil

The mean maximum depth of each species root penetration for flooded treatments was roughly half that of the drained treatment (Table 4.1). Picea mariana and P. glauca showed shallower root penetration under drained and undrained conditions in comparison to the pines and tamarack.

The depth of root penetration below a water table for P. contorta has been reported at 18.5 cm when roots are grown at 10°C and 12.5 cm when grown at 20°C (Coutts and Philipson, 1978b). My results show a mean depth of penetration of 11.3 cm at a median temperature of 22.5°C. Thus, deeper rooting depth in P. contorta seems to be correlated with cool soil temperatures. Lower root temperature decreases root respiration, and as a consequence the demand for O_2 along the diffusion pathway in the root is

Table 4.1. Mean depth (cm) of root mass for each species under flooded or drained conditions. Standard error is given in brackets and n = sample size.

Species	n	Flooded	n	Drained
P. banksiana	6	10.8 (1.2)	6	22.2 (0.4)
P. contorta	6	11.3 (1.1)	6	24.0 (0.3)
L. laricina	6	13.6 (1.4)	6	22.3 (1.4)
P. glauca	6	6.7 (0.8)	6	16.5 (3.0)
P. mariana	6	7.0 (0.6)	6	12.9 (0.6)

decreased (Armstrong and Read, 1972; Ando et al., 1983). This in turn extends the potential distance over which O_2 can diffuse down the root and determines the distance to which the root will grow into a flooded soil.

Levan and Riha (1986) reported root penetration into a water table by P. mariana and P. glauca to depths of 7.0 and 2.0 cm respectively. My P. mariana and P. glauca roots penetrated 7.0 and 6.7 cm into the water table. Pinus banksiana roots grew to 10.8 cm below the water table. Pinus resinosa, which had less reduction of growth than P. banksiana in response to flooding (Tang and Kozlowski, 1983), showed water table root penetration to a depth of 7.0 cms (Levan and Riha, 1986). Larix laricina showed the greatest mean depth of root penetration (13.6 cm) in this experiment.

The spruces showed less root penetration than the Pinus spp. or L. laricina under both flooded and drained conditions. Under drained conditions this was probably a reflection of these species shallower rooting habit rather than a physical limitation imposed by restriction of O_2 diffusion rates. Under waterlogged conditions, spruce root length was probably restricted by the availability of O_2 diffusing through the root from the shoot (Coutts and Philipson, 1978b) or from the soil surface (Lieffers and Rothwell, 1986), although it is possible that the shallower rooting habit may also play a role in determining root length in the spruces. It is unlikely that the time period between harvesting species would have affected the length of flooded roots by allowing the last species harvested to grow its roots deeper. This is because rooting depth under flooded condition is determined by features controlling oxygen diffusion to the root (Justin and Armstrong, 1987), not the age of the root.

Pore space volume measurements

Only L. laricina seemed to show a mean root prorosity increase in response to flooding (Table 4.2). All other species demonstrated small decreases in average porosity and this corresponded with Levan and Riha's (1986) data which showed no porosity increase in northern conifers in response to flooding. In addition, mean porosity values from my experiment were low in comparison to substantial increases seen under flooded conditions in several wetland species (up to 52.8%) (Justin and Armstrong, 1987) and willow (42%) (Levan and Riha, 1986). A large porosity value is correlated with deep rooting in flooded soils because of lowered resistance to internal O_2 diffusion (Justin and Armstrong, 1987). Conversely, lower porosity values are associated with increased resistance to diffusion and shallower rooting of plants in flooded soils. Low porosity values are also correlated with decreased shoot dry weight.

Root anatomy

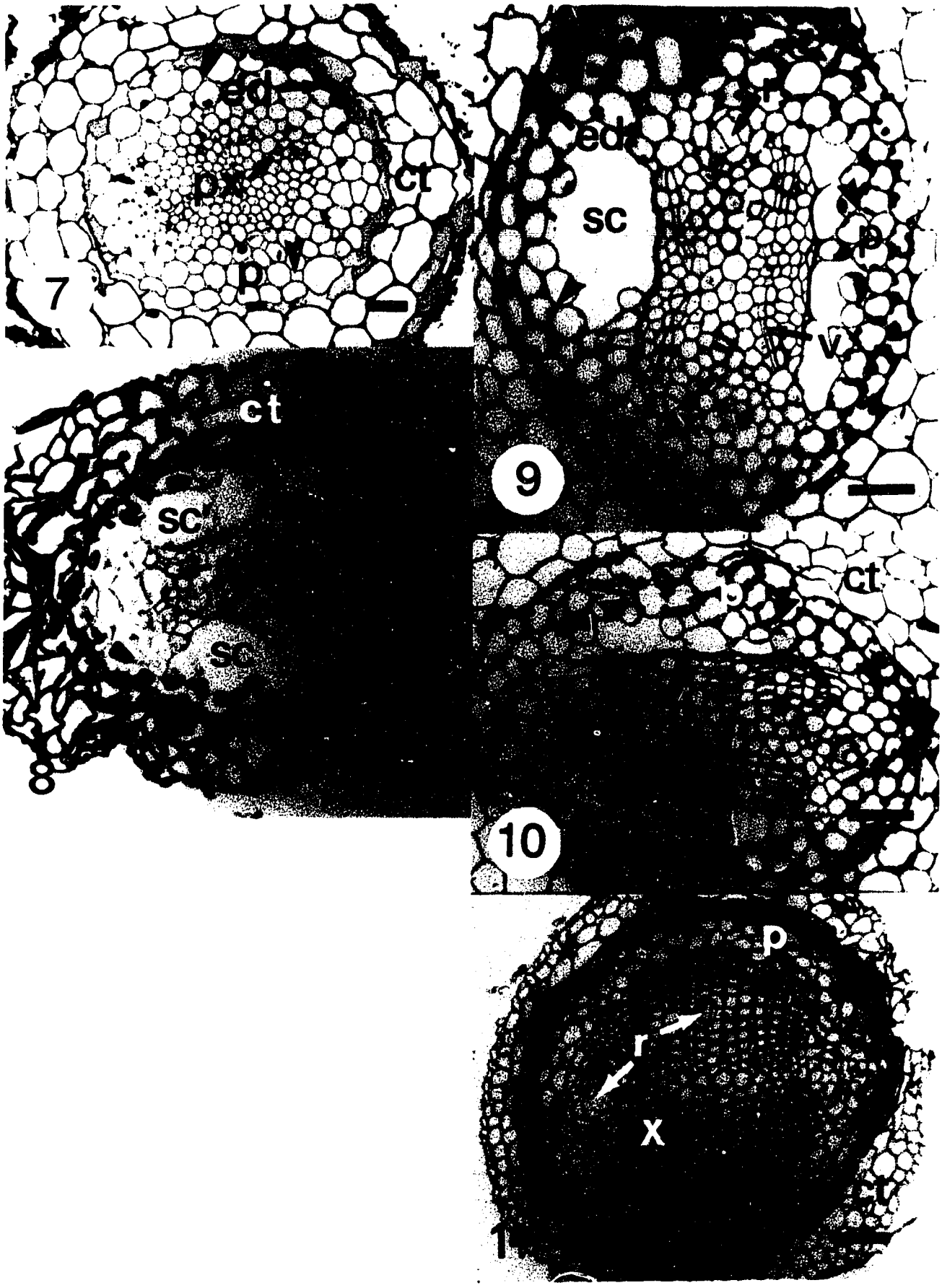
None of the five species showed any gross anatomical changes (e.g. lysigenous air space formation) in response to flooding. Steler air cavities were seen in both flooded and drained roots of P. banksiana (Plates 4.1 - 4.6) and P. contorta (Plates 4.7 - 4.11). Comparison of

Table 4.2. Mean percent values of root pore space using pycnometer measurements of whole root mass for each species. Standard error is given in brackets and n = sample size.

Species	n	Flooded	n	Drained
P. banksiana	3	2.4 (0.7)	3	8.8 (2.3)
P. contorta	3	3.1 (1.2)	3	5.5 (1.6)
L. laricina	3	5.4 (3.4)	3	3.4 (1.9)
P. glauca	3	5.5 (1.4)	3	7.3 (0.5)
P. mariana	3	6.9 (2.1)	3	11.4 (4.6)

Plates 4.1. through to 4.6. 1. Transverse cross-section of drained P. contorta root taken 2.0 cm from root tip; ed - endodermis, ct - detriorating cortex, p - pericycle, px - protoxylem. All scale bars 50 µm unless otherwise noted. 2. Closer view of pericycle tissue in Plate 1. Tailless arrows indicate intercellular spaces in all micrographs unless otherwise noted. Symbols same as Plate 1. Scale 20 µm. 3. Transverse cross-section of drained P. contorta root taken 6.0 cm from root tip; tailess arrows represent steler cavities. Cortical tissue has collapsed. 4. Flooded P. contorta root transverse cross-section 2.0 cm from root tip. Scale bar 100 µm. 5. Closer view of the stele in Plate 4. sc - steler cavities, px - protoxylem, v, initial development of vascular cambium. 6. Closer transverse view of cortical tissue from Plate 4. Scale bar 20 µm.

Plates 4.7 through to 4.11. 7. Transverse cross-section of drained P. banksiana root 2.0 cm from root tip. ed - endodermis, px - protoxylem, p - pericycle, ct - cortex. All scale bars 50 µm unless otherwise noted. 8. Transverse cross-section of wax-embedded drained P. banksiana root 3.0 cm from root tip. sc -steler cavities. 9. Transverse cross-section of flooded P. banksiana root 3.0 cm from root tip. v - vascular cambium, r - resin duct. 10. Transverse section of same root as Plate 9, but at 4.0 cm distance from root tip. 11. Transverse cross-section 1.0 cm from base of same root as in Plate 9. x - xylem tissue. Scale bar 150 µm.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steler air cavities as a percentage of the steler area showed little difference between treatments in P. banksiana and P. contorta (Table 4.3). Formation of these cavities in Pinus spp. seemed to be due to the lysing of pericycle cells in the layer of tissue parallel to the plane of initiation of 2° growth (Plates 4.5, 4.9 and 4.10). This allowed room for growth of secondary vascular tissue into the spaces created by these lysing cells. The steler cavities became filled with xylem tissue in the older, basal portions of the root (Plates 4.9, 4.10, 4.11). Steler air cavities were not observed in roots of P. mariana, P. glauca or L. laricina (Plates 4.12 - 4.23).

The creation of steler cavities in P. contorta has been presented as a root response to flooding stress (Coutts and Armstrong, 1976; Coutts and Philipson 1978). However, it could also be hypothesised that these cavities develop with the initiation of a vascular cambium. This latter hypothesis is supported by the fact that these steler cavities are soon filled with growing vascular tissue; the cavities then become useless for air transport. This would suggest that O_2 flow through these cavities is limited, since the growth of the vascular cambium will eventually prevent continuous flow of air into these cavities in any maturing pine root. Roots of all species from both treatments contained intercellular air spaces in the 1° cortex. However, under drained conditions, roots showed collapse of the primary cortex much closer to the apex than under flooded conditions. Collapse began to occur shortly after 2° development began in drained roots. Flooded roots, on the other hand, retained their cortical layers after endodermal suberization and onset of 2° growth (Plates 4.1, 4.4, 4.11, 4.16). Unlike the continuous intercellular air spaces found in the cortex of Oryza sativa ("columnar aerenchyma") (Ando et al., 1983) and other wetland plants (Armstrong, 1979; Conlin, 1986), cortical intercellular air spaces in P .banksiana and P. contorta, P. glauca and P. mariana and L. laricina were not continuous and did not extend to the root apical meristem.

Intercellular air spaces were also observed in the pericycle adjacent to the plane of developing 2° vascular cambium and in the pericycle of older roots with 2° development (Plates 4.2, 4.7, 4.13, 4.17, 4.19, 4.21, 4.22 and 4.23). Pericycle tissue also did not have continuous air spaces. However, pericycle tissue was continuous throughout the length of the roots and would seem the most likely tissue for O_2 transport throughout the length of conifer roots.

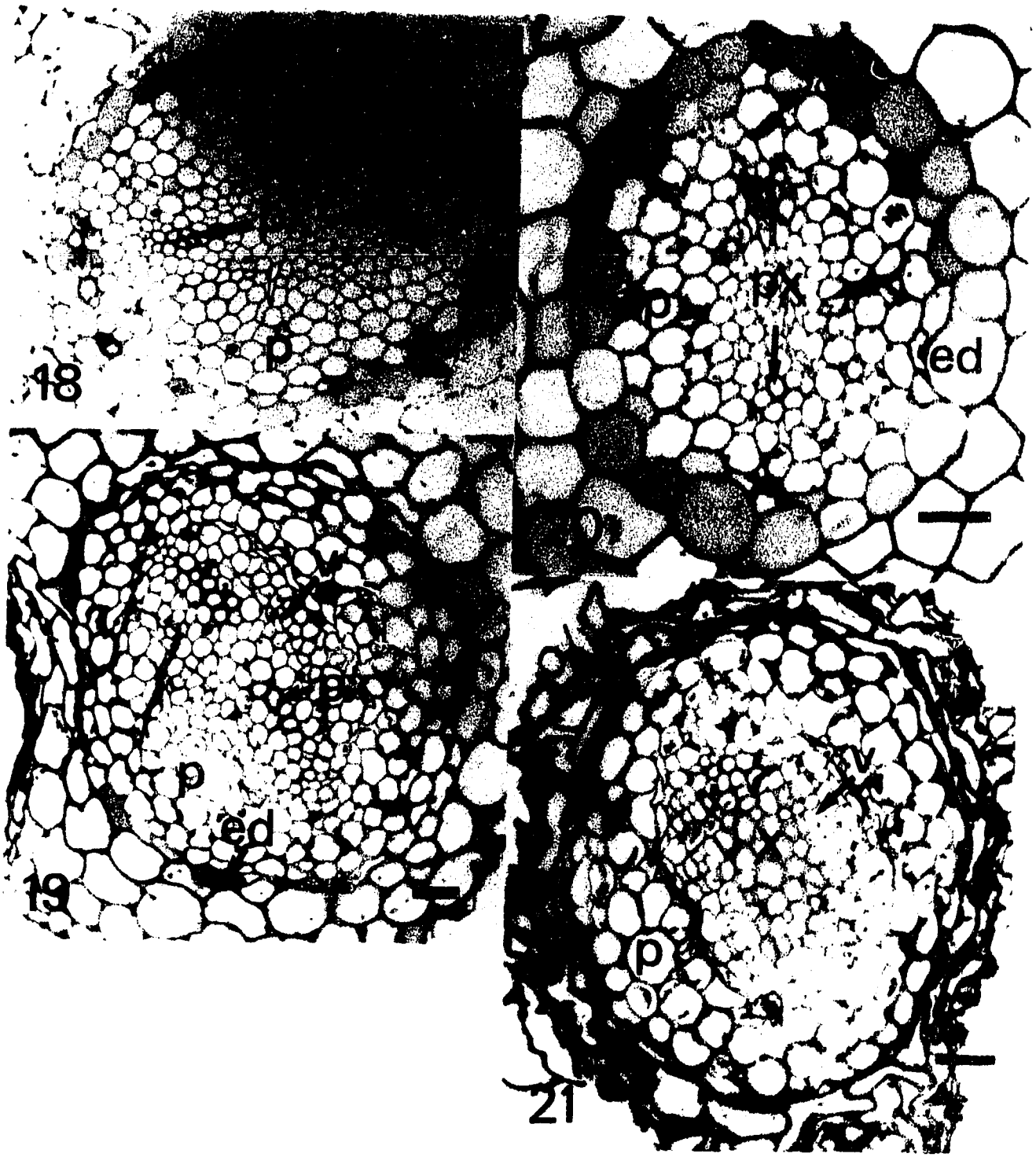
Comparison of flooded spruce and pine roots with drained roots showed evidence that the development of flooded roots were retarded in our experiment. These included differences in distance from the quiescent centre to initiation of vascular cell elongation, pericycle elongation, cortical cell elongation and endodermis formation (Table 4.4). This retardation of 2° development could influence the diffusive transport of O_2 down the roots of these species. For example, if the roots of the Pinus spp. are delayed in their anatomical development, this could suspend the progression of 2° growth and allow the roots to retain their steler air cavities. Retention of the 1° cortex would also ensure a direct tissue connection from the base of the root to the growing apices. Mangrove roots do not show 2° growth after they penetrate flooded substrate. Rhizophora mangle tend to keep a porous cortical layer that facilitates air flow from larger, woody roots above the flooded layer (Gill and Tomlinson, 1977).

Table 4.3. Average area of steler cavities as percent of total stele area in Pinus contorta and Pinus banksiana. Root sections were taken from the third and fourth cm root segments and samples contain material from both experiments. Standard error given in brackets and n = sample size.

Species	n	Flooded	n	Drained
P. contorta	5	20.1 (1.9)	5	19.7 (7.0)
P. banksiana	5	14.9 (2.8)	5	18.3 (1.9)

Plates 4.12 through to 4.17. 12. Transverse cross-section of drained P. mariana root stele 2.0 cm from root tip. ed - endodermis, px - protoxylem, p - percycle, ct, cortex. All scale bars 50 µm unless otherwise noted. 13. Closer transvers view of the pericycle-endodermis-cortical region 2.0 cm from drained P. mariana root tip. Scale is 10 µm. 14. Transverse cross-section of drained P. mariana root base. Remenants of collapsed cortical tissue are seen around the periphery of pericyle. 15. Transverse cross-section of flooded P. mariana root 2.0 cm from root tip. 16. Transverse cross-section taken from base of flooded P. mariana root. 17. Closer view of transverse cross-section of vascular cambium-pericycle-endodermis-cortex in 16. v - vascular cambium. Scale is 20 µm.

Plates 4.18 through to 4.21. 18. Transverse cross-section of flooded P. glauca stele 2.0 cm from root tip. ed - endodermis, px - protoxylem, p - pericycle. All scale bars 50 µm. 19. Transverse cross-section root base of flooded P. glauca showing the stele. v - vascular cambium. 20. Transverse cross-section of drained P. glauca stele. 21. Transverse cross-section of drained P. glauca root base. Remenants of collapsed cortical tissue are seen around the periphary of pericycle.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Plates 4.22 and 4.23. 22. Transverse cross-section of drained L. laricina root base. ed - endodermis, x - xylem, v - vascular cambium, p - pericycle. All scale bars 50 µm. 23. Transverse cross-section of flooded L. laricina root base.

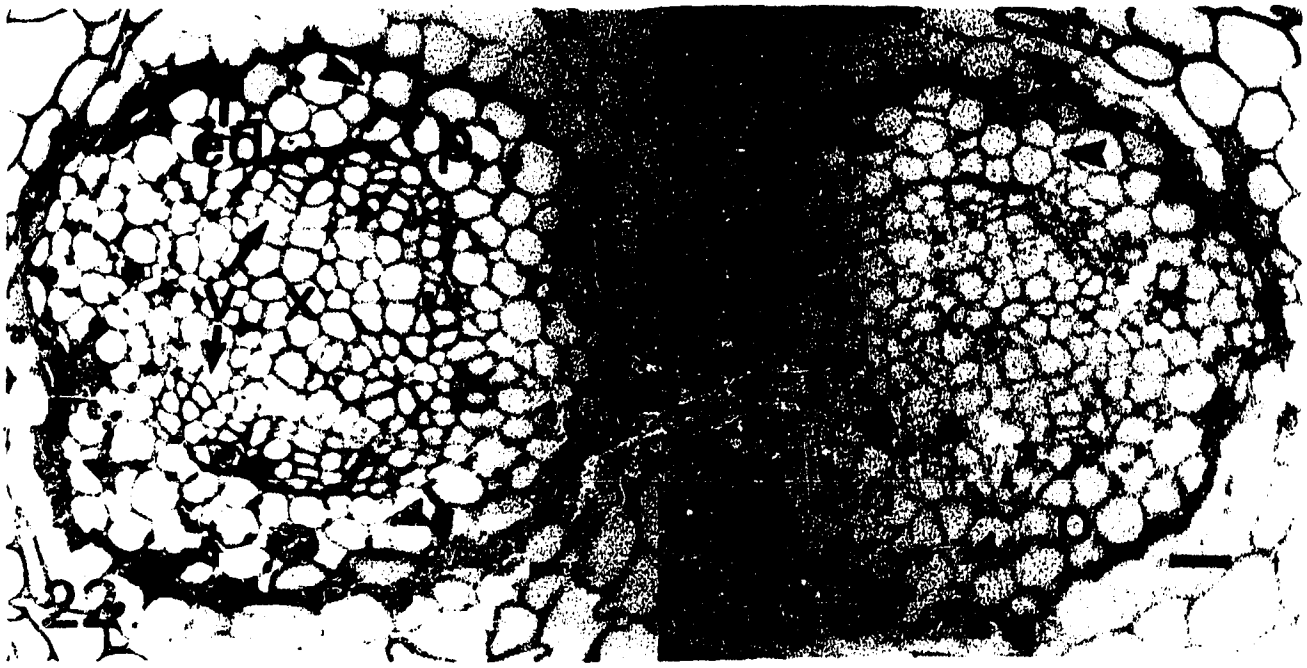
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Table 4.4. Measurements of distance from quiescent centre to start of endodermal formation, vascular tissue elongation, pericycle cell elongation, cortical cell elongation and protoxylem formation in P. contorta and P. banksiana and P. mariana and P. glauca. All measurements in millimeters. Standard error in brackets denotes a sample size of two to three roots. Numbers without a standard error represent a single measurement only. F - flooded treatment, D - drained treatment.

	Endodermal Formation	Vascular Cell Elongation	Pericycle Cell Elongation	Cortical Cell Elongation	Protoxylem Begins
			Pinus contorta		
F	1.454 (0.154)	0.667 (0.173)	0.939 (0.216)	0.727 (0.187)	0.953 (0.389)
D	0.238 (0.0)	0.113 (0.004)	0.137 (0.013)	0.250 (0.093)	1.874 (0.989)
			Pinus banksiana		
F	1.136 (0.096)	0.159 (0.016)	0.295 (0.048)	0.795 (0.048)	1.309 (0.186)
D	0.574 (0.237)	0.069 (0.015)	0.144 (0.026)	0.413 (0.158)	2.636
			Picea mariana		
F	0.576 (0.069)	0.318 (0.077)	0.394 (0.069)	0.651 (0.146)	2.561 (0.774)
D	0.282 (0.049)	0.237 (0.072)	0.284 (0.054)	0.609 (0.146)	2.773 (0.079)
			Picea glauca		
F	0.318 (0.032)	0.218 (0.006)	0.341 (0.016)	0.955 (0.096)	2.727
D	0.280 (0.139)	0.143 (0.017)	0.143 (0.017)	0.244 (0.055)	2.000

Endodermal formation occurred at a much greater distance from the quiescent centre in flooded roots than in drained roots. Mean distance of endodermis formation corresponded to mean distance of cortical cell elongation under drained conditions in the pines and white spruce. Roots of Pinus spp. from flooded treatments showed endodermal formation which lay behind cortical cell elongation, while endodermal development occurred before cortical cell elongation in flooded P. glauca and with cortical cell elongation in flooded P. mariana (Table 4.4).

Only L. laricina showed staining of a casparian strip on the inner face of endodermal cells (Plates 4.22 and 4.23). The pines and the spruces, on the other hand, showed complete suberization of entire endodermal cells very early in their root development. The casparian strips of all species from both treatments stained with both toludine blue and sudan black. L. laricina differed from the other species in that it showed very little ability to incorporate toludine blue into cell walls of both endodermal and xylem tissues.

Differences were also noted in the number of cortical cell layers from flooded and drained treatments (Table 4.5). Flooded roots had a higher mean number of cortical cell layers than did drained roots. However, cortical cell sizes (length and width) were the same in both treatments. The larger number of cortical layers resulted in a tumid appearance to flooded roots when compared to drained roots. Primary tissues of flooded roots also tended to be more brittle than that of drained roots. Swollen, brittle roots develop in response to flooding in Nyssa sylvatica (Keeley, 1979).

Yamamoto et al. reported that P. halepensis stem tracheids from flooded plants were rounded in comparison shape to those seen in drained plants. These rounded tracheids created intercellular air spaces between the tracheid cells. My results show no rounding of tracheid elements in flooded roots of any of the species, nor is their any indication that any intercellular air spaces are formed between tracheids in response to flooding.

Although most root x-sections showed a diarch development of secondary vascular tissue, triarch growth was observed in P. contorta and P. banksiana from flooded and drained treatments. Our observations differed from the observations made on the roots of Pseudotsuga menziesii (Mirb.) Franco, which showed diarch development restricted to lateral roots and triarch and tetrarch development restricted to primary roots (Bogar and Smith, 1965). All secondary vascular meristem development in Picea spp. and L. laricina roots were diarch.

Amyloplasts were found in both the root cap column and in the pericycle cells of all species from drained and flooded treatments. Treatment did not have an effect upon the presence or absence of amyloplasts in root tissues.

Summary

The results of my experiments showed no definitive anatomical adaptations (aerenchyma formation) to flooding by roots of P. banksiana, P. contorta, P. glauca, P. mariana and L laricina. Steler air space cavities were found in roots of both pine species from flooded and drained treatments, while intercellular air spaces were found in the cortex and

Table 4.5. Average number of cortical cell layers from the apical root segment. Samples contain material from both experiments. Standard error is given in brackets and n = sample size.

	n	Flooded		n	Drained	
P. banksiana	3	5.7	(0.5)	3	3.7	(0.5)
P. contorta	3	6.7	(0.3)	3	3.7	(0.3)
L. laricina	3	5.3	(0.3)	3	4.7	(0.3)
P. glauca	3	6.0	(0.5)	3	3.7	(0.3)
P. mariana	3	6.3	(0.3)	3	5.3	(0.3)

pericycle tissue of roots of all species grown under both treatments. Tracheid shape in the transverse plane were not affected by flooding, as reported for other pine species, and no inter-tracheid air spaces were observed. Measurements of percent air space tissue in roots from flooded and drained treatments using the pycnometer method corroborated the results of the anatomical studies.

I hypothesized that the creation of steler cavities in pine roots has more to do with the development of the vascular cambium and the growth of 2° tissue than it does with the flow of air into the root. This hypothesis is reinforced by the observations that steler cavities become filled with 2° tissue as roots matured and that cortical and pericycle air space tissues are not continuous with the root meristem.

Flooded roots do show features which indicate that root growth and development is retarded by flooding. These features include a shorter root length and regions of root cell differentiation and elongation which take place at a greater distance from the quiescent centre in flooded roots than in drained roots. Retardation of 2° development may confer an advantage to pine 1° roots by prolonging the lifespan of stelar cavities, thus maintaining them as air channels to respiring root tissues. However, this advantage would be of little use to woody roots. Delay of 2° growth may also prevent the loss of 1° cortical tissue in all the species examined in this experiment, thereby providing a continuous tissue through which air might diffuse to growing apices.

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CHAPTER FIVE

TRANSLOCATION OF ^{14}C TO ROOTS OF CONIFERS EXPOSED TO ANOXIA AND COLD TEMPERATURES.

Introduction

Oxygen diffusion has been observed from root surfaces of Pinus contorta, indicating that internal O_2 transport occurred in this species (Armstrong and Read, 1972; Coutts and Philipson, 1978; Philipson and Coutts, 1978). The results of these experiments indicated that it was possible for small seedlings to survive soil anoxia via cortical O_2 diffusion, but did not show what happened in intact older seedlings with longer roots or roots with secondary tissues. Philipson and Coutts did show oxygen diffusion from woody root segments cut from trees. However, severing the roots may have obviated the interference that stem tissues would have had upon movement of O_2 from the atmosphere to roots, or, could have killed root tissues and allowed oxygen normally used in respiration to diffuse from root surfaces, or, may have altered any variable barrier to O_2 diffusion like those found in root nodules of legumes (Streeter, 1993).

Species known to have efficient internal O_2 transportation also showed increased root porosity after plants were grown in flooded soil (Smirnoff and Crawford, 1983; Justin and Armstrong, 1987). In contrast, Levan and Riha (1985) showed no increase in root porosity of several North American conifer species grown in flooded soils. My work (Chapter four) also showed no root porosity increase in P. contorta, Pinus banksiana, Picea mariana, Picea glauca and L. laricina grown in flooded soil. However, chapter four also showed that roots of P. contorta and Pinus banksiana contained stelar spaces which might be conduits for O_2 diffusion and Conlin and Lieffers (1993) showed O_2 diffusion from basal regions of P. contorta, P. banksiana and L laricina root surfaces. These spaces were found in roots from flooded and drained treatments and therefore did not appear to be adaptations to flooding. No surface diffusion was observed in roots of Picea mariana or Picea glauca.

Thus the roots of both pine species showed evidence of non-adaptive aerenchyma while the spruces and L. laricina did not. The pines and L. laricina also demonstrated evidence of internal O_2 diffusion, while the spruces did not. Ecologically, both pine species are restricted to drained sites while Picea mariana and L. laricina are found in flooded areas (Van Cleve et al., 1983; Corns and Annas, 1986; Lieffers and Rothwell, 1987). This conflicting information makes it difficult to determine whether O_2 transport plays a role in the survival of conifer roots exposed to soil anoxia, especially during spring and summer inundations seen in peat fens of northern Alberta.

The interruption of translocation of photoassimilated ^{14}C to anoxic organs and through anoxic stems and petioles has been demonstrated by several authors (Sij and Swanson, 1973; Geiger and Sovonick, 1975; Fensome et al, 1984). Decreased transport of ^{14}C to roots after exposure to anoxia has also been reported and it has been hypothesized that this is a response to lack of internal O_2 transport to roots (Nuritdinov and Vartapetyan, 1980; Schumacher and Smucker, 1985). It has also been shown

by Webb and Armstrong (1983) and Waters et al. (1991a) that growth and viability of roots are prolonged under anoxia when an exogenous supply of glucose are added to the culture medium. These authors concluded that anoxia reduced the flow of carbohydrate through the phloem and that added glucose partially replaced a shortfall in translocated sugar.

I decided to use ^{14}C to test the translocation response of intact P. banksiana, P. contorta, P. mariana, P. glauca, and L. laricina exposed to anoxic rooting media. I expected to see translocation maintained in roots of pines exposed to anoxia, which showed evidence of possible internal O_2 diffusion, but not the spruces, which showed no evidence of internal O_2 diffusion. Uninhibited transport to anoxic roots would support the hypothesis that internal O_2 diffusion sustains respiration linked translocation (Nuritdinov and Vartapetyan, 1980; Webb and Armstrong, 1983; Schumacher and Smucker, 1985; Waters et al., 1991a).

Materials and Methods

Plant Culture

Seeds of Pinus banksiana, Pinus contorta, Picea glauca, Picea mariana and Larix laricina were germinated in autoclaved 2:2:1 sand-terralite-peat mixture in flats. After emergence of cotyledons, seedlings were transplanted to a hydroponic system described in Conlin and Lieffers (1993).

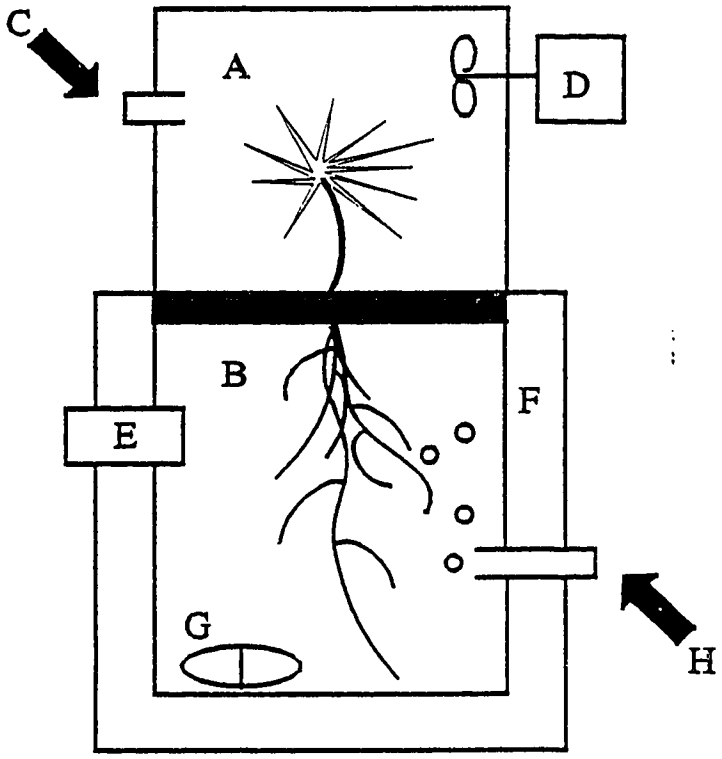
Experimental Apparatus

A robust seedling of approximately 6 weeks age (8 weeks for black spruce) was selected and its stem embedded in terrostat putty and mounted in a split rubber cork. The cork with its plant was mounted in a two chamber airtight Plexiglas cuvette containing nutrient solution in the lower chamber (Figure 5.1). Temperature of the nutrient solution was maintained at 5 or 12°C by a surrounding water jacket which was connected to a Haake F3-C digital Refrigerated Bath and Circulator. Nutrient solution temperature was monitored using a copper-constantan thermocouple. A stream of nitrogen gas or industrial air (21% O_2 and 79% N_2 was constantly bubbled through the nutrient solution in order to make the solution anoxic or oxic. Solution anoxia was monitored using a YSI oxygen probe. Gentle circulation of the cuvette solution was accomplished through the use of a magnetic stirrer.

Seedlings were exposed to ^{14}C by introducing 2.5 µCi of $^{14}CO_2$ into the upper chamber. The $^{14}CO_2$ was created by reacting ^{14}C-sodium carbonate (Nordion International Inc.) with HCl inside a syringe and pumping the resulting gas into the upper chamber through a rubber septum. The upper chamber was illuminated with 1200 µmoles m^{-2} s^{-1} of photosynthetically active radiation.

Each seedling was maintained under one of four treatment combinations for 22 hours. Immediately following treatment,
the roots of each seedling were rinsed in deionized water and the seedling was divided into foliage, stem, roots andterminal 2 cm of root including apices, dried at 60°C and weighed. Samples were then prepared for scintillation counting using a Harvey Biological Materials Oxidizer.

Figure 5.1. Schematic of experimental apparatus. A, upper Plexiglas chamber enclosing seedling foliage; B, lower chamber enclosing nutrient solution and roots; C, $^{14}CO_2$ injection port; D, electric motor and impeller which mixed upper chamber atmosphere; E, oxygen probe; F, water jacket which controlled root temperature; G, stirring bar used to circulate nutrient solution; H, airline which bubbled N_2 or O_2 gas through nutrient solution.


C
A
D
B
F
E
G
H

Experimental Design and Analysis

Seedlings were subjected to a factorial treatment combination of oxic or anoxic (gas treatment) nutrient solution at either 5 or 12°C temperature (temperature treatment). Each treatment combination was replicated three times. Analyses of transport of ^{14}C to roots were based upon ^{14}C activity of root tissue as a percent of total plant activity.

The results were analyzed statistically, after transforming percentages using arc sin square root, using a two-way analysis of variance on each species for temperature x oxygen effects and three-way analysis of variance of species x temperature x oxygen effects.

Percent activity of root tips was plotted against dry weight of the root tip over dry weight of the rest of the root mass. This was done in order to determine if there were any noticeable effects of the total root mass upon translocation into apices.

Results

Picea mariana showed a large drop in percent activity after exposure to anoxia when compared to the oxic treatment.
Pinus contorta also showed some decrease in activity after being subjected to anoxia (Figure 5.2). Two-way analysis of variance demonstrated that P. contorta was the only species to show a significant gas x temperature interaction; at 5°C the
anoxic treatment had very low translocation compared to the oxic treatment. Pinus contorta, Picea glauca and Larix laricina did not show significant differences between the treatments or significant interactions.

Three-way analysis of variance indicated a borderline significant (0.07) response to temperature increase over all species (Table 5.1). Even more significant were the translocation differences between species ($p<0.01$). Overall, Pinus banksiana showed a higher rates of translocation than that seen in the spruces and tamarack.

There were no significant differences between treatments in transport of ^{14}C to root tips even after correcting for differences in total root mass.

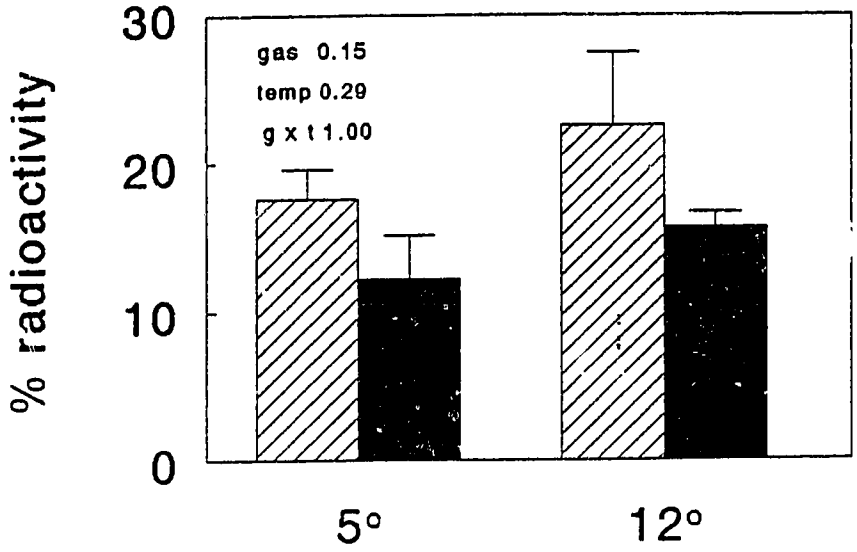
Discussion

The hypothesis that translocation will be maintained under anoxia only in those species with internal O_2 transport does not appear to hold true for all species. For example, P. contorta showed reduced translocation under anoxia, while at 12°C there was little difference in translocation between anoxic and oxic conditions. Decreasing temperature leads to reduced root respiration and O_2 consumption in conifers (Armstrong and Read, 1972; Weger and Guy, 1991). This is manifested as increased O_2 diffusion from roots of P. contorta and other species, presumably because internal diffusion sources supply O_2 in surplus to respiratory needs (Armstrong and Read, 1972; Ando et. al., 1983). Thus, roots of P. contorta should have been better supplied with O_2 at 5°C than at 12°C and should have been able to maintain translocation. The fact that it could not suggests that O_2 does not play a major role in translocation in this

Figure 5.2a and b. ^{14}Carbon activity within roots of Pinus banksiana and P. contorta as a percentage of total plant radioactivity under oxic (stippled bar) and anoxic conditions (solid bar). Probability of F is given for each treatment factor and the interaction.

a)

Pinus banksiana



b)

Pinus contorta

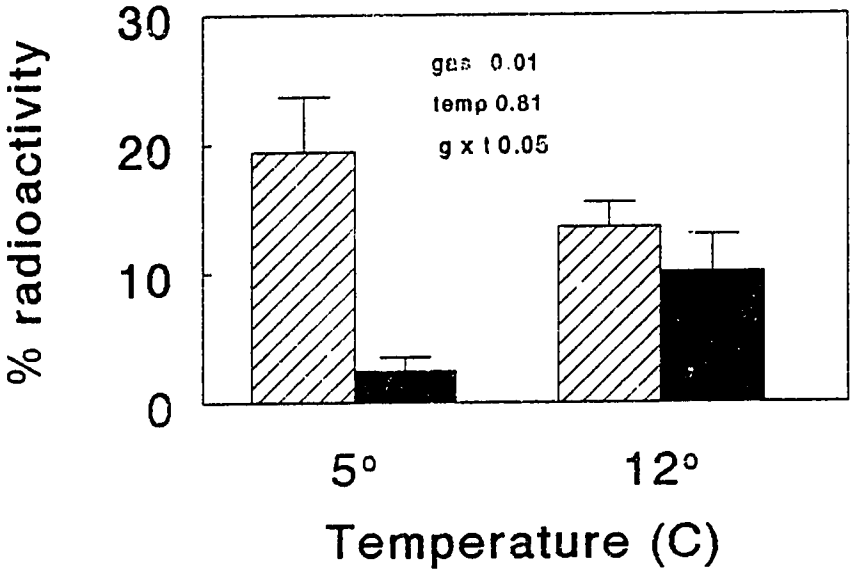
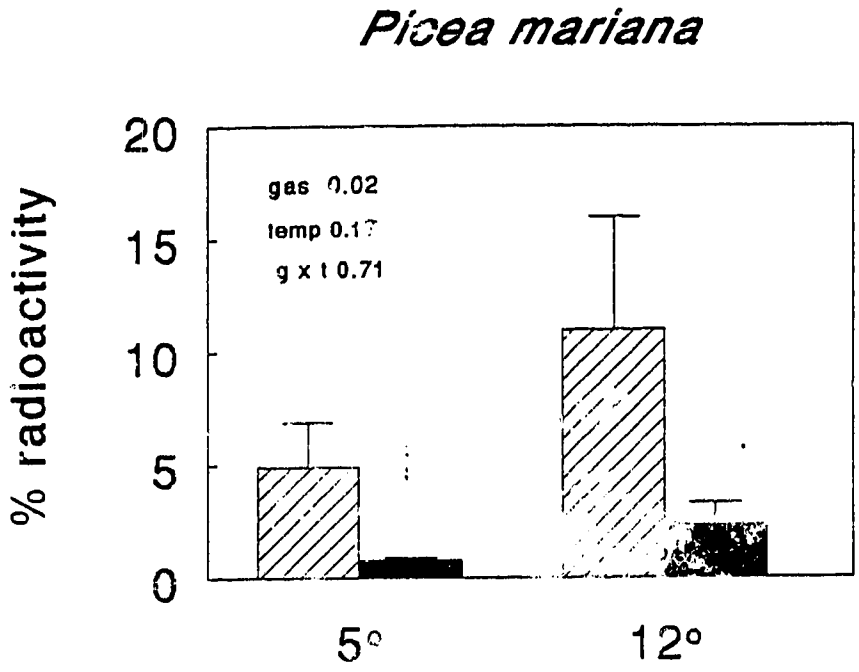


Figure 5.2c and d. ^{14}Carbon activity within roots of Picea mariana and P glauca as a percentage of total plant radioactivity under oxic (stipple bar) and anoxic conditions (solid bar). Probability of F is given for eac treatment factor and the interaction.

c)


Picea mariana
20
15
10
5
0
% radioactivity
gas 0.02
temp 0.1?
g x t 0.71
5°
12°

d)

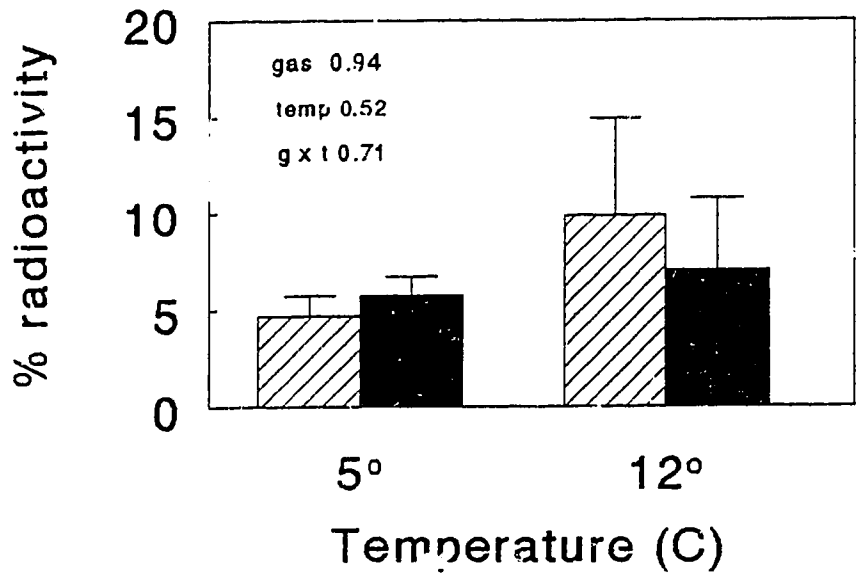

Picea glauca
20
15
10
5
0
% radioactivity
gas 0.94
temp 0.52
g x t 0.71
5°
12°
Temperature (C)

Figure 5.2e. ^{14}Carbon activity within roots of Larix laricina as percentage of total plant radioactivity under oxic (stippled bar) a anoxic conditions (solid bar). Probability of F is given for ea treatment factor and the interaction.

e)

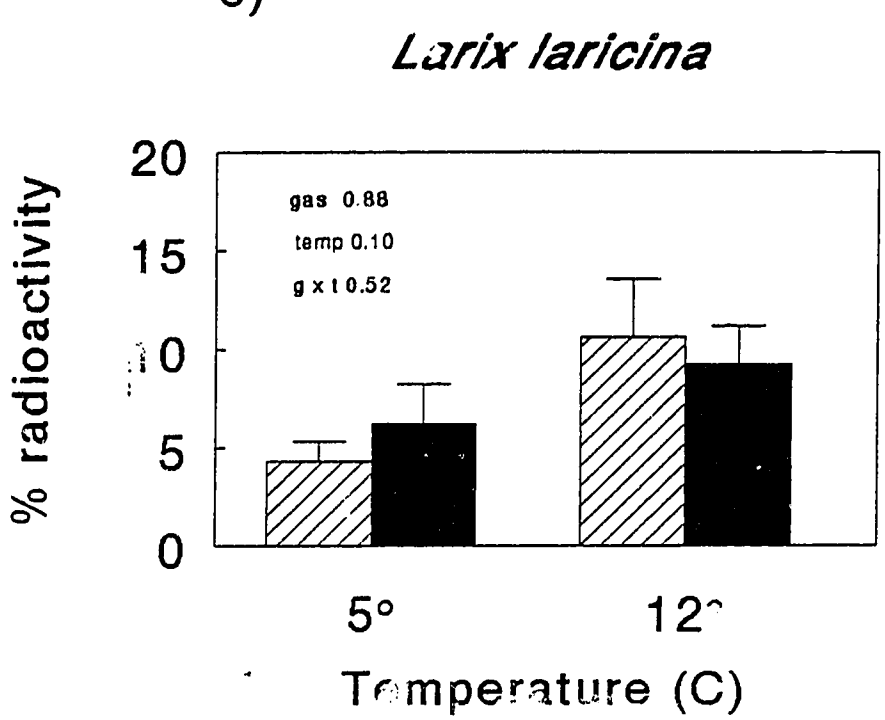

Larix laricina
% radioactivity
20
15
10
5
0
gas 0.88
temp 0.10
g x t 0.52
5°
12°
Temperature (C)

Table 5.1. Three-way analysis of variance table for all five species.

	df	ss	ms	f	sig. of f
gas	1	436.43	436.43	12.23	.00
temperature	1	122.14	122.14	3.42	.07
species	4	1241.92	310.48	8.70	.00
gas x temp.	1	21.75	21.75	0.61	.44
spp. x gas.	4	443.94	110.98	3.11	.03
spp. x temp.	4	72.45	18.11	0.51	.73
sp. x gas x temp.	4	330.49	82.62	2.32	.07
within	39	1391.44	35.68		
total	58	4060.56			

species. Instead, the predominant influence controlling translocation in P. contorta may be temperature.

A possible explanation for no difference between anoxic and oxic treatments in P. contorta at 12°C may originate in Armstrong and Beckett's (1987) hypothesis that stelar anoxia enhances O_2 transport to growing tissues in roots. However, their model of internal aeration of root tissues is based upon the assumption that air diffusion passages are found in the cortical tissues, resulting in aerated cortex, and that the endodermis acts as a barrier to O_2 diffusion into the stele. The only evident and continuous diffusion pathways in roots of P. contorta are restricted to the pericycle (Coutts and Philipson, 1978; Chapter four). These pathways should ensure that cortical anoxia occurs before steler anoxia, a feature of root aeration not taken into account by Armstrong and Beckett.

Picea mariana also showed reduced translocation of ^{14}C under anoxic conditions. This was expected since P. mariana had not shown evidence of root aerenchyma (see Chapter four) or evidence of internal diffusion of O_2 into its roots (Conlin and Lieffers, 1993). The other three species did not show any statistically different ^{14}C translocation rates. This was an unanticipated response for P. glauca because it had not shown root aerenchyma and evidence for internal [illegible] of O_2. The results seen in Larix laricina were also unexpected [illegible]d also shown no aerenchyma and less rhizospheric oxidation than [illegible] (Chapter four), indicating reduced internal O_2 diffusion. Pinus [illegible]a translocation rates under both treatments were similar. This was [illegible]ted because the presence of air space tissue in the stele of 1° root tissue and the rhizospheric oxidation seen in roots of this species indicated internal O_2 diffusion.

It could be argued that cessation of growth by roots of P. mariana and P. contorta and the resulting reduction in sink force was interpreted as diminished translocation under anoxia. However, the existing consensus (Geiger and Sovonick, 1975; Webb and Armstrong, 1983; Armstrong and Beckett, 1987; Thomson et al, 1989; Waters et al., 1991a; 1991b) supports the hypothesis that growth is reliant upon a carbohydrate supply via the phloem, which is sensitive to O_2 supply, or upon available carbohydrate reserves in respiring tissue.

If it is assumed that ^{14}C activity is proportional to the amount of photosynthate moving to roots, it would appear that the pines have higher aerobic rates of translocation than the spruces or L. laricina. This is contrary to expectations based upon Conlin and Lieffers (1993) findings showing that L. laricina had greater rates of respiration at 5°C than either pine species. These rates should have been correlated with larger translocation activity (Hatrick and Bowling, 1973). In addition, the limited evidence examining carbon flow to fermentative metabolism indicates that translocation is not enhanced by the Pasteur effect (Thomson et al., 1989) and the results in this chapter suggest that this is the case for these conifer species.

The results contained in this chapter suggest translocation within roots could be motivated by forces other those directly linked to the internal transport of O_2 and its sustenance of respiration. These forces may be governed by response of root carbohydrate metabolism to temperature (Marshall and Waring, 1985; Delucia, 1986), anoxia (Barclay and Crawford,

1983; Raskin and Kende, 1984; Waters et al., 1991b), apoplastic versus symplastic unloading of carbohydrate from phloem (Oparka, 1986; Lemoine et al., 1988) or energy dependent phloem transport mechanisms (Ford and Peel, 1967; Coulson and Peel, 1971). Investigation of these aspects of carbohydrate translocation should be undertaken in order to determine the physiological role of oxygen diffusion in roots of conifers as it pertains to flooding tolerance.

Summary

A combination of low temperature and anoxia in solution culture caused a significant decrease in percent translocation of ^{14}C to Pinus contorta roots. Increasing the solution temperature eliminated this reduction in translocation. Anoxia, but not low temperature, decreased translocation in Picea mariana roots. Neither anoxia or an interaction of anoxia and low temperature reduced translocation in Pinus banksiana, Picea glauca, or Larix laricina. Although examples exist where shoot-root carbohydrate translocation is halted by rhizospheric anoxia, the results of this chapter demonstrated that this is not a universal phenomenon. This chapter also showed that apparent internal diffusion of O_2 within the root does not guarantee sustenance of translocation during periods of solution anoxia, or that it is necessary for translocation under anoxia. Other factors, such as cold soil temperatures in combination with anoxia may be equally important in influencing translocation of carbohydrate to roots.

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CHAPTER SIX

SEASONAL GROWTH OF *Picea mariana* AND *Larix laricina* ROOTS*

Preamble to Chapter Six

I felt that a study of root growth of *Picea mariana* and *Larix laricina* under field conditions was needed to compliment my lab and greenhouse experiments. This study would indicate whether root growth took place during the season when soils were warm and drained or during periods when soil conditions seemed less then ideal. If root growth occurred only under the most ideal conditions, then all the adaptive features thought to confer flooding tolerance to these species may be in place to simply help these trees survive flooding, not to help them actively exploit flooded soil habitat. Given the literature on flooding tolerance, this would indicate that survival of flooding by these plants should be based on metabolic adaptations rather than on anatomical adaptations. The latter maintain root respiration in flooded soils and promote active growth while rooted in anoxic soils.

Introduction

Measurements of seasonal root growth have been made on various conifer tree species. However, these studies have been restricted to nurseries (Langlois et al., 1983), plantations (Deans, 1979), arboretums (Tryon and Chapin, 1983) or well drained, mineral soil habitats (Persson, 1978; 1983; Tryon and Chapin, 1983). Little work has been done on seasonal root growth of species found on natural peatlands.

Picea mariana and *Larix laricina* are the dominant tree species found on boreal peatlands (Lieffers and Rothwell, 1987a). Lieffers and Rothwell (1987b) showed that in peatlands these species have root systems which are restricted in depth of penetration by height of the water table in peatlands. Since the water tables in these peatlands are often near the soil surface, it could be inferred that seasonal root growth of these species is also influenced by fluctuations in the water table. However, Tryon and Chapin's (1983) data suggest that seasonal pattern of growth of Alaskan lowland black spruce roots and their distribution in the soil profile are also governed by soil temperatures.

Greenhouse experiments showed that root:shoot ratios were greater in *P. mariana* and *L. laricina* grown in peat under combined high water table and low temperatures (median value of 9°C) than when they were grown with a high water table and high temperatures (median value of 18.5°C)(Lieffers and Rothwell, 1986). Lieffers and Rothwell (1986) thought that these larger root:shoot ratios could be attributed to measured increases in O_2 diffusion rates into the cold peat. However, it could also be hypothesized that metabolic adaptations or internal O_2 diffusion were also responsible for higher root:shoot ratios. Internal O_2 diffusion promotes root growth in anoxic soils (Philipson and Coutts, 1978), whereas metabolic adaptations do not (Coutts and Armstrong,

* A version of this manuscript has been published in the 1993 issue of the Canadian Journal of Botany, Vol. 71:359-360.

1976). In addition, literature on O_2 diffusion in roots suggests that cold soils should be ideal for species which depend upon internal diffusion to support respiration (Armstrong and Read, 1972; Philipson and Coutts, 1978; Ando et al., 1983). Therefore, cold peatland soils should facilitate O_2 diffusion throughout roots and as a consequence promote root respiration and growth. Growth of roots in peatlands during periods of high water table and low soil temperature could be taken as evidence for internal O_2 transport in roots of P. mariana and L. laricina. Conversely, the lack of root growth in the presence of a high water table and cold soil temperature could seen as evidence for the lack of internal O_2 diffusion in these species.

This chapter reports on the phenology of root growth of L. laricina and P. mariana during the 1989 growing season. In it I discuss possible influences of soil temperature and soil flooding upon growth in these species.

Materials and Methods

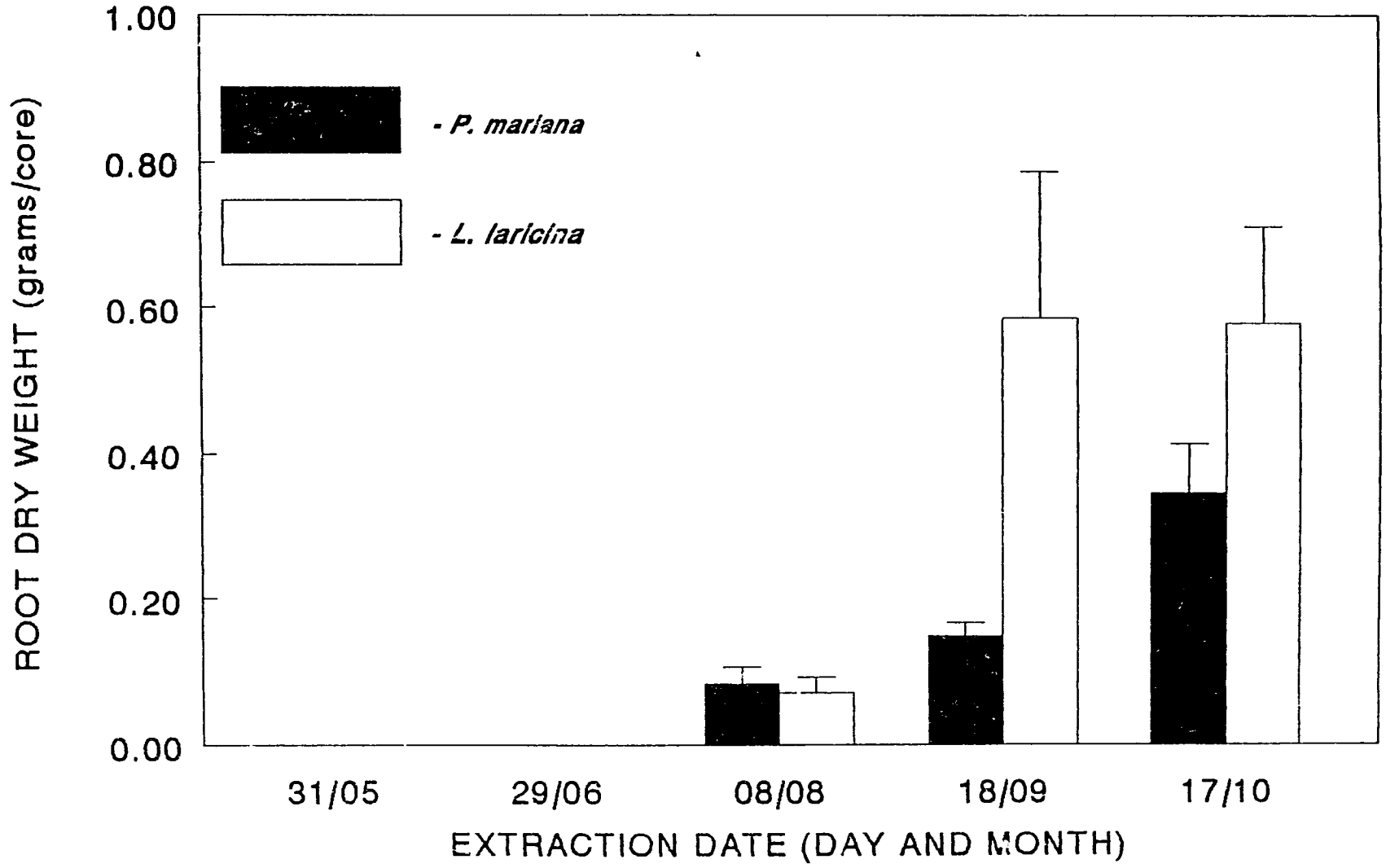
This experiment was initiated during late October, 1988. At this time ten P. mariana trees and ten L. laricina trees were selected in the undrained section of the Saulteaux River study site (Lieffers and Rothwell, 1987a; 1987b) in the province of Alberta, Canada (55° 3' N; 114° 15' W). This site is characterized by an open canopy P. mariana - L. laricina forest (mean dbh and height are 4.03 cm and 2.2 m and 5.38 cm and 4.3 m, respectively) with a Betula pumila L. shrub layer and ground cover composed of Carex spp., Ledum groenlandicum Oeder, Equisetum fluviatile L. and Andromeda polifolia L. Estimation of seasonal root growth activity was based upon a method used by Persson (1983). In October 1988, five holes 30 cm deep and 15 cm in diameter were bored in the surrounding peat approximately 1 meter from the trunk of each tree (0.8 to 1.2 meters range in distance, due to the hummocky nature of the peat). The corer used to bore the holes was constructed from a 50 cm length of 15 cm diameter steel pipe fitted with a piece of band saw at one end. The core from each hole was discarded and the hole repacked with 5.3 dm^3 of wet, milled peat.

Cores were lifted sequentially from around each of the trees at a rate of one core every four to six weeks during the 1989 growing season and new roots which had grown into these cores were separated from the peat, dried and weighed.

Results and Discussion

No conifer roots were found in the cores extracted in late May and June (Figure 6.1). Roots of P. mariana and L. laricina began to appear in peat cores during the third extraction date in early August. The average dry weight for both species increased for the next two extractions in mid-September and October. Root mass of L. laricina increased more rapidly than P. mariana and reached a maximum mean dry weight of about 0.6 grams $core^{-1}$ in the September sample. Picea mariana average root weight continued to increase through September and into October. This data suggests that L. laricina roots had ceased growing by September, while P. mariana roots continued growth during this month.

Figure 6.1. Mean root mass (gram dry wt. of roots per 5.3 dm^3 core) of *Picea mariana* and *Larix laricina* over the 1989 growing season. ($\bar{x} \pm$ s.e.; n=10).


1.00
0.80
0.60
0.40
0.20
0.00
ROOT DRY WEIGHT (grams/core)
- P. mariana
- L. laricina
31/05
29/06
08/08
18/09
17/10
EXTRACTION DATE (DAY AND MONTH)

All roots were found in the top 5.0 cm of the peat cores.

Lyr and Hoffman's (1972) review of tree root growth and Deans' (1979) work on Picea sitchensis showed that low soil temperature and unfavourable soil moisture (too dry or too wet) are two major factors which limit root growth of trees. Historically, soil temperatures recorded at 10 cm depth at the Saulteaux river site show a peak in mean values late in July and early August (Lieffers and Rothwell, 1987a; Lieffers, 1988; Rothwell and Silins, 1990). Although it is not possible to correlate root growth in this study to past observations of soil temperature trends, the fact that growth did take place during a period when the soil appears to be warmest could be used as a basis for further investigation of temperature as a factor determining phenology of root growth in these species.

This site experienced soil flooding during the 1989 season, with the water table fluctuating between -5 and -40 cm before dropping to a fluctuating level between -50 and -70 cm early in August and throughout September (Rothwell and Silins, 1990). High water tables encourage soil saturation and diffusion of oxygen into waterlogged soils is reduced. These low oxygen diffusion rates, when coupled with respiration of soil micro-organisms and roots, result in anaerobic soils (Drew, 1983). Low oxygen tensions have been shown to prevent root growth (Geisler, 1965; Atwell, 1985) and unless roots develop internal gas pathways, root growth will not occur (Coutts and Armstrong, 1976). Since root growth was correlated with a large drop in water table, this could indicate that internal diffusion was not involved in the growth of roots of P. mariana or L. laricina.

Summary

Root growth of Picea mariana and Larix laricina into peat cores did not begin until August of the 1989 growing season. Picea mariana showed continued root dry weight increase late into the growing season, while L. laricina dry weight stopped increasing after mid-September. Initiation of root growth in both species was correlated with a seasonal peak in soil temperature at -10 cm depth and a decrease in the water table level to at least -50 cm. The lack of root growth in both species during a period of high water table and low soil temperature may indicate that internal diffusion of O_2 to roots does not support root growth.

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CHAPTER SEVEN

CONCLUSIONS

The central issue of this thesis is confirmation of the processes which might allow survival of any one of the five conifer species under flooded conditions. Is the survival process a result of root respiration sustained through internal diffusion of O_2, is it the result of increased fermentation, or is it the result of several processes, anatomical and metabolic, functioning together? I attempted to answer this question by pursuing several avenues of investigation including a study of possible increase in root air space tissue in response to flooding and the metabolic responses of roots to anoxia.

My experiments investigating root anatomy showed little conclusive evidence to suggest that air space tissue forms in roots of Pinus contorta, Pinus banksiana, Picea mariana, Picea glauca and Larix laricina in response to flooding (see Chapter Four). No increase in porosity were seen in roots grown in flooded soils and microscopic examination of root anatomy showed no changes between flooded and drained treatments. Steler cavities, which have been described in the literature as anatomical adaptations to flooding in P. contorta, were seen in roots of both pine species grown in flooded and non-flooded treatments. These cavities were ephemeral in nature because they were quickly filled with xylem tissue as roots matured. I hypothesized that these cavities are linked to 2° tissue development because they were associated with appearance of 2° xylem tracheid.

Although no direct evidence suggested anatomical changes which would enhance internal O_2 movement in roots, use of redox dyes showed that internal O_2 diffusion did take place. For example, Chapter Three demonstrated diffusion of O_2 from root surfaces of P. contorta, P. banksiana, and L. laricina. Chapter Four also showed that flooded pine seedlings and L. laricina have the ability to root at a greater depth than do flooded spruces and this would be consistent with greater internal O_2 diffusion. I hypothesized that 2° growth was delayed in roots of flooded P. contorta and P. banksiana, thereby ensuring the continuity of steler cavities for gas diffusion.

Results of experiments measuring rates of anoxic and oxic CO_2 efflux rates at 5°C showed that P. mariana had a higher anoxic to oxic CO_2 efflux ratio than P. contorta, P. banksiana, P. glauca and L. laricina (see Chapter Three). Results also showed that L. laricina had the highest anoxic and oxic CO_2 efflux rates in comparison to the pines and spruces at this temperature. High anoxic to oxic CO_2 efflux ratios are features found in roots of plants which show a high tolerance to flooding and are indicative of high fermentation rates. High oxic CO_2 efflux rates are indicative of high respiration rates. High fermentation and respiration rates and a high fermentation to respiration ratio at 5°C are features which set L. laricina and P. mariana aside from the pines and P. glauca in Chapter Three. Elevated fermentation rates may confer a metabolic tolerance of flooding in P. mariana, while increased respiration rates coupled with internal O_2 diffusion may benefit L. laricina when exposed to waterlogged soils, especially in cold soils.

Anoxic nutrient solutions caused decreased translocation rates in the

P. contorta and P. mariana while no significant reduction was observed in P. banksiana, P. glauca and L. laricina. This was despite the external diffusion patterns seen in the pines and L. laricina and the absence of external diffusion observed in the spruces and reported in Chapter Three. Furthermore, although decreased translocation in P. mariana could be attributed to anoxia, the decreased translocation to roots of P. contorta occurred only at lower temperatures. These observations led me to hypothesize that the absence of rhizospheric O_2 has little effect on the transport of assimilated ^{14}C from shoots to roots in the pines, P. glauca and L. laricina. In addition, assimilate translocation in these species did not appear to be dependent upon the internal diffusion of O_2. Temperature, however, seemed to have a major effect upon carbon translocation, especially in P. contorta at 5°C. This could be attributable to a number of physiological factors that could influence translocation and should be studied more closely.

Finally, the results of the field study in chapter six showed a correlation between growth of roots of L. laricina and P. mariana and a drop in the water table with accompanying increases in soil temperature. This indicates that internal O_2 diffusion alone is not sufficient to promote root growth during periods of soil flooding in peatlands.

The results of my research lead me to conclude that tolerance to flooding in P. contorta, P. banksiana, L. laricina, P. glauca and P. mariana cannot be attributed exclusively to internal diffusion of O_2. Evidence suggests a metabolic adaptation to flooding in P. mariana through increased rates of fermentation. However, sustenance of this increased rate is problematic given the decreased assimilate translocation rates observed in this species after imposition of rhizospheric anoxia at 5 and 12°C. This species may rely upon stored carbohydrate in the roots to sustain fermentation. Decreased translocation at 5°C under anoxia in P. contorta implies that this species may also have difficulty in providing carbohydrate for fermentative metabolism in roots exposed to low soil temperature. This could be a factor determining the exclusion of this species from peatlands in the boreal forest. Low rates of fermentation may be a factor in exclusion of P. glauca and P. banksiana from flooded soils. Roots of L. laricina have high rates of fermentation and respiration which appear to be adaptations to low soil temperatures. This species also appears able to provide photosynthate to the roots for fermentative and respiratory metabolism under cold rooting temperatures. Together, these physiological characteristics may contribute to distribution of L. laricina on boreal peatlands. The observation of rhizospheric oxidation in this species indicates that it may be the only species of the five where internal diffusion of O_2 may be an adaptive feature.

If further studies were to be undertaken on the flooding tolerance of these conifers, they should concentrate upon the role of carbohydrate metabolism in these species. Both temperature and anoxia could play a key role in the regulation of energy metabolism in these conifers via carbohydrate metabolism. Design of experiments should centre around changes in soluble carbohydrate levels of roots in response to anoxia and cold temperature. These studies will eventually, out of necessity, include examination of alcohol dehydrogenase activity, but study of this activity should not be restricted to a single tissue or isozyme in order to isolate the specific action of the isozyme. It should also involve

examination of both translocation of ethanol in the transpiration stream and utilization of alcohol as a carbohydrate in respiration.

VITA

Name:	Timothy Shaun Stafford Conlin.
Place and Year of Birth:	Roblin, Manitoba, 1960.
Education:	VII Form, Bishop's College School, 1978, Lennoxville, Quebec.
	Brandon University, 1978-83 B.Sc. (Specialist, Environmental Science), 1983.
	School of Graduate Studies, Queen's University, 1983-1986.
	M.Sc. (Biology), 1986.
	Faculty of Graduate Studies, University of Alberta, 1988-1993.
	Ph.D. (Forest Science), 1993.
Experience:	Laboratory Instructor, Queen's University, 1983-1986.
	Research Associate, Agriculture Canada, Brandon Research Station, 1986-1987.
	Summer Youth University Instructor, University of Alberta, 1988-1992.
	Marker, Silviculture, 1991.
Awards:	Gus Hendzel Memorial Award in Botany, 1980.
	NSERC Undergrad Summer Fellowship, 1982.
	Queen's Graduate Award, 1983.
	Queen's Graduate Award, 1984.