AN ABSTRACT OF THE THESIS OF

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Title: <u>Xylem Cavitation in Western Hemlock (*Tsuga heterophylla* (Raf.) Sarg.) Seedlings Following Planting.</u>

Abstract approved: _____

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Reforestation of Pacific Northwest forest-lands with western hemlock has been hindered by unexplained plantation failures. The purpose of this thesis is to provide information that will help in determining the cause of seedling damage and mortality. The studies focused on the relationship between xylem water potential, xylem cavitation and the seedlings subsequent ability to transport water.

The initial experiment illustrated that following planting and exposure to water stress newly planted western hemlock seedlings were unable to transport sufficient water to meet transpirational demand. Hydraulic conductance declined from 1.01ug cm^{-2} s^{-1} MPa^{-1} to 0.16ug cm^{-2} s^{-1} MPa^{-1} over an 11 day period.

In the second study xylem cavitation was detected almost immediately following planting in moist soils and in one case rapidly escalating diurnal cavitation rates ended in seedling

mortality. There did not appear to be any correlation between cavitation rates and ambient temperature and relative humidity.

In addition it was determined that xylem cavitation was initiated when xylem water potentials reached -1.5MPa. A peak rate of 90 events per minute was experienced at -3.6MPa declining thereafter as the number of water filled tracheids decreased. A similar pattern of cavitation rate and water potential was experienced when monitoring intact potted seedlings in a greenhouse. The cavitation rates followed a diurnal pattern however which declined with increasing xylem water potentials.

The final experiment in this series measured the impact of xylem cavitation on hydraulic conductivity. A 30% loss in hydraulic conductivity was experienced following a water potential between -2.5 and -3.0MPa. A complete loss was experienced following water potentials of -4.0MPa.

Following planting, western hemlock seedlings experience water stress due to the inability of the root system to supply sufficient water to meet transpirational demand. This water stress results in water potentials which are sufficiently negative to cause xylem cavitation and embolism formation. The embolized tracheids result in a decline in hydraulic conductance and subsequent adjustments in stomatal aperture and leaf area. Without adjustments to reduce transpirational demand the seedling can experience severe enough cavitation to result in mortality.

Xylem Cavitation in Newly Planted Western Hemlock *(Tsuga heterophylla* (Raf.) Sarg.) Seedlings

by

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A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the degree of

Doctor of Philosophy

Completed July 1, 1993

Commencement June 1994

APPROVED:

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Date thesis is presented_____July 1, 1993_____

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ACKNOWLEDGMENTS

could not have imagined completing this degree without your love and support. Isn't it wonderful when love flows in both directions.

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INTRODUCTION

The Coast Range of the Pacific Northwest contains some of the most productive forest lands in the world and reforestation of these lands is of ongoing concern to the land managers and the public. The primary species of interest is Douglas-fir (*Pseudotsuga menziesii*) which has an excellent record in terms of reforestation success and growth productivity. A second coniferous species, western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) has received increasing attention due to its high potential productivity (Wiley, 1976), and as an alternative to Douglas-fir on certain sites. Western hemlock is a native species of the Coast and Cascade Ranges that is found on relatively moist sites and in areas of western Oregon and Washington from 500-4500' in elevation. It has greater tolerance to shading from competitive vegetation than Douglas-fir and is better adapted to some types of soils (Packee, 1990).

With increasing demands on our forest resource for ecological diversity and market flexibility it is desirable to have species other than Douglas-fir to broaden management options. Recent interest has focused on the contribution of western hemlock to structural and species diversity on Coastal Range and Cascade

Mountain sites. However forest management practices and periodic fire have eliminated much of the hemlock from these areas.

Reforestation with western hemlock is slowly gaining acceptance but unexplained plantation failures have discouraged extensive use of hemlock. The majority of the research done on planted western hemlock seedlings has attempted to modify seedling culture and planting techniques to improve reforestation success. Still, puzzling observations of high mortality and poor growth continued to be made on vigorous, sun-adapted hemlock seedlings planted in well moistened soils.

This thesis research examined the water relations of western hemlock following planting and demonstrated some parallels between damage to the water conducting system and subsequent morphological changes and mortality.

Each of the four chapters in this thesis describe the procedure and outcome of separate experiments exploring the rate and impact of xylem cavitation in western hemlock seedlings. Chapters I and IV are to be published as separate papers while chapters II and III are more exploratory dealing primarily with methodology.

In Chapter I I analyzed the response of western hemlock seedlings to planting in the context of water stress and subsequent impacts on water transport. The initial question

asked concerned the root systems impact on the seedlings subsequent ability to transport water.

In Chapter II my goal was to explore the possibility of detecting xylem cavitation in newly planted seedlings as well as to determine if cavitation occurred following planting.

Chapter III was a continuation of methodology exploration with the goals of determining the water potential at which xylem cavitation occurs in western hemlock as well as the best location on the seedling to detect cavitation.

The final chapter dealt with the vulnerability of western hemlock to cavitation, quantifying both the water potential at which cavitation occurs and the resultant impact on the seedlings ability to transport water.

LITERATURE REVIEW

Western hemlock

Brix (1978) measured photosynthetic rates of a variety of coniferous species under different moisture stress conditions. He found that in western hemlock death occurred at water potentials around -40 bars. In contrast, Douglas-fir, white spruce (*Picea glauca*) and lodgepole pine (*Pinus contorta*) do not die until water potential fell below -110 bars.

Maximum stomatal conductance rates have been measured at 0.31 centimeters per second (cms^{-1}). Compared to $0.44 cms^{-1}$ for Douglas-fir (Livingston and Black, 1987). In the same study stomatal conductance on planted seedlings was found to be related to average solar irradiance, air temperature, vapor pressure deficit, soil water and number of hours from sunrise. By combining all the above values 85% of the variation in stomatal conductance was explained. Stomatal aperture in western hemlock is especially sensitive to vapor pressure and a modest 10mb vapor pressure deficit can cause stomata to close (Marshall and Waring, 1984) whereas in Douglas-fir the same degree of closure did not occur until 20mb (Waring and Franklin, 1979).

Changes in morphological and physiological characteristics in sun and shade grown western hemlock has contributed to our understanding of ecophysiological adaptations. Sun adapted western hemlock seedlings have a lower photosynthetic rate than shade adapted seedlings, and they have the ability to maintain needle relative water content even under conditions of high evaporative demand (Keller and Tregunna, 1975). Keller and Tregunna (1975) also found that shade adapted seedlings have higher rates of stomatal conductance and reduced relative water content under increased evaporative demand. In addition, the inability of the shade adapted seedlings to reduce stomatal conductance when exposed to increasing light intensities enhanced photosynthetic efficiency but it also caused severe moisture stress during periods of high evaporative demand.

Western hemlock needle morphology also changes with exposure to different light intensities. Leaves grown under shade have a greater ratio of needle width to thickness, a thinner palisade mesophyll and a greater ratio of surface area to weight when compared to sun-grown needles. (Tucker and Emmingham, 1977) It was also found that the number of stomata per mm^2 increased on the sun-grown foliage. The fact that the shade grown needles had fewer stomata but increased rates of stomatal conductance indicates stomatal aperture is not very well controlled under increasing illumination. The inability of hemlock foliage to adjust to changing growing conditions has been cited as a cause of needle mortality in understory seedlings and saplings following

removal of an overstory canopy (Tucker and Emmingham, 1977). They found that shade adapted foliage died soon after exposure to full light and the ability of the tree to survive was dependent on the growth of new foliage

Reforestation

Survival rates of hemlock following operational planting are inconsistent and unpredictable. Arnott (1975) reported three year survival rates of 50, 53, 65, 45 and 29% when comparing various western hemlock stock types planted on Vancouver Island. In studies of western hemlock wildlings, Newton (1978) had mean survival rates of 33-66% depending on the month planted, number of days heeled in and the amount of competing vegetation. In this study the highest survival rates (66%) occurred in seedlings planted during the month of February in an area without competing vegetation. The use of shade cards and irrigation was only able to improve survival rates of container grown western hemlock seedlings to 64% whereas Douglas-fir planted on the same site had survival rates of 95% (Livingston and Black, 1987). However, Newton *et al.* (1987) reported 98% survival in western hemlock bare root seedlings planted in February.

Correlation with the date that nursery stock is lifted has shown that the best survival results are with seedlings dug in mid-winter and stored in a cold room for 4-6 weeks before planting (Nelson, 1980). Dunsworth (1988) recommended lift dates, cold

storage guidelines and targets for determining western hemlock seedling quality. By following these guidelines he was able to obtain survival of at least 88% under research conditions in raised beds in Vancouver British Columbia.

The main cause of mortality and poor growth following planting of western hemlock is drought stress (Arnott, 1975; Newton, 1978; and Livingston and Black, 1987). Because of the relatively high survival rates of containerized stock, further research was undertaken to improve cultural practices and describe morphological and physiological features that resulted from different cultural regimes. O'Reilly et al. (1989) focused on hemlock shoot growth, morphology, and the formation of cuticle and epicuticular wax following a variety of cultural pretreatments. They concluded that short days were most effective in promoting bud formation and there was no treatment effect on cuticle and epicuticular wax. O'Reilly et al. (1988) continued this research project by tracking the seedling growth performance during the first year of field establishment. They concluded that seedlings pretreated with long days and those lifted in March had the longest shoots. Survival data following planting was not reported in this study but it was noted that sample sizes were reduced due to poor survival.

I suspected that nursery practices and subsequent planting techniques affect root water uptake which results in moisture stress and subsequent plantation failure. Bare root seedlings are

often root pruned to meet a maximum 8" root length, removing most of the unsuberized root tips. Containerized stock suffers less root damage but it, as well as the bare root stock, must grow new roots into the soil to reestablish good root-soil contact. It is speculated that water stress and subsequent stomatal closure can inhibit new root growth in that western hemlock seedlings do not have high starch reserves and are highly dependent on current photosynthate to initiate root growth (Marshall and Waring, 1985).

Although many of the experiments cited above focused on water relations and the role of increasing water stress on seedling mortality they did not determine the actual cause of the observed water stress. Further exploration on the effect of root morphology on the newly planted western hemlock seedlings ability to obtain sufficient water to meet transpirational demand was needed.

Root uptake

A better understanding of the mechanisms limiting water uptake in newly planted hemlock and the resultant impacts on the entire hydraulic system should help us understand the inability of western hemlock seedlings to survive planting and thrive. Water movement into, out of and within the tree encounters a series of hydraulic resistances imposed by the soil, roots, bole, branches, and leaves which in turn influence the water status of the leaves (Kramer 1969). Running (1980) described the water flow system

as a summation of flows and resistances through small segments
e.g.,

$$q = \frac{dY_{soil\text{-}root}}{R_{soil\text{-}root}} + \frac{dY_{root\text{-}xylem}}{R_{root\text{-}xylem}} + \frac{dY_{xylem\text{-}leaf}}{R_{xylem\text{-}leaf}} + \frac{dY_{leaf\text{-}air}}{R_{leaf\text{-}air}} \quad (1)$$

Where q equals the water flow through the entire system; dY is
the water potential gradient and R is the resistance to flow.
Equation 1 illustrates that maintenance of a constant flow rate (q)
requires and increase in the water potential gradient (dY) to
compensate for an increase in resistance (R). Following planting,
root resistance can increase due to a reduction in the number of
unsuberized roots (Johnson *et al.* 1988), poor soil-root contact
(Sands, 1984; Grossnickle, 1988) and/or low soil temperatures
(Running and Reid, 1980; Lopushinsky and Kaufmann, 1984).
Any or all of these factors can result in the inability of the xylem
to meet transpirational demand and consequently in more
negative xylem water potentials.

Successful planting in the Pacific Northwest relies on the ability
of seedlings to endure a certain amount of moisture stress with
the expectation that the seedling will later recover. Endurance,
however, appears to be lacking in western hemlock. No research
has been done to date on the long-term impact of moisture stress
incurred during the planting process. In order to understand
how a reduction in water uptake can have a long term impact on
the plants response to the environment, we must first examine
water transport and the effect of water deficit.

Water transport

Water is transported from the soil into the roots, through the xylem, to the leaves, and into the atmosphere under tension (Dixon, 1914). Evaporation at the cell wall surfaces in the leaf causes the air-water interface to retreat into the minute spaces between the cellulose fibers in the cell wall. A consequence of surface tension i.e. capillarity, tends to draw the air-water interface back up to the surface of the minute pores. This tendency to draw the air-water interface upward places a tension (or negative pressure) on the mass of water behind it. This negative pressure is transmitted the entire length of the water conducting column in the plant. As evaporative demand increases, water recedes into smaller pore spaces resulting in increased tension on the water column and more negative pressures.

Transport of water in the plant under tension requires that water remains liquid at sub-atmospheric (negative) pressures. In order to maintain the continuity of the water column the introduction of air, which can provide a nucleus for vaporization, must be prevented. Water under sub-atmospheric pressures is in a metastable state and the introduction of even a um sized bubble can result in vaporization. Xylem conduits are water filled from inception and initially contain no entrapped air bubbles that could nucleate cavitation (Tyree and Sperry, 1989).

Water flow in trees occurs through the lumen of vessels and tracheids and through the lateral pits that interconnect the conduits (Esau, 1965). Coniferous trees lack vessels and are entirely dependent on the less efficient tracheids for water transport. Tracheid walls are perforated with numerous lateral pits which are thin porous depressions in the cell wall where secondary thickening is not present. Water flows into and out of the tracheid through these pits, each of which include a membrane across the orifice.

Many of the physical properties of a tracheid allow for water transport under tension. The rigid cell wall prevents collapse as tension on the water column increases. The hydrophilic cell walls decrease the possibility of entrapped air bubbles at the wall-water interface. If air is introduced into the system it can be contained in a single tracheid by the surface tension effects at the pit membrane between adjoining tracheids (providing tensions on the water column are not too great). Built in pathway redundancy allows for limited damage to individual tracheids to occur without disrupting water conduction in the whole plant.

Quantifying water flow

Water flow through a plant has often been equated with Ohm's law (Van den Honert, 1948). The amount of water flowing through the plant is treated as a connected series, analogous to the current in an electric circuit composed of a series of

conductances (or the reciprocal, resistance). This can be written in equation form to describe water flow from point A to point B.

$$F = k(Y_A - Y_B) \qquad (2)$$

Where $F(kg\ s^{-1})$ is the flux, $k\ (kg\ s^{-1}\ MPa^{-1})$ is the hydraulic **conductance**, and $Y_A - Y_B$ is the water potential drop across the segment in MPa. Equation 2 is similar to equation 1 except that Running (1980) chose to use the reciprocal of conductance, resistance.

Hydraulic conductance is often computed by measuring the flux of water through a plant (transpiration) while simultaneously measuring soil and leaf water potential (Blizzard and Boyer, 1980; Running and Reid, 1980; and Mattson-Djos, 1984).

Hydraulic **conductivity** (k_h) describes the porosity of the wood and its relative ability to transport water. Hydraulic conductivity is different from conductance in that path length (dx) is included in the calculation i.e.

$$F = k_h \left(\frac{Y_A - Y_B}{dx} \right) \qquad (3a)$$

-OR-

$$k_h = \frac{F}{\dfrac{Y_A - Y_B}{dx}} \qquad (3b)$$

The denominator in equation 3b ($\frac{Y_A - Y_B}{dx}$) is often referred to as the pressure gradient, the change in pressure over a given distance, abbreviated as $\frac{dP}{dx}$.

When k_h is divided by the sapwood cross-sectional area, specific conductivity (k_S) is obtained. k_S is a function of the number of conduits per unit area and the average diameter of the conduits. To summarize, **conductance** (k) describes the volume of water that moves through a given segment at a given pressure differential but since it is not normalized by segment length or diameter it does not provide information about the physical properties of the segment. **Conductivity** (k_h) on the other hand, is an indication of the porosity of the materials contained in the segment. **Specific** conductivity (k_S) gives further information about the properties of the conducting segment since cross-sectional area is taken into account making it easier to compare different samples.

Table i.1. Hypothetical data of water flow in three stem segments.

	Seg. A	Seg. B	Seg. C
Length (cm)	20	15	10
Cross-sec area (cm^2)	0.79	3.1	7.1
YA-YB (MPa)	2	2	2
Flux (kg s^{-1})	4	4	4
Conductance (kg s^{-1} MPa^{-1})	2	2	2
Conductivity(kg cm s^{-1} MPa^{-1})	40	30	20
Specific Cond (kg s^{-1} MPa^{-1} cm^{-1})	51	9.5	2.8

Table i.1 shows data from three hypothetical stem segments with different physical properties. Their respective lengths are 20, 15, and 10cm and cross-sectional areas are 0.78, 3.1, and 7.1cm^2 respectively. All three segments are capable of transporting the same volume of water (flux) per unit pressure (i.e. conductance). Although the capacity of the stem segments to transport water can be quantified nothing is known about the physical properties of the materials involved in the process. When the segment length is taken into consideration and conductivity calculated we

obtain values of 40, 30 and 20 respectively. It is evident from the data that segment C has a much lower rate of conductivity indicating it is made of materials that are not as efficient in the transport of water when compared to other samples. By dividing the conductivity values by the respective cross-sectional areas it becomes even more apparent that segment A is comprised of tracheids or vessels which are highly efficient in water transport. Segment A is deemed more "efficient" hydrologically in that it required a relatively small investment of xylem to transport a volume of water at a given pressure gradient.

Tyree and Ewers (1991) modified Poiselles law to model xylem transport based on the number and size of xylem conduits demonstrating the importance of material properties to conductivity (k_h).

$$k_h = \frac{p\,r}{128z} \cdot \sum_{i=1}^{n} d^4 \qquad\qquad (4)$$

Where r is the density of the fluid, z is the viscosity of the water, d is the diameter of the ith tracheid and n equals the number of tracheids in the cross-section.

Poiselles' law illustrates the importance of conduit diameter to conductivity since the diameter of the tracheid is raised to the 4th power. Therefore a large tracheid 4 times the median size would conduct as much water as 256 (4^4) median size tracheids (Zimmermann, 1983). Plants with a larger average diameter

tracheid are more efficient transporters of water (i.e. they can transport a greater volume per unit investment in sapwood).

All of these equations illustrate the trade-offs which are possible in designing a plant hydraulic system. High rates of flux can result from an increased conductivity or an increase in the pressure gradient. Xylem conductivity has been found to be influenced by cross-sectional area, number of tracheary elements which retain transport function, and the size of tracheary elements (Zimmerman, 1983).

Throughout this discussion of the hydraulic system it must be remembered that the function of the water conducting system is to supply moisture to the leaves. As discussed below the more efficient the transport of water, the more leaf area supported, the greater the potential plant productivity.

Sapwood/leaf area

A strong relationship exists between stem hydraulic conductivity and leaf area (Ewers and Zimmerman, 1984). This relationship was first quantified by calculating Huber values (HV) which are defined as the sapwood cross-sectional area (A) divided by the leaf area distal (LA_d) to it (Huber, 1928 as described by Zimmermann, 1983). The theory is that a more efficient transporter of water (i.e. one having a higher hydraulic conductivity) will be able to support an increased leaf area for a given investment in sapwood cross-section. A more recent

method used to describe the relationship between hydraulic conductivity and leaf area is leaf specific conductivity (LSC), which is obtained by dividing hydraulic conductivity (k_h) by the leaf area distal (LA_d) to it (Zimmermann, 1978).

LSC is a good measure of the ability of the stem to supply water to the leaves distal to the stem because the evaporative flux (E) from the leaves supplied by the stem is related to the pressure gradient (dP/dx) and hydraulic conductivity (Tyree and Ewers, 1991).

LSC is calculated by dividing hydraulic conductivity by leaf area distal to the segment i.e.:

$$LSC = k_h/LA_d \qquad (5a)$$

Flux is calculated by multiplying hydraulic conductivity by the pressure gradient i.e.:

$$F = k_h \ (dP/dx) \qquad (5b)$$

Maximum evaporative flux of water out of the leaf is equal to the flux in the stem segment, as calculated above, divided by the leaf area supported by the stem i.e.:

$$E = F/LA_d \qquad (5c)$$

Therefore by combining the above equations it can be shown that:

$$E/LSC = F/k_h = dP/dL \qquad (6)$$

LSC values indicate the pressure potential required to maintain transpiration at a given rate as illustrated by equation 6. The higher the LSC value, the lower the pressure gradient required to supply a given amount of water to the leaves.

The next set of equations describe the relationship between LSC, HV and specific conductivity (k_S).

Specific conductivity (k_S) is calculated by dividing stem conductivity by cross-sectional area(A) i.e.:

$$k_S = k_h / A \qquad (7)$$

Huber value is calculated by dividing stem cross-sectional area by leaf area distal to the segment (LA_d):

$$HV = A/LA_d \qquad (7a)$$

Leaf specific conductivity is calculated by dividing conductivity by leaf area distal to the segment i.e.:

$$LSC = k/LA_d \qquad (7b)$$

Therefore by combining the above equations it can be shown that:

$$LSC = HV(k_S) \qquad (8)$$

The question arises as to which factor, HV or specific conductivity, has more influence on the LSC value. The structural basis for a reduction in LSC in *Abies balsamea* was found to be

due to reduced HV rather than to a reduction in tracheid diameter (Ewers and Zimmerman, 1984).

LSC values vary widely between species and within the bole and crown of an individual tree. Within tree LSC values for a number of gymnosperms have been examined including *Tsuga canadensis, Abies balsamea,* and *Thuja occidentalis* (Ewers and Zimmermann, 1984). LSC was found to be higher at the base and decrease substantially with increasing elevation and distance from the bole. Most of the resistance (lowest conductivity) to flow is located in the last meter or less of the pathway from the base of the tree to the leaf bearing branches (Tyree and Ewers 1991). This form of hydraulic architecture serves two functions: 1) to assure all branches equal access to water supplied by the bole and 2) to create a water potential gradient such that xylem cavitation will be more likely to occur in minor branches than in the bole while transpiration is occurring (Zimmermann, 1983).

Xylem cavitation

Hydraulic conductivity is reduced when water potentials become sufficiently negative to introduce air into the water conducting column, causing xylem conduits to cavitate and become embolized. The cavitation process occurs as follows: Water molecules in xylem tracheids are under tension. As tension increases a large number of hydrogen bonds can break, leaving a void filled with water vapor. This leads to the further introduction of air, which comes out of the surrounding tissues,

forming an embolism which restricts water transport (Tyree and Sperry, 1988). Conifer tracheids have been shown to cavitate and become air-filled and nonfunctional at water potentials ranging from -0.5MPa to -5.0MPa (Tyree and Ewers, 1991).

Reductions in the hydraulic conductivity of the sapwood due to cavitation have been found to cause a subsequent reduction in leaf area. Tyree and Sperry (1988) modeled the dynamics of water flow from data collected on four widely differing tree species. Their model predicts that trees operate near the point of catastrophic xylem failure due to cavitation. "Runaway cavitation" occurs following periods of xylem cavitation when additional tension is placed on the remaining water columns, due a lower conductivity, causing a steeper pressure gradient to develop to maintain transpirational demand (equation 3a). This cycle can be halted by reducing transpiration demand (flux) through stomatal closure or by leaf loss.

Tyree and Sperry's catastrophic model supports an earlier model proposed by Zimmermann (1983) known as the "plant segmentation hypothesis" which suggests that plants are designed hydraulically to sacrifice minor branches first as water potential decreases. Minor branches were found to have a lower leaf specific conductivity (LSC) which means that a steeper pressure gradient is required to maintain water flow in their xylem (equation 6), and are therefore first to cavitate during periods of high transpirational stress. The subsequent loss of

non-essential branches results in an improved water balance, protecting the more irreplaceable organs from runaway cavitation. Both of these models demonstrate that under extreme drought conditions leaf area adjustments are made by the plant resulting in an improved hydrologic status.

By avoiding xylem cavitation events a tree also avoids possible reductions in leaf area and death. Vulnerability curves (a plot of percent-loss in hydraulic conductivity verses xylem water potential) illustrate a species' ability to avoid effects of cavitation events. Tree species have been found to vary in their ability to avoid xylem cavitation and it has been demonstrated that the more vulnerable a species is to cavitation the lower the xylem tensions it can withstand in natural conditions (Sperry *et al.* 1988). According to Zimmermann's (1983) air-seed hypothesis, vulnerability to cavitation is a function of cell wall pore or border pit diameter. The size of the cell wall or border pit pore is critical in determining the surface tension forces that must be overcome to introduce an air bubble into a tracheid. Zimmermann's hypothesis has since received considerable experimental support, (Crombie *et al.*, 1985; Sperry and Tyree 1988).

It would seem to be an adaptive advantage to have larger conduits therefore improving hydraulic conductivity. However, Zimmermann (1983) pointed out that there are drawbacks to larger diameters: 1. the loss of a single large conduit would result in a loss of a large percentage of hydraulic capacity. 2. Larger

conduits, within a species, have been found to be more vulnerable to xylem cavitation probably due to decreased cell wall thickness and subsequently larger border pit pores. As plants have evolved they have had to balance the need for increased tracheid diameter and enhanced hydraulic conductivity with an increase in vulnerability to cavitation.

Measuring cavitation

During a cavitation event, breakage of the water column results in a vibration of the xylem vessel wall as the tension on water column is relieved. This vibration results in an acoustic emission (AE). AEs' can be detected in both low (Milburn and Johnson, 1966) and ultrasonic (Tyree and Dixon, 1983; Tyree *et al.* 1984) frequencies. Detection in the ultrasonic frequency has advantages due the lack of background noise interference and the ability to make more than one measurement, such as plant water potential, on the same plant concurrently.

Results reported in the literature have established a clear relationship between cavitation events and AE frequency. Tyree and Dixon (1983) found AE occurred in *Thuja occidentalis* only when water potentials exceeded -1.0 MPa and the rate of AEs increased as xylem pressure became more negative. In addition, AEs were found to stop following rewetting and a less negative value of xylem pressure potential. Loss in hydraulic conductance was related to the initiation of AEs in *Thuja, Tsuga* and *Acer* (Tyree and Dixon, 1986). They also found that the last vestiges of

hydraulic conductance in conifers corresponded to the cessation of AEs. The cumulative number of AEs has been found to correspond to the number of tracheids in small samples of Thuja (Tyree *et al.* 1984). Although Sandford and Grace (1985), using a less efficient AE counting system, found only 16% of the expected AEs from a sample of *Chamaecyparis* wood.

Xylem cavitation and embolism formation can also be studied using indirect measures such as: (1) percent loss of hydraulic conductivity (Sperry *et al.*, 1988), (2) loss of water conducting cross-sectional area (Salleo and LoGullo, 1989) and (3) changes in xylem density to gamma rays (Dixon *et al.*, 1984; Pena and Grace, 1986).

Many of the initial studies on xylem cavitation have involved the used of detached woody stems which are debarked then dehydrated and rehydrated rapidly under laboratory conditions. More recent applications have involved the use of whole plants under controlled environmental conditions. Jones *et al.* (1989) utilized AE monitoring equipment to quantify the relationship between apple root stock variety and the initiation and severity of embolism formation during water stress. A 70% reduction in hydraulic conductance was found in intact potted *Picea abies* seedlings due to embolism formation following 3 drought stress periods (Borghetti *et al*, 1989). They also showed that the effect of the embolism formation was cumulative and the plants did not recover fully even following rewatering.

Refilling

No discussion of xylem cavitation would be complete without considering refilling of the embolized tracheids or vessels with water. The majority of the early work on the repair of embolized conduits focused on xylem vessels. To dissolve air in embolized vessels the xylem pressure must return to positive values. Surface tension puts the bubble of air under pressure equal to $2T/r$ where T = surface tension and r is the bubble radius. Assuming that the vessel diameter is measured in microns then the air pressure in kilopascals is equal to $140/r$; therefore a bubble $10\mu m$ in size would be at 14kPa above atmospheric pressure. (Tyree and Sperry, 1989).

Spring refilling was documented in sugar maple (*Acer saccharum*) (Sperry *et al.* 1988) and wild grape vine (*Vitis labrusca* and *Vitis riparia)* (Sperry *et al.* 1986). Grape vines were found to generate very large spring root pressures (>0.1 MPa) which not only dissolved air bubbles but expelled the air out of the vine through leaf and inflorescence scars. Sugar maple belongs to a unique genus that is capable of generating positive stem pressure as a consequence of spring sap flow. Sperry *et al.* (1986) measured positive xylem pressures of 16kPa at the time refilling of vessels was occurring. The ability of vessel bearing species that do not generate positive root pressures to refill has not been examined thoroughly. Many have reported evidence

that refilling occurs on a seasonal basis but the mechanism for such a repair is not well understood.

Refilling of conifer tracheids is made more difficult by the aspiration of the bordered pits following cavitation. The bordered pits act as a valve and the positive pressures generated by the air bubble deflect the pit membrane, covering the pit opening with the torus (which is non-porous). Sperry and Tyree (1990) were unable to restore hydraulic conductivity in embolized branch segments of several conifer species even after applying pressures of 0.175 MPa. But Borghetti *et al.* (1991) demonstrated refilling in whole plants and detached stems of *Pinus sylvestris*. Moreover the refilling was shown to occur when the uptake of water was under tension. This phenomena was explained by the fact that water may be trapped in the tapered ends of the tracheid and thus provide a significant capillary potential to suck water from the surrounding cells. Following the entry of water into the tracheid the air bubble comes under positive pressure and dissolves, restoring the water column. Further explanation of this refilling mechanism was explored by Sobrado *et al.* (1992) and they concluded that reversal of embolisms were possible depending on the water potential of the source, severity of the embolism and hydraulic conductivity.

The question of refilling of embolized tracheids in *vivo* is still unanswered. Most of the work done to date has been on detached segments of whole seedlings which were connected to a

direct source of water (i.e. not root uptake). Further work needs to be done to demonstrate conclusively whether refilling in conifers occurs on a daily or seasonal basis.

CHAPTER I
HYDRAULIC CONDUCTANCE IN WESTERN HEMLOCK FOLLOWING PLANTING

Introduction

The ability of newly planted western hemlock seedlings to avoid water stress is critical to their survival. Following planting, root conductance can decrease due to poor soil-root contact (Sands, 1984; Grossnickle, 1988), a reduction in the number of unsuberized roots (Johnson *et al.*, 1988), and low soil temperatures (Running and Reid, 1980; Lopushinsky and Kaufmann, 1984) and possibly root breakage and desiccation. Any or all of these factors can result in the inability of the xylem to meet transpirational demand resulting in an increase in water stress. Changes in hydraulic conductance of western hemlock root systems following planting and the resultant effect on the soil-plant-atmospheric continuum (SPAC) have, however, not been well characterized.

Since it is difficult to measure root conductance in intact seedlings, total hydraulic conductance from the soil to the atmosphere is commonly measured. Using a null-balance porometer and pressure chamber, it is possible to measure the flux (transpiration) and pressure differential (leaf water potential), allowing conductance to be calculated (Scholander *et al.*, 1965; Beardsell *et al.*, 1972; Roberts and Knorr, 1977; Running and Reid, 1980; and Mattson-Djos, 1984). By examining values of

hydraulic conductance for a variety of root treatments it is possible to evaluate the impact of root treatment on total plant hydraulic conductance. The relative differences in conductance should indicate treatment effects.

In this experiment five root treatments/stock types were used. The objective of this experiment was to quantify the impact of root treatment on hydraulic conductance. I hypothesized that the root treatments/stock types with the greatest amount of unsuberized tips will be the most effective in maintaining conductance, when planted in well watered soils, resulting in a reduction in water stress.

Methods

Plant material

All seedlings were from Coast Range sources and grown in a nursery as bare root or containerized stock. The bare root stock were plug-1's (seedlings grown one year in a container and one year in the nursery bed) which were obtained from the Industrial Forests Association Nursery in Canby, Oregon. The large containerized stock (D-Cells) were obtained from the Environmental Protection Agency in Corvallis, OR where they had been grown for 3 years. The 1-yr old containerized stock were grown at Champion International Nurseries in Lebanon, Oregon. The root treatment/stock types were: (1) plug-1 non-pruned; (2) plug-1 pruned to 8 in; (3) plug-1 pruned to 4 in; (4) 3-yr old

containerized stock-undisturbed root system;   (5) 1-yr old containerized stock-undisturbed root system.   The container grown seedlings were characterized as having undisturbed root systems because they did not undergo the nursery procedures for lifting and storing bare-root seedlings.

Site

The field planting site was located on a north-east facing slope owned by Starker Forest Inc. in the central Coast Range approximately 5 kilometers northeast of Eddyville, Oregon.   The site had been clearcut and burned the proceeding fall leaving it practically devoid of above ground vegetation at the time of planting.   The seedlings were planted March 8, 1990 in a randomized block design using 2ft x 2ft spacing and the entire planting was surrounded by a two tree border row.   Thirty seedlings per treatment were planted in one of three blocks for a total of 450 seedlings.   The weather immediately preceding, during and following planting was characterized by 10°C temperatures, heavy cloud cover and steady rain.   The soils were fully charged at the time of planting, but drained well.

Measurements

Fifteen randomly chosen seedlings (1 per treatment per block) were measured on each sample date.   Measurements of plant water conductance began on the fourth day following planting when the foliage was sufficiently dry to make porometer

measurements and continued on every third day when weather conditions permitted. Measurements were made on: March 12, 16, 21, 24, 27, April 3, 7, 10 and May 11.

Predawn xylem water potential measurements were made between 4:00 and 4:30am on each sample seedling using a pressure chamber (PMS Instrument Co., Corvallis, OR). This measurement was used as an indicator of soil water potential. Stomatal conductance and transpiration (F) were recorded between 8:00 and 10:00 am (depending on when the foliage dried) on intact needles with a steady state porometer (LiCor, Model 1600) Needles contained in the cuvette during porometer measurements were removed, placed in a plastic bag, labeled and returned to the lab where projected leaf area was measured using a leaf area meter (LiCor, Model 3100). Simultaneous temperature and humidity measurements were made and the data used to compute water vapor pressure deficit (VPD). Leaf water potential (Y_B) of detached stems was also measured at the same time as stomatal conductance.

Calculations

Hydraulic Conductance

Stomatal conductance and transpiration measurements were divided by the leaf area in the cuvette to calculate values on an area basis. Transpiration rates per unit leaf area (F) and xylem water potential (Y_B) were used to calculate hydraulic

conductance (k) on a leaf area basis i.e. the amount of water (ug/sec) that flows per unit leaf area (cm^2) per pressure differential (MPa):

$$F = k(Y_B)$$

Where F is transpiration in ug sec^{-1} cm^{-2}, k is conductance in ug sec^{-1} cm^{-2} MPa^{-1} and Y_B is xylem water potential in MPa.

Note the distinction between this value, hydraulic **conductance** on a leaf area basis, and hydraulic **conductivity** on a leaf area basis. In order to calculate conductivity, path length must be known. By computing hydraulic conductance on a leaf area basis I was able to compare values from seedlings with varying amounts of leaf area.

Soil water potential was not used to determine the pressure differential because soil moisture did not vary significantly within the site and the saturated soils were not a limiting factor in water uptake. Predawn water potential did not vary significantly between treatments.

Vapor Pressure Deficit

VPD is the difference between the ambient water vapor pressure (AVP) in the atmosphere and the saturated water vapor pressure (SVP_t). VPD was calculated from the product of the saturation vapor pressure, which is temperature dependent, and $\frac{RH}{100}$. This gives the AVP at a given humidity and temperature. The

ambient vapor pressure is then subtracted from the SVP$_t$ to obtain VPD.

$$AVP = SVP_t \left(\frac{RH}{100}\right)$$

$$VPD = SVP_t\text{-}AVP$$

Statistical Analysis

Predawn water potential, stomatal conductance and specific leaf conductance were analyzed using Analysis of Variance. A randomized complete block design with a 9x5 factorial treatment structure was applied to determine the significance of effects by root treatment and days following planting. Pairwise comparisons were made among means using Tukeys test with α=0.05.

Results

There was no significant variation in predawn water potential by treatment. As is illustrated in Fig. I.1, the value for treatment 5 appears to be lower but the data are skewed by an unexpectedly low set of readings on Mar 21 (See explanation below).

Predawn water potential did not vary significantly throughout the experimental period with the exception of March 21 when the average value (n=15) was -1.9 MPa (Fig I.2). The predawn water potential measurements taken on Mar 21 were abnormally low due to the unusual weather conditions that morning. At 4:00am there was no dew on the foliage and there was a
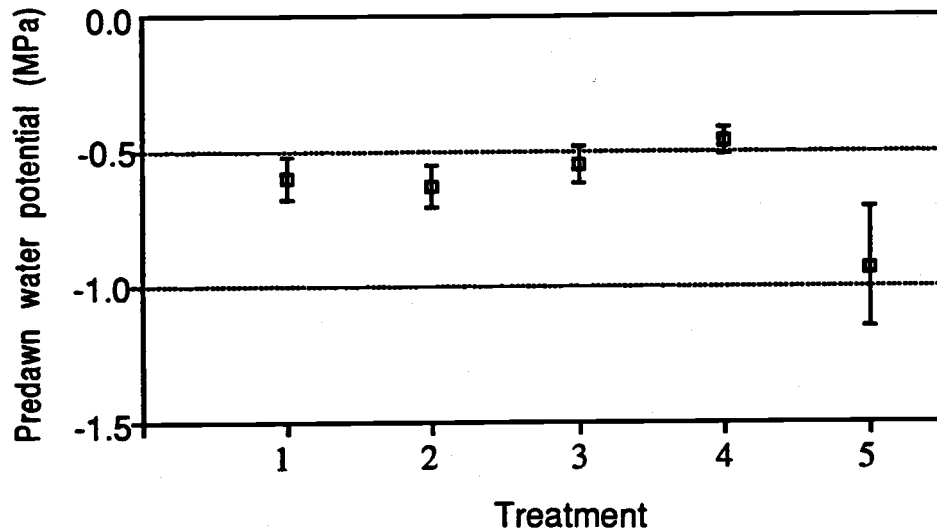
Figure I.1.   Predawn water potential of western hemlock seedlings by treatment. Treatment (1) = plug-1 roots nonpruned; (2) = plug-1 with roots pruned to 8"; (3) = plug-1 with roots pruned to 4"; (4) = 3yr old containerized stock, (5) = 1yr old containerized stock. Each point is a mean of 27 values. Vertical bars represent the standard error of the mean.

relatively dry east wind. The unusually low predawn water potentials are an indication that the stomata were open and the seedlings were transpiring; therefore the readings were not an accurate reflection of soil water potential.
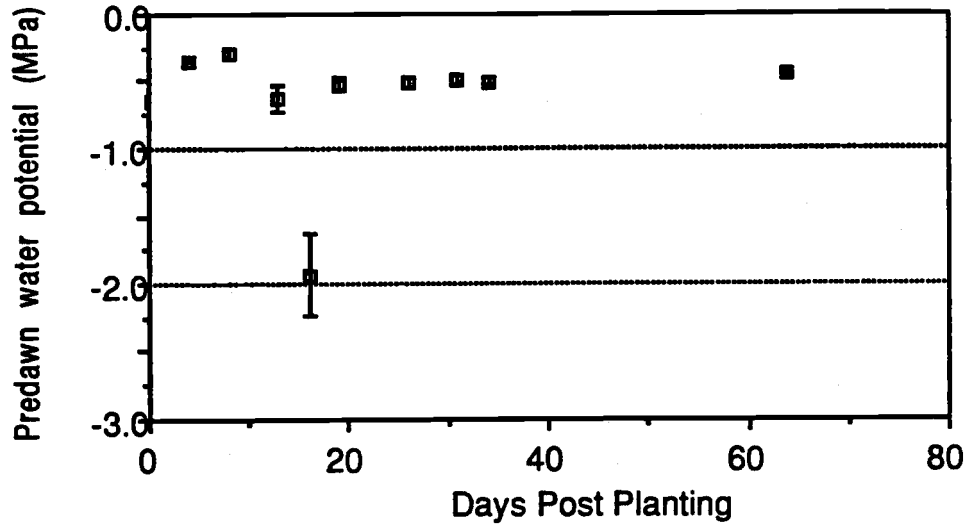
Figure I.2. **Predawn water potential of western hemlock seedlings following planting.** Each point is the mean of 15 values (3 per treatment). Vertical bars represent the standard error of the mean.

Conductance did not change significantly with root treatment (Fig I.3) but as illustrated in Fig I.4 there was a very strong effect attributable to days following planting.
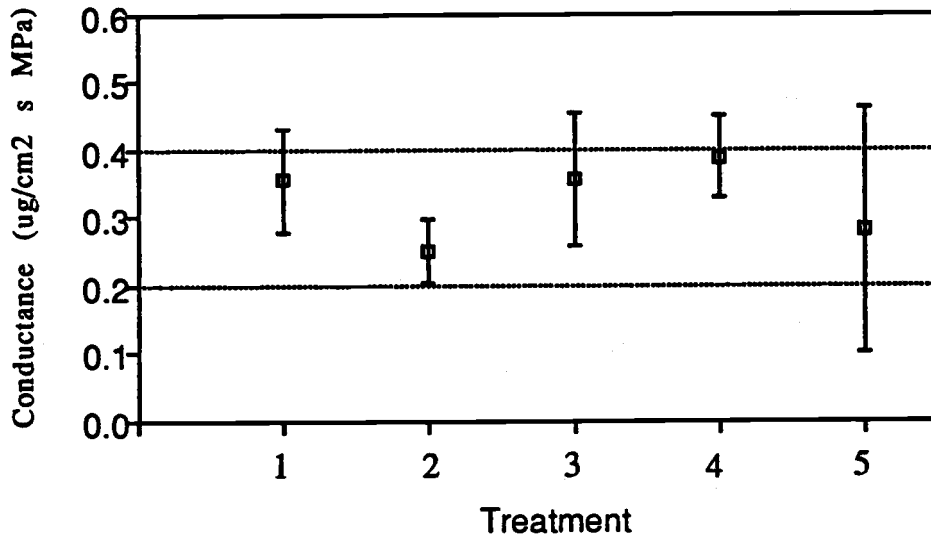
Figure I.3. Mean leaf specific hydraulic conductance following planting by treatment. Treatment (1) = plug-1 roots nonpruned ; (2) = plug-1 with roots pruned to 8" , (3) = plug-1 with roots pruned to 4"; (4) = 3yr old containerized stock, (5) = 1yr old containerized stock. Vertical bars represent the standard error of the mean (n=27).
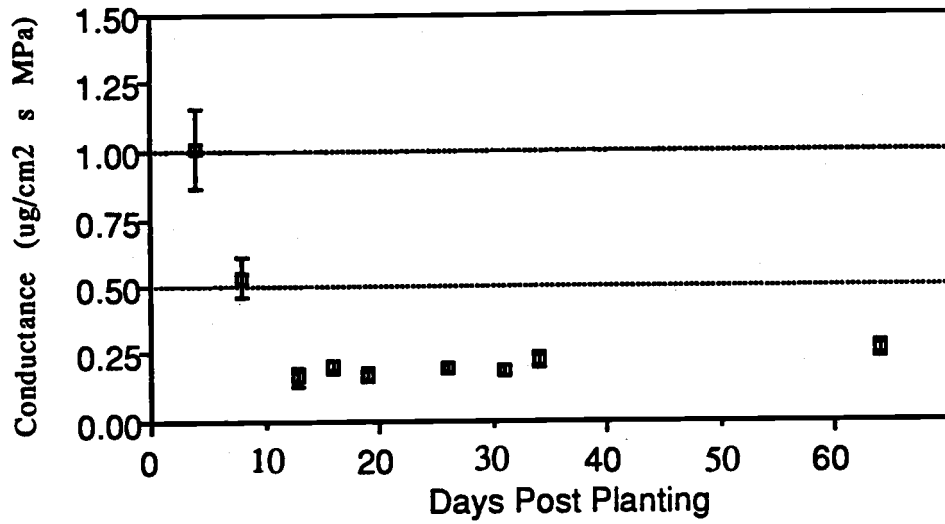
Figure I.4. **Mean leaf specific hydraulic conductance for western hemlock seedlings following planting.** Each point represents a mean of 15 measurements (3 per treatment). Bars represent the standard error of the mean.

As illustrated in Fig. I.5 vapor pressure deficit varied from 0.47-
1.13 KPa and stomatal conductance ranged from a maximum of
0.32cm s^{-1} to a minimum of 0.05cm s^{-1}.
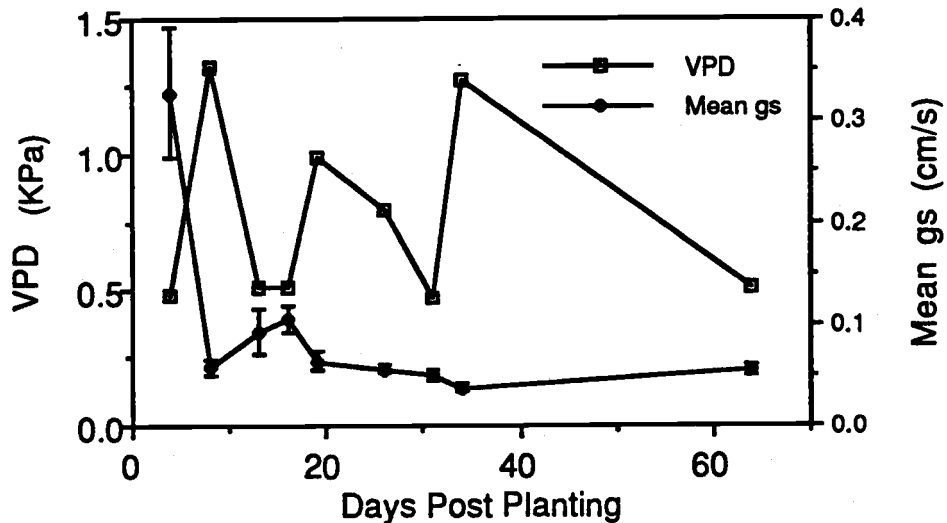
Figure I.5. Mean stomatal conductance(g_s) and VPD
following planting of western hemlock seedlings. Both
readings taken at the same time in mid morning after the
foliage had completely dried (8:00-10:00am). Bars represent
standard error of the mean (n=15).

Initially this experiment was designed to examine the difference
in root treatments but following preliminary data analysis it
became evident that the more interesting aspect was in the
change in hydraulic conductance in the SPAC over time. The
rapid drop in conductance following planting masked any
changes that could be explained by root treatments. Reduction in
the calculated value of hydraulic conductance was caused by a

combination of reduced stomatal conductance, subsequent reductions in transpiration as well as an initial increase in leaf water potential.

The sufficiency of the hydraulic system to meet demand can be quantified by the ability of the plant to maintain transpiration without a decrease in water potential. As transpirational demand exceeds supply, water retreats further into stomatal pores and the tension on the water column increases. The results in Fig. I.6 illustrate a change in hydraulic sufficiency over time. By plotting conductance against transpiration it is possible to visualize the impact of water potential. The slope of the line declines as water potential (Y_B) become more negative for a given transpiration rate since conductance is calculated by dividing transpiration by water potential.
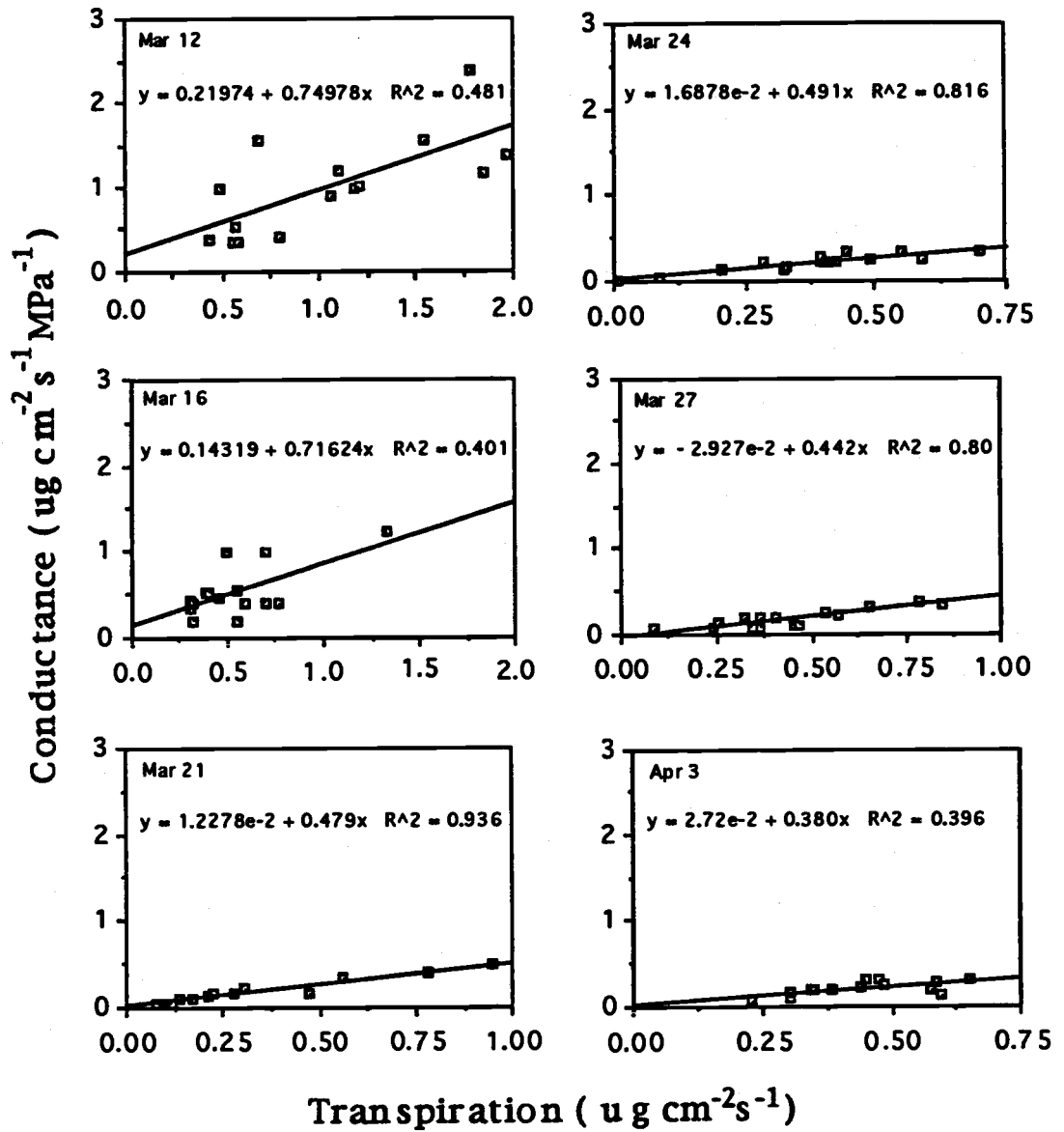
Figure I.6. Transpiration vs leaf specific conductance by date. Each point is a single measurement. n=15.
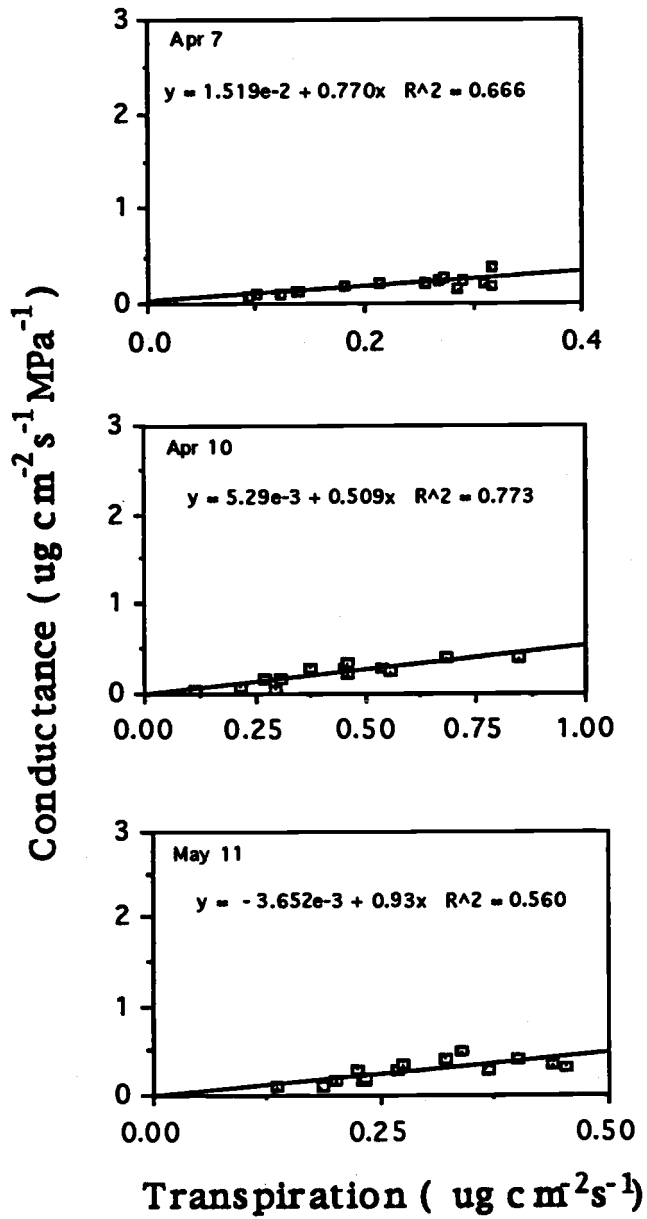
Figure I.6. cont.

Initially (Mar. 13th and 16th) water potential was not the only factor influencing stomatal conductance so the relationship between transpiration and conductance was not very strong (r^2=0.43). Starting with March 21st the relationship between transpiration and conductance became very strong and water potential was able to explain over 90% of the variation (r^2=0.94). This trend continued for the remainder of the experimental period with the exception of a few outliers on 4/3 and 5/11.

Discussion

Stomatal aperture and therefore conductance respond to a variety of environmental stimuli including light, VPD, soil water potential and wind (Smith and Holinger, 1991). Transpiration rate is a function of stomatal conductance and the driving force, VPD. I selected five dates from my field measurements when all environmental variables were roughly equivalent. The VPD was well below the 1.0 Kpa (10mbar) level at which Marshall and Waring (1984) demonstrated stomatal closure in western hemlock. It is evident from the values in Table I.1 that the loss in stomatal conductance and therefore transpiration was not a function of VPD. Initially a leaf water potential of 1.17 MPa was sufficient to supply water at a flux of 1.06 ug cm^{-2} s^{-1} but by the following week a 1.98 MPa differential was required to supply a lower water at a flux of 0.31 ug cm^{-2} s^{-1}. Hydraulic conductance had been reduced severely, resulting in more negative water potentials and stomatal closure.

Table I.1. Selected field measurements of stomatal conductance in cm s^{-1} (gs), vapor pressure deficit in KPa (VPD) Transpiration in ug s^{-1}(F), morning xylem water potential in MPa (Y_B) and calculations of conductance in ug s^{-1} MPa^{-1}(k) on western hemlock following planting.   n=15; standard errors are in parentheses.

Date	VPD	Mean g_s	Mean F	Mean Y_B	Mean k
Mar 12	0.48	0.33 (0.06)	1.1 (0.14)	1.17 (0.11)	1.01 (0.15)
Mar 21	0.50	0.09 (0.02)	0.31 (0.07)	1.98 (0.12)	0.16 (0.03)
Mar 24	0.51	0.10 (0.01)	0.38 (0.05)	2.06 (0.17)	0.20 (0.03)
Apr 7	0.47	0.05 (0.05)	0.22 (0.02)	1.23 (0.07)	0.17 (0.03)
May 11	0.51	0.05 (0.05)	0.29 (0.09)	1.20 (0.09)	0.26 (0.03)

Stomatal conductance in conifers has been shown to be responsive to changes in VPD (Waring and Franklin 1979). Thirteen days following planting stomatal conductance of the western hemlock ceased to be responsive to changes in VPD (Fig. I.6).   Again it appears that stomatal aperture was being limited by the ability of the internal hydraulic system to deliver sufficient water to meet transpirational demand.

It is evident that there has been a reduction in hydraulic conductance following planting that cannot be attributed solely to a loss in ability of the roots to take up water. Growth of new root tips was observed approximately 20 days following planting but

as is illustrated in figure I.4 conductance did not recover. Rather, there seems to be a significant loss in conductivity in some other portion of the SPAC. Borghetti *et al.* (1992) recorded a similar phenomenon in *Picea abies* following water stress and xylem cavitation. Stomatal conductance and F rates were lower for cavitated seedlings when compared to non-cavitated controls at the same water potential. Transpiration rates of *Pinus sylvestris* following xylem cavitation remained below those of non-cavitated controls even though the treatments were measured at a similar Y_B (Pena and Grace 1986). In both of these examples xylem cavitation was implicated in a reduction of hydraulic conductivity which resulted in an increased leaf water potential for a given F rate or as a corollary, a reduced rate of F for a given value of leaf water potential.

In this experiment, a maximum leaf water potentials of 4.0 MPa, measured in the morning 13 days following planting, is within the range of water potentials (0.5 MPa to 5.0MPa) which were found to result in cavitation in conifers (Tyree and Ewers 1991). Since all water potential measurements in this experiment were made in the morning it is assumed that water potential levels negative enough to cause xylem cavitation were frequently reached by mid-afternoon.

To reduce tension on the water column following xylem embolism, adjustments are made to leaf area and/or stomatal aperture therefore preventing runaway cavitation and death

(Tyree and Sperry, 1989; Zimmerman 1983). Foliar adjustments were made by the western hemlock following planting. No measurements were made to quantify foliage loss but by March 21st it was noted that 10-20% of the seedlings had suffered foliage loss and/or branch mortality. By March 24th 10 seedlings had not only died but had completely dropped all foliage and on April 7th it was noted that almost 40% of the planted seedlings had suffered visible needle loss.

Conclusion

Due to reduced stomatal conductance, transpiration and increasing water potentials as well as foliage loss, I further hypothesized that the western hemlock seedlings suffered from severe enough water stress to cause xylem cavitation. The cavitation resulted in a reduction in hydraulic conductivity and therefore conductance.

The emboli had a long-term impact on the seedlings ability to respond to the environment and subsequent productivity. All of the evidence thus far is circumstantial and it was determined that further experimentation was warranted. The following chapters report on the direct measurements of cavitation following planting, the Y_B at which xylem cavitation occurs in western hemlock, and the relationship between xylem cavitation and reduction in hydraulic conductivity.

CHAPTER II
MONITORING CAVITATION FOLLOWING PLANTING

Introduction

There are numerous publications outlining techniques for assessing cavitation events. The three main methods summarized by LoGullo and Salleo (1991) include staining of the sapwood, percentage loss in hydraulic conductivity and acoustic emission detection. Only one of these techniques, acoustic detection of cavitation, is non-destructive. There is a large body of research on acoustically monitoring cavitation events in the laboratory using detached plant segments from a variety of species. Monitoring cavitation events on intact potted *Picea abies* seedlings (Borghetti *et al.*, 1989) and *Pinus sylvestris* seedlings (Pena and Grace, 1986) has been useful in assessing the impact of soil drought on plant hydraulic conductance.

No research had been done to non-destructively monitor cavitation events on seedlings in the field following planting. In addition, no research had been done measuring cavitation rates on planted seedlings which have not been subjected to soil moisture deficit but instead are responding to a reduction in root hydraulic conductance due to planting.

One problem with measuring cavitation in *vivo* was the lack of suitable instrumentation to monitor cavitation rates in the ultrasonic acoustic range. The recent development of an

ultrasonic acoustic emission detector, the Drought Stress Monitor, (Physical Acoustic Corporation, Princeton, NJ), which is not only field portable but also able to store data for several days, has given scientists the ability to measure cavitation non-destructively under a variety of conditions (Tyree and Sperry, 1988). A second limitation to field measurements is the inability of the Drought Stress Monitor to record cavitation events from more than one transducer at a time. The only way to switch between samples is to physically unplug a transducer from the Drought Stress Monitor and plug in a different one or to physically move the individual transducer between samples. These techniques are not realistic for continuous long-term monitoring and severely limit sample size and experimental design.

The sensor that is clamped to the exposed seedling xylem which receives, amplifies and transmits the acoustic emission from the cavitation event is an R15 transducer (Physical Acoustic Corporation, Princeton, NJ). The transducer must receive power from an outside source to function and transmit acoustic emissions to the Drought Stress Monitor. A programmable interface was constructed which controls power to as many as eight transducers making it possible to monitor either a number of seedlings or numerous locations on a single seedling without physically switching connectors. The programmable interface was developed at Oregon State University by Bob Kaser, Joe Zaerr and Kathleen Kavanagh. It is designed to deliver power to a

different transducer on a continuous programmed time interval. Since only one sensor receives power at a time and the Drought Stress Monitor is able to store data on a time dependent basis, it is possible to collect data from each sensor for a given interval (i.e. one minute) and then store it in the Drought Stress Monitor. The data was collected in series without physically moving the sensors between locations. The data were then downloaded into a personal computer where a SAS program was used to compile it. This equipment design does not allow for simultaneous monitoring of samples but it does enable the researcher to obtain a sample of nondestructive measurements of cavitation from a variety of locations over a very short or extended time period.

The objective of this experiment was to monitor cavitation events in western hemlock seedlings following planting in moist soils in a an ambient spring environment (i.e. relatively low vapor pressure deficits)

Methods

Plant Material

Western hemlock plug-1 seedlings from a Coast Range seed source were obtained from the Industrial Forestry Association (IFA) Nursery. The seedlings were donated by Starker Forests Inc. Corvallis, OR.

Forty western hemlock seedlings were planted on April 16, 1991 in well watered sandy loan forest soil contained in covered raised

beds at the Forest Research Lab in Corvallis, OR. All seedlings were watered every 2 days since the raised beds had a fiberglass roof over them which intercepted rainfall. Light levels on a sunny day ranged from 800-1000uE at mid day.

Cavitation detection

The day following planting (April 17th) a R15 transducer was attached to the main stem of each of five randomly chosen seedlings approximately 1 cm above the root collar. The transducers were clamped to a $1cm^2$ patch of xylem which was exposed by carefully removing the bark and phloem. The exposed xylem was covered with petroleum jelly to prevent localized desiccation and improve signal transmission. A sixth sensor was left unattached to serve as a control on the timing of data collection verifying that the Drought Stress Monitor and programmable interface were synchronized. The programmable interface was used to switch between the six transducers once every minute, 24 hours a day; so data were collected in series for one minute out of six per transducer. The programmable interface was connected to an acoustic detector, 4615 Drought Stress Monitor (Physical Acoustic Corp. Princeton NJ.). Total amplification of the signal was 80dB.

There was no statistical analysis of the data since there was not sufficient replication due to equipment limitations. There were only six transducers and one Drought Stress Monitor available.

Therefore the data is descriptive and intended only to indicate wether or not cavitation had occured.

Results

The weather data recorded at the Hyslop Farm weather station in Corvallis, OR (Figure II.1) illustrates that the time period between April 17 to 30th was characterized by high relative humidity(RH) and seasonal temperatures. Hyslop Farm is approximately 5 miles from the research site and is representative enough of the local weather to be chosen as the offical weather station for the Corvallis vicinity. Daytime temperatures averaged 15°C and relative humidity never fell below 80%.
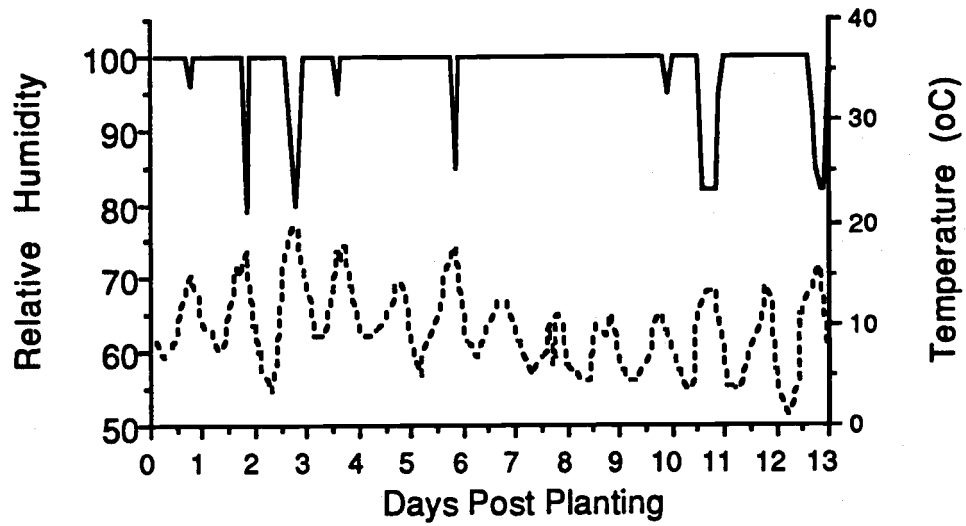
Figure II.1   Weather data collected at the Hyslop farm weather station Corvallis, OR from April 17th to 30th. Relative humidity (———) and Temperature (- - -) measurements were recorded every 2 hours using a Hygrothermograph.
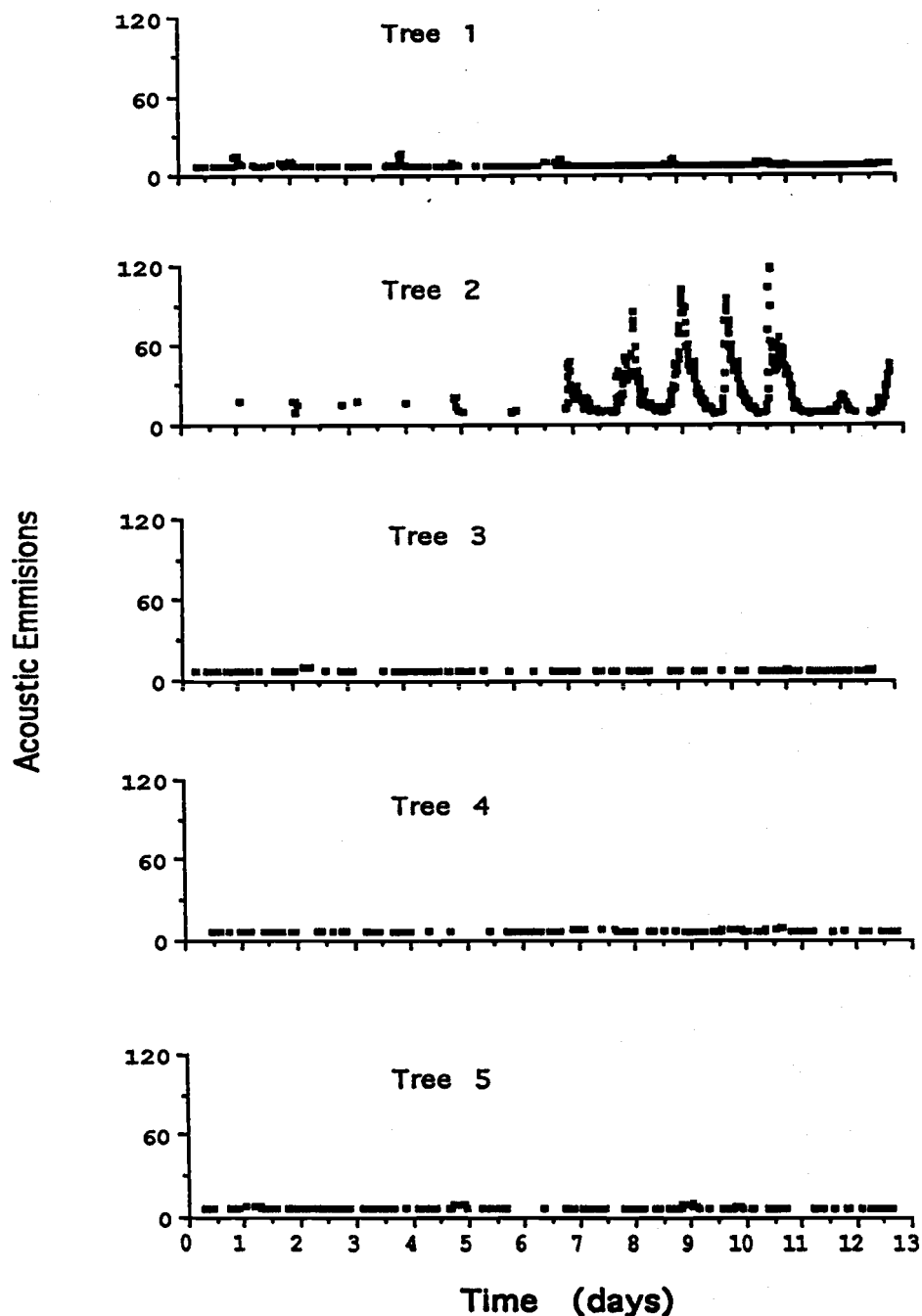
Figure II.2   Acoustic emissions recorded every 6 minutes on 5 Plug-1 western hemlock seedlings following planting.   Acoustic emissions were recorded using a R15 transducer and a model 4615 Drought Stress Monitor (Physical Acoustic Corporation, Princeton, NJ).   Periods with no emissions are not plotted.

There were no AEs recorded by the control sensor. Of the five seedlings monitored only tree #2 experienced severe cavitation in the vicinity of the transducer. Seven days following planting acoustic emissions escalated during the day and decreased in evening and night hours. This diurnal pattern continued for the next 5 days until acoustic emission levels tapered off and the seedling exhibited severe foliar loss and death. Tree # 1 also exhibited a diurnal pattern of acoustic emissions but it was much less severe than tree #2 and the seedling did not succumb. Trees #3, 4 and # 5 experienced very low levels of cavitation with events never exceeding 3 events per minute (Fig. II.2). Foliage and branch loss occurred in trees #1, 3, 4 and 5 during the one-month period following planting. All trees monitored with the exception of #2 were alive 2 months following planting. Eight of the original 40 trees planted succumbed in the two month period following planting.

There did not appear to be any correlation between the weather data collected at Hyslop Farm and the level of cavitation. However, field notes show that commencing seven days following planting, which coincides with the onset of severe cavitation, there were winds which lasted for a total of four days.

Discussion

A model developed by Tyree and Sperry (1988) for a number of coniferous and deciduous species, predicts that when loss of conductivity due to embolism exceeds 10 to 20% then catastrophic xylem embolism can occur. As the number of embolized tracheids increases then a higher pressure gradient is required to deliver sufficient water to the leaves. This increasing pressure gradient (i.e. decreasing water potential) results in an escalation of the cavitation rate which puts additional strain on the remaining tracheids and leads to a vicious cycle referred to as "runaway cavitation" (Tyree and Sperry 1988). Runaway cavitation is illustrated in tree #2 in Fig. II.2. As the water potential increased on a diurnal basis cavitation became severe enough to limit water transport. So on subsequent days the seedling continued to cavitate as water potential increased. The seedling appeared to be unable to halt the diurnal "runaway" cavitation cycle by reducing transpiration and subsequently water potentials during the day. In the evening, when stomatal closure occurred and xylem water potential recovered, the seedling ceased to emit acoustic emissions. This recovery period does not appear to be sufficient to restore the water column to a level where transpiration can be maintained.

It is further illustrated by Tyree and Sperry's (1988) model that trees operate often near the point of catastrophic xylem failure and that under normal conditions stomatal closure occurs prior to

xylem failure. This pattern did not appear to be the case in tree #2 following planting in the present experiment (Fig. II.2). Apparently the tension on water column was not relieved sufficiently to halt cavitation.

Not all the seedlings in this experiment recorded cavitation events but they experienced some foliage loss, indicating the possibility that localized cavitation events may have occurred throughout the crown. The location within a seedling that experiences cavitation initially and/or more severely is dependent on the type of water stress that occurs. Cochard (1992) monitored cavitation events in detached conifer branches from a variety of species. He found that larger diameter, more hydraulically efficient branches were more vulnerable to cavitation. The larger branches were shown to have larger diameter tracheids and were therefore more efficient in water transport, but they were also more vulnerable to cavitation ("efficiency vs. safety"). Initially these findings appear to be in direct disagreement with the Zimmermann's (1983) plant segmentation hypothesis but there is one major underlying difference between the theory and the experiment. Zimmermann's findings are based on "dynamic water stress", water stress that occurs due to transpiration. As a plant transpires, the xylem must transport enough water to meet the demand, if the demand is not met, more negative water potentials result; increasing the gradient or driving force of water transport. Smaller branches which contain less efficient tracheids

must reach a more negative water potential (when compared to the larger more efficient branches) to meet transpirational demand. It is this increase in water potential in the smaller branches which causes "runaway cavitation" to occur in the smaller branches first thereby reducing transpirational demand on the remainder of the plant. Cochard's (1992) findings are based on detached branches which were allowed to desiccate on a lab bench and therefore are assumed not to be transpiring and therefore not experiencing dynamic water stress.

In this experiment it is believed that the seedlings were undergoing "dynamic " water stress leading tree 2 to experience a "runaway" cavitation event in the bole resulting in death. Tree 1 also experienced some cavitation in the main bole but it was not severe enough to cause mortality but there was needle and branch death. Trees 3, 4, and 5 also suffered foliar loss but very few cavitation episodes were recorded in the vicinity of the transducers indicating that they may have been better able to localize cavitation in the more "expendable". branches and needles as outlined by Zimmermann's segmentation hypothesis.

Monitoring cavitation events at the base of the seedling, as done in this experiment, may not be sufficient to detect runaway cavitation events on a smaller scale which result in branch and foliage loss. The ability of the R15 transducer to monitor cavitation events is limited by the propagation of the signal from the tracheid which has embolized. Cochard (1992) determined

that the maximum distance a tracheid could be located from the transducer and still have the acoustic signal recorded was 2 to 3 cm. Therefore by monitoring cavitation events at the base of the seedling it was not possible to detect cavitation and xylem failure in small branchlets.

The ability of plants to avoid cavitation is not only a function of the water potential at which embolisms form but also a function of the hydraulic efficiency of the water conducting system which influences the water potentials required to meet transpirational demand. The seedling observed undergoing runaway cavitation in this experiment appeared to be unable to conduct sufficient water to meet transpirational demand following root damage due to planting. The other seedlings appeared to have been able to compartmentalize their cavitation in the more expendable branches and needles.

Summary

Western hemlock seedlings may experience cavitation events following planting. In some cases the cavitation events are severe enough to result in "runaway cavitation" and seedling mortality. In many other cases it is hypothesized that although runaway cavitation does not occur on the entire seedling, localized cavitation may result in branch and foliage loss. Monitoring cavitation events at the base of the seedling is not sufficient to record such events. In the following chapters the best location for monitoring cavitation will be defined more

precisely and the water potential at which cavitation occurs in western hemlock will be determined.

CHAPTER III
DETERMINING WATER POTENTIAL AT WHICH CAVITATION OCCURS IN WESTERN HEMLOCK

Introduction

Tracheids cease to conduct water when they become air-filled or embolized. The causes of cavitation in xylem conduits include water stress (Tyree and Dixon, 1986; Sperry and Tyree, 1988, 1990), freezing (Sauter, 1984; Sperry *et al.* 1988) aging, (Sperry *et al.*, 1991) and pathogens (Zimmermann, 1983; Kuroda, 1991) and physical injury. Cavitation during water stress occurs when the xylem water potential (Y) reaches a level negative enough to pull air into water filled trachieds from adjacent air filled tracheids. The pulling force (tension) of the water column must be great enough to overcome the surface tension forces at the air-water interface in the inter-tracheid pit membrane (Zimmermann, 1983; Crombie *et al.*, 1985; Sperry and Tyree, 1988, 1990).

Differences in vessel and tracheid anatomy influence the water potential at which cavitation occurs. Initial studies suggested a strong relationship between xylem conduit diameter and vulnerability to cavitation, with more xeric adapted species having smaller less vulnerable vessels or tracheids (Carlquist, 1975). More recent work has demonstrated that this relationship holds up well within a species but between species there is little correlation between xylem conduit diameter and vulnerability to

cavitation (Tyree and Sperry, 1989). It has been determined however that the more vulnerable a species is to cavitation the lower the xylem tensions it can withstand under natural conditions (Sperry et al., 1988) or as a corollary: drought tolerant species are less vulnerable than drought avoiding species (Cochard, 1992). Vulnerability to cavitation in conifers covers a wide range of water potential values. *Abies balsemea* and *Picea rubens* have been shown to embolize at tensions between -2.0 to -3.0MPa and become completely non-conducting between -3.0 to -4.0MPa whereas *Juniperius viginiana* didn't begin to cavitate until it reached values below -4.0 MPa (Sperry and Tyree, 1990). Significant embolisms occurred in *Pinus sylvestris* at -2.5 MPa, *Pseudotsuga menziesii* and *Abies alba* embolized starting at -3.0MPa but the more drought tolerant *Cedrus atlantica* initiated significant cavitation at -4.0 MPa (Cochard, 1992).

The level of water stress that western hemlock can incur without suffering xylem cavitation has not been determined. The objectives of the following experiment were to: (1) Determine the optimum location for monitoring cavitation events with an ultrasonic detector in western hemlock seedlings and (2) determine the leaf water potential at which xylem cavitation occurs in western hemlock.

Methods

Experiment 1

Branches, 20-30cm in length, were cut from a western hemlock sapling growing adjacent to the Forest Sciences Lab in Corvallis, OR. Three branches were sampled from December 1 to 7, 1990.

Immediately following harvest each sample was brought into the laboratory where a small foliar sample was removed and initial water potential (Y) measured using a pressure chamber apparatus (PMS Instrument Co., Corvallis, OR). To monitor cavitation events the sample was then connected to an acoustic emission transducer (R15, Physical Acoustics Corp., Princeton, N.J. USA) which was connected to an acoustic detector, 4615 Drought Stress Monitor (Physical Acoustic Corp. Princeton, N.J. USA). The transducer amplified the acoustic emission following cavitation by 20dB and the detector further amplified the signal by 40dB, for a total of 60dB. The transducer was clamped to a $1cm^2$ patch of xylem which was exposed by carefully removing the bark and phloem. The exposed xylem was covered with petroleum jelly to prevent localized desiccation and improve signal transmission. The acoustic emission data was collected by a dedicated microcomputer built into the detector.

The branch was left exposed on the lab bench and allowed to desiccate. Y was measured, removing a small foliar sample, as soon as acoustic emissions (AEs) were detected then every 3

hours thereafter. If needed, the sample was covered with a moist towel and plastic bag to prevent further desiccation overnight.

Experiment 2

Western hemlock plug-1 seedlings were obtained from the Industrial Forestry Association (IFA) Nursery. The seedlings were donated by Starker Forests Inc., Corvallis, Oregon.

In July 1991, three seedlings were removed from cold storage and planted in the same pot using potting soil, vermiculite and perlite (2/1/1). The seedlings were then placed in a greenhouse maintained at a day/night temperature of $28/20^{\circ}C$. Light levels averaged 700umol with a range of 300-1200umol. The pot was watered to field capacity.

One hour following planting an ultrasonic detector (Drought Stress Monitor, Model 4615 Physical Acoustic Corporation, Princeton, NJ) was attached to an individual seedling via six acoustic transducers. The signal amplification settings were the same as above except the detector amplification was set at 60dB, for a total amplification of 80dB. Three sensors were placed on the main stem of the seedling (low, mid, and high levels) and three sensors were placed on lateral branches (low, mid and high laterals). The sensors were attached as above.

A programmable interface was used to switch between the six sensors every minute, 24 hours a day; so data were collected for

one minute out of six for each sensor. The data were stored in the Drought Stress Monitor and later downloaded into a PC.

On the second day of the experiment Y measurements were made as above on the seedling being monitored for acoustic emissions. Measurements were made at 4:15, 9:00, 11:00, 12:00, 13:00, 15:00, and 17:00. On the third day it was no longer possible to obtain a water potential measurement due to excessive readings and the experiment was terminated.

Results

Experiment 1

Descriptive statistics were not used in the analysis of this data due to the unequal branch diameter having an influence on the actual number of acoustic emissions (AE) detected. Therefore any variability described would be due to diameter changes and not relevant to the objective. The pattern of cavitation that is illustrated in Figure III.1 is more descriptive in this case.

Initial water potential measurements were -1.0MPa on all branches sampled. The first AEs were detected at approximately -1.5MPa with rates rapidly escalating after the branches reached approximately -2.8MPa. Reaching a peak of 90 EPM at 3.6MPa (Fig. III.1). The rate then decreased rapidly at more negative water potential. The pattern of cavitation and water potential was similar for each branch but the actual number of events

varied due to changes in branch diameter where the transducer was attached.
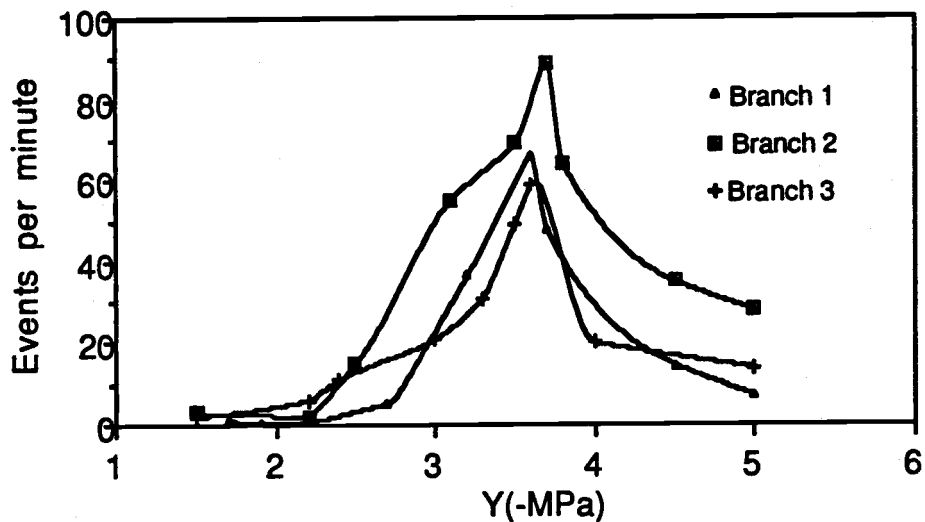

Figure III.1. Xylem cavitation rates related to xylem water potential (Y) in 3 detached western hemlock branches dehydrated on a laboratory bench.

Experiment 2

The acoustic sensor placed at the base of the seedling stem recorded at least five times more acoustic emissions than any other sensor throughout the day (Table III.1).

Table III.1.--Number of acoustic emissions recorded over a 9 hour period by six sensors placed on a single plug-1 western hemlock seedling.

Time	Seedling stem			Lateral branch		
	low	mid	top	low	mid	top
(hour)	--------*AE per minute*[1]--------					
8:00	0	0	0	0	0	0
9:00	57	7	8	1	1	0
10:00	253	17	31	4	3	0
11:00	246	33	62	9	4	1
12:00	407	46	84	17	6	2
13:00	479	40	86	7	7	2
14:00	172	18	34	9	3	1
15:00	135	9	30	2	2	1
16:00	30	6	13	1	0	0
17:00	0	0	0	0	0	0

[1] Acoustic emissions per minute were averaged over the one hour period.

By computing a cavitation index, the mean number of cavitation events per minute divided by the total number recorded for the entire time period, it is possible to assess the pattern of cavitation throughout the day (Fig III.2).
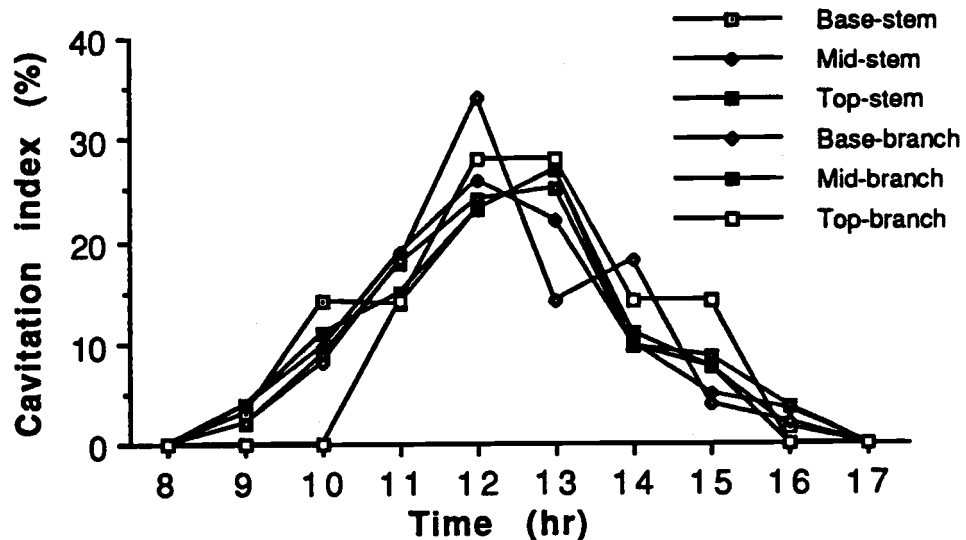
Figure III.2 Cavitation index (mean number of events per minute divided by the total number of events recorded over the 9 hour period) expressed as a percent. Data was obtained by attaching 6 sensors to a western hemlock seedling planted in a greenhouse.

On the day following potting, predawn water potential was -1.2 MPa. The number of acoustic emissions detected increased as water potential became more negative with a decrease in acoustic emissions in the afternoon as water potential became less negative (Fig. III.2). Acoustic emissions began at a xylem water potential of -1.9MPa, reached a peak at -3.4MPa around noon, then became less negative. Note that the water potential the seedling experienced was not a result of soil water availability but rather a result of short term exposure to an environment with a high vapor pressure deficit. In the early afternoon water potential became less negative, perhaps because of stomatal

closure and/or a reduction in VPD due to shading by a tree adjacent to the greenhouse.

By comparing the rate of acoustic emissions between experiment 1 and 2, maxima of 60 vs. 460 EPM respectively, it is evident that the signal amplification rate has a profound effect on the number of emissions recorded per minute. Therefore the amplification rate should be maintained at a constant dB level.
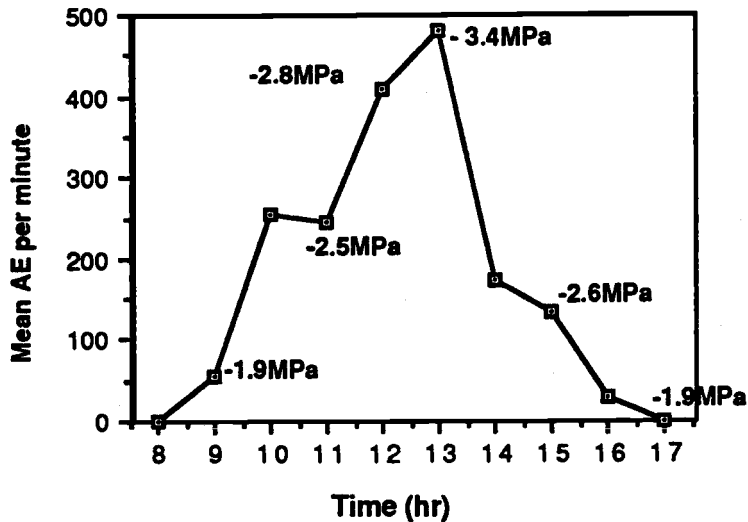

Figure III.3.--Acoustic emissions (AE) per minute (averaged over one hour). Data was obtained from a single sensor placed at the base of a plug-1 western hemlock seedling. Water potential measurements are shown alongside the data points.

Discussion

It is evident from the results that western hemlock seedlings initiate cavitation at relatively low levels of water stress. This is not an unusual result since the native range of western hemlock is generally limited to cool, moist coastal and moist upland Cascades sites. These results agree with the hypothesis of Sperry *et al.* (1988) that the more vulnerable a species is to cavitation the lower the xylem tensions withstood under natural conditions.

The rate of cavitation was found to be relatively high at -2.5MPa; a water potential which was frequently exceeded in the planted seedlings monitored in the experiment described in Chapter I. Sufficiently negative water potentials for significant cavitation were found to occur by 9:00am on sunny days following planting in moist soils.

Acoustic emission detection was highest at the base of the seedling for a variety of reasons: (1) The larger stem diameter increased the contact area with the sensor allowing for more signals to be recorded. (2) An increased diameter also increases the number of tracheids close enough to the sensor to transmit a signal. (3) Due to basal taper a higher percentage of larger diameter tracheids are located at the base of the seedling.

It is interesting to note that the cavitation index did not change significantly between sensor location(Fig III.2). I hypothesize from this pattern that the seedling experienced a major

"runaway" cavitation event resulting in emboli formation throughout the crown and stem. Had segmentation occurred I would have expected a higher percentage of cavitation to occur in the branches initially.

Within a species, early wood tracheids have been shown to be more vulnerable to cavitation than late wood. (Tyree *et al.* 1984, Dixon *et al.* 1984, Sperry and Tyree 1990, Logullo and Salleo 1991). Earlywood tracheids are characterized by a larger radial diameter, thin cell wall, increased size of lumen, larger pit aperture and number of pits. Late wood which provides a majority the mechanical strength in the xylem, is characterized by thick cell walls, narrow lumen, fewer pits and pits with a small diameter (Panshin and DeZeeuw, 1980)

Since flow rate through a tracheid is proportional to the 4th power of the radius of the tracheid (Zimmermann, 1983) the initial loss of large tracheids due to cavitation may have a significant effect on water conductivity. Zimmermann (1983) calculated that 96% of the water transported by the xylem passes through the earlywood and any change in the proportion of early to latewood would have an impact on the conductivity of the hydraulic system. During a "runaway event as illustrated in Fig. III.3 the loss of larger earlywood tracheids at the base of the seedling signifies a significant loss to water conduction at a location where further sacrifice (as in the segmentation

hypothesis) is not possible. In addition the loss of these larger conduits in western hemlock under relatively low water stress (-1.9MPa) had a significant affect on the ability of the seedling to conduct water to the foliage and is an indication of the susceptibility of western hemlock to water stress.

Not all cavitation episodes are as severe as recorded in this experiment. By placing the seedlings in a hot relatively low humidity greenhouse it was possible to record a "runaway" event. Since the environmental conditions following planting are not usually as severe as described in this experiment, I believe that the segmentation model would better describe the typical foliage loss and morphological changes experienced following a cavitation episode in a newly planted seedling. The seedling must make an adjustment to the new hydraulic conditions. This adjustment may come in the form of foliar loss, particularly of lower lateral branches and/or a reduction in current year's needle growth. If the seedling is unable to make such adjustments then the water potential may reach a level where cavitation is rampant resulting in seedling mortality. It is possible that following planting and the onset of a warm, dry east wind runaway cavitation can occur.

Summary

Western hemlock seedlings were found to cavitate at water potentials well within the range experienced following planting. There is evidence that during a runaway cavitation event initial

cavitation occurs throughout the crown and stem resulting in a severe impact on hydraulic conductance. Further studies need to be done to determine the impact of xylem cavitation on stem hydraulic conductivity and conductance by measuring the percent loss of hydraulic conductivity that is experienced following different levels of exposure to water stress and xylem cavitation.

CHAPTER IV
WESTERN HEMLOCK VULNERABILITY CURVE

Introduction

The percent loss in hydraulic conductivity following a cavitation event is directly linked to the diameter of the conduit that is embolized. According to Zimmermann's' (1983) "efficiency vs safety" theory the larger more efficient tracheids are the first to cavitate. Therefore, simply measuring cavitation events does not fully evaluate the impact of embolism formation on the plant's water conducting system. The loss of larger tracheids in the early stages of a xylem cavitation event has a proportionately greater effect on conductivity than the loss of numerous small tracheids.

Vulnerability curves illustrate the percent loss of hydraulic conductivity plotted against xylem water potential (Y). When a plant's xylem water potential reaches a level where cavitation occurs (see Chapter III) reduction in hydraulic conductivity can be expected. Vulnerability profiles can be used to estimate the effect of moisture stress on water transport in the xylem by illustrating the water potential at which the impact of cavitation is realized as well as the range of water potentials that result in decreasing water transport (Tyree and Sperry, 1989).

Vulnerability curves have been developed for several coniferous species with a range of vulnerability profiles. No curve has been

developed for western hemlock seedlings. It was the objective of this experiment to develop a vulnerability curve for western hemlock seedlings so that the relationship between water potential, level of cavititation, and subsequent loss in hydraulic conductivity can be fully described.

Methods

Plant material

Western hemlock plug-1 seedlings were obtained from the Industrial Forestry Association (IFA) Nursery. The seedlings were donated by Starker Forests Inc., Corvallis, Oregon.

Initial Preparation

A sample seedling was prepared by severing the root system at soil level. A 5 cm long stem segment was cut from the base of the seedling, wrapped in plastic and refrigerated to prevent desiccation for up to one hour. The segment was used to measure initial hydraulic conductivity (see below) prior to cavitation. The exposed surface of the seedling was immediately sealed with paraffin to prevent localized desiccation.

To monitor cavitation events an R15 transducer was attached to the main stem approximately equidistant from the tip to the base. The transducer was connected to an acoustic detector, 4615 Drought Stress Monitor (Physical Acoustic Corp., Princeton, N.J.). Total amplification of the signal was 80dB. The transducer was

clamped to a 1cm^2 patch of xylem which was exposed by carefully removing the bark and phloem. The exposed xylem was covered with petroleum jelly to prevent localized desiccation and improve signal transmission. The acoustic emission data was collected by a dedicated microcomputer built into the detector.

Following attachment of the acoustic detector the seedling was placed in a growth room . Due to the removal of the roots and subsequent lack of water, dessication occurred. Periodic measurements of xylem water potential(Y) and hydraulic conductivity were made concurrently.

Measuring hydraulic conductivity

The excised stem segment was submerged under water and the bark removed. The segment ends were then trimmed to eliminate air-filled tracheids caused by initial cutting and exposure. Since conifer tracheids are approximately 3 mm long (Zimmerman, 1983) one centimeter of each end of the stem segment was excised. While still submerged the proximal end of the stem section was securely attached to tygon tubing, then removed from the water. A measuring solution of 10$u$mol/l oxalic acid was prepared with double distilled water then filtered to 0.22um and degassed by sparging with helium. A syringe and needle was used to remove the water in the tubing and replace it with the measuring solution. The tubing with the segment attached was then connected to a reservoir containing the

measuring solution being careful to prevent any air bubbles from forming in the tubing. The solution in the tubing was pressurized to 10kPa by gravity.

The amount of solution passing through the stem segment was measured by attaching a pre-weighed vial containing filter paper to the distal end of the segment. The vial was removed and weighed at 60 second intervals.

Hydraulic conductivity(k_h) of each segment was computed utilizing the following relationship:

$$F=k_h \left(\frac{dP}{dx}\right)$$

Where F is the measured flow rate, dP is 10kPa and dx is the segment length. In order to compare segments with varying diameters, specific hydraulic conductivity (k_s) of each segment was calculated by dividing k_h by the sapwood cross-sectional area of that segment.

Vulnerability curve

Following the initiation of cavitation events due to desiccation a second 5 cm stem segment was removed from the base. The cut end of the seedling was immediately resealed with paraffin. The detached segment was immediately placed in water and the ends trimmed leaving a 3 cm segment which was used to measure hydraulic conductivity as above. In conjunction with the removal of the stem segment, measurements of water potential were

made on a small foliar sample with a pressure chamber apparatus (PMS Instruments, Corvallis OR).

A third, sometimes fourth segment (depending on seedling size) was sampled along with water potential as desiccation proceeded. This experimental procedure was repeated for 10 seedlings with each seedling providing 3-4 segments for hydraulic conductivity and water potential measurements.

To insure that no change in specific conductivity (k_S) occurred within the area sampled for hydraulic conductivity the main stem of 3 seedlings were dissected, prior to cavitation, into 4 adjacent segments and k_S measured as above.

To check for leaks in the tubing and/or confirm cavitation the segment was perfused, under 10kPa of pressure, with 0.2% w/v safranin dye filtered to 0.22um. Any seedling whose initial segment showed signs of previous cavitation was eliminated from the population used to construct the vulnerability curve.

Calculations

Percent loss in specific hydraulic conductivity was calculated using the following relationship:

$$\%loss = \frac{k_S - k_Y}{k_S} \times 100 \tag{1}$$

where k_S is initial specific conductivity prior to cavitation and k_Y is the specific conductivity at a given water potential (Y). Percent

loss was plotted against the water potential measured at the time of segment removal.

Statistical Analysis

Using the data generated above a line representing a fitted logistic regression of the data was calculated (i.e. Log ($\frac{loss}{1-loss}$ = $B_0 + B_1 * Y$). A t-test of the parameters Bo and B_1 was also done to determine if the line was significantly different from zero.

Results

As illustrated in Table IV.1 specific conductivity did not vary significantly between adjacent segments on a non-cavitated seedling.

Table IV.1   Specific hydraulic conductivity for adjacent stem segments of western hemlock seedlings.

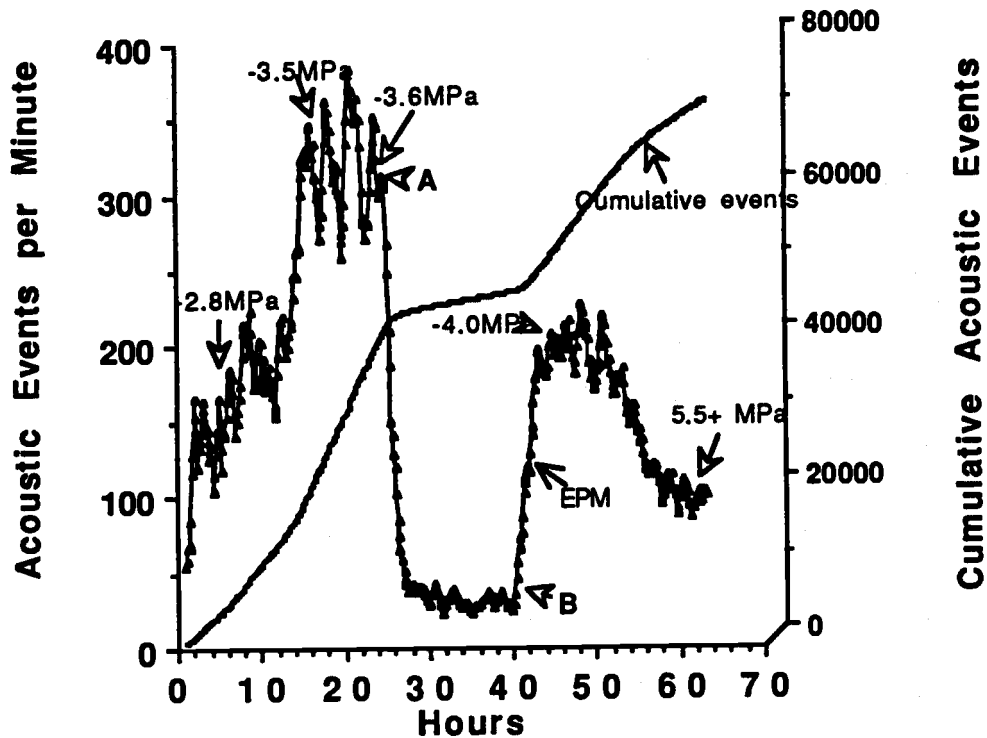|	Specific Hydraulic Conductivity(ug/s KPa cm^2)			
	Segment 1	Segment 2	Segment 3	Segment 4
Seedling				
1	0.059	0.061	0.062	0.053
2	0.044	0.042	0.041	0.042
3	0.065	0.066	0.064	0.064

Figure IV.1 Example of acoustic emission data collected during western hemlock seedling desiccation. Acoustic events per minute were measured continuously 1 out of every 8 minutes. Approximately 24 hours after the initiation of data collection the seedling was covered (Point A) with a moist plastic bag which was removed the following morning (Point B). Water potential readings are shown along appropriate data points. These data are for a single seedling.

Vulnerability to cavitation for coastal western hemlock seedlings is shown in Figure IV.2 where percent loss in specific hydraulic conductivity is plotted against xylem water potential.
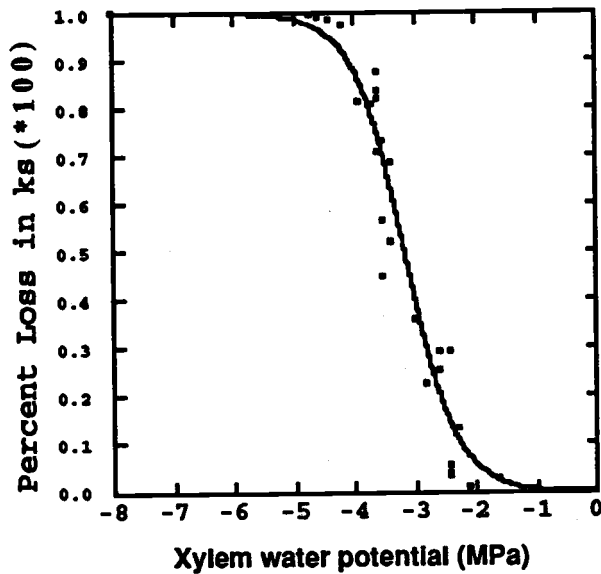

Figure IV.2   Percent loss in specific conductivity (ks) with decreasing water potential in western hemlock seedlings.   Each point represents one water potential (Y) and k_S measurement.   Line represents a fitted logistic regression of the data (p<0.0001).

Discussion

By examining the vulnerability curve one is able to deduce the relative ability of a species to withstand cavitation and subsequent embolism formation. Vulnerability curves have been developed for numerous species and there is a correspondence between the ability to conduct water following exposure to low water potentials and drought resistance (Tyree and Ewers, 1991). Among the coniferous species examined *Juniperus virginiana* was found to be least vulnerable with a 20% loss of hydraulic conductivity at -5.0 to -6.0 MPa compared with *Abies balsemea* which experienced a 50% loss between -2.0 and -3.0 MPa (Tyree and Ewers, 1991). Cochard (1992) examined a variety of coniferous species with similar results. The more drought tolerant species such as *Cedrus atlantica* were the most resistant to cavitation and the more drought intolerant *Pinus sylvestris* the least.

The vulnerability curve for western hemlock seedlings (Fig. III.2) illustrates an intolerance of water stress. A 30% loss in hydraulic conductivity was experienced following a water potential between 2.5 and 3.0MPa. A 50 % loss was measured following water potentials of 3.0MPa. There was a complete loss of conductivity after reaching water potentials of 4.0MPa. The relative steepness of the vulnerability profile illustrates the rapid loss of hydraulic conductivity following exposure to low water potentials.

There is not much data on the ability of conifer species native to the Pacific Northwest to avoid xylem cavitation. Cochard (1992) developed a vulnerability profile for *Pseudotsuga menziesii* (Douglas-fir) branches from trees growing in France. Reduction in hydraulic conductivity began at 3.0 MPa with 50% loss occurring at 3.6MPa. Total loss occurred at 5.0MPa. Douglas-fir was shown to be less vulnerable than the western hemlock with cavitation and subsequent loss in hydraulic conductivity starting at a more negative value of Y. These results agree with the species distribution in the Pacific northwest. Western hemlock has a more limited distribution than Douglas-fir with populations found on more moist coastal and upper Cascade Range sites.

Conclusions

Following planting western hemlock seedlings experience water potentials where xylem cavitation and loss in hydraulic conductivity can occur. This loss in conductivity is reflected by a decrease in conductance (i.e. requiring an increasing pressure gradient to meet transpirational demand). The seedling may respond to changing xylem conductance by reducing stomatal aperture and/or by reducing leaf area.

CONCLUSIONS

The purpose of this thesis research was to provide an answer to the question of unexplained mortality and/or foliar loss in western hemlock following planting. In the initial experiment, observations of water potentials in excess of -3.5MPa as well as a reduction in hydraulic conductance indicated that water stress was implicated. The water stress experienced in the first few days following planting had a long-term affect on the seedling's ability to transport sufficient water to meet transpiration demand.

The loss in hydraulic conductance was further explored by considering the relationship between water potential, xylem cavitation and embolism formation. It was determined that xylem cavitation can occur in western hemlock following planting with events starting at -1.9MPa and with maximum rates recorded at -3.5MPa. These values were well within the range experienced by the seedlings planted in experiment 1. To further clarify the relationship between cavitation rates and loss in hydraulic conductivity a vulnerability curve was constructed for western hemlock seedlings. A 30% loss in hydraulic conductivity was measured following a water potential between -2.5 and -3.0MPa. A complete loss of water movement capability was experienced following water potentials of -4.0MPa.

The loss of foliage can be explained by Zimmermann's (1983) plant segmentation hypothesis which states that during periods of water stress xylem cavitation will occur initially in secondary branches to protect the more valuable bole from embolism formation. Mortality on the other hand is indicative of a "runaway" cavitation event as proposed by Tyree and Sperry (1988). The strong relationship between sapwood conductivity and leaf area lends further support to these models.

Further research needs to be done to examine a more direct cause and effect relationship between loss in hydraulic conductivity and subsequent changes in leaf area. In addition, anatomical and physiological studies need to be done to determine why western hemlock seedlings cavitate at relatively high xylem water potentials and why there seems to be a lack of sufficient stomatal control to prevent water stress severe enough to initiate cavitation following planting. Although I did not have sufficient data to build a strong case for the importance of temperature, relative humidity, light levels and wind in setting up the conditions in which cavitation is experienced, I believe these factors play an important role through stomatal function and flux. Further research needs to be done to characterize the importance of environmental variables in initiating cavitation events following planting.

As outlined above development is required before this technology can be applied. Future directions anticipated include

using hydraulic conductance as an indicator of seedling vigor. If a seedling has experienced water potentials during culture and handling in excess of levels required to cause cavitation it may be predisposed to failure following planting. It may be possible to develop a method quantifying percent loss in hydraulic conductivity with plant performance. It is possible that "transplant shock" i.e. reduced growth, internode and needle elongation may be a response of the seedling to a change in xylem hydraulic conductivity caused by water stress.

Although this research does not explain all the causes of western hemlock seedling mortality it does propose a model which is helpful in diagnosing plantation failures.

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