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Jose M. Montano for the degree of Doctor of Philosophy

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Title: Postharvest Water Relations and Needle Abscission in Cut Trees of Douglas-fir (Pseudotsuga menziesii Mirb. Franco)

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William M. Proebsting

At room temperature, cut Douglas-fir trees reached a critical water potential (ψ_L) of -3.5 MPa within 12 days. About one-half of the trees dried to this ψ_L lost significant quantities of needles, while the remaining one-half lost from zero to less than 5% of the needles. Trees at this stage of water stress appeared comparable to the control and did not exhibit further damage if rehydrated. Foliage of trees allowed to dry below -3.5 MPa became brittle and did not recover after rehydration. Comparison of various methods to study water relations in cut trees indicates that ψ_L is an appropriate single measurement of the water status of cut trees and the damage threshold.

Water potential was maintained above the damage threshold for at least 1 month if the trees were stored under lath house conditions with wet foliage. Under indoor conditions, watered trees maintained a ψ_L above the damage threshold for at least 2 weeks, but unwatered trees dried to -5.8 MPa within the same time.

The ability of antitranspirants to retard tree moisture loss was related to the storage environment. Under outdoor conditions,

ψ_L was maintained above the damage threshold for at least one month. Under greenhouse conditions, water stress of antitranspirant treated trees was nearly equivalent to untreated trees within 4 to 6 days.

Formation of the abscission zone is an integral part of Douglas-fir needle ontogeny. The separation layer was formed by late July, shortly after complete needle elongation. The separation layer was formed from a layer of cells proximal to the intercalary meristem. The protective layer was formed shortly afterwards and was situated proximal to the separation layer.

Abscission appeared to be the result of mechanical tearing of separation layer cells induced at a ψ_L of -3.5 MPa. During drying, normally thick secondary walls of the separation layer shrank, eventually tearing on all planes. A ligno-suberized layer of cells (protective layer) proximal to the separation layer remained attached to the bark after abscission.

POSTHARVEST WATER RELATIONS AND NEEDLE ABSCISSION IN CUT TREES OF DOUGLAS-FIR (PSEUDOTSUGA MENZIESII MIRB. FRANCO)

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Jose M. Montano

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Chapter 1

INTRODUCTION

Desiccation of cut trees is a problem that plagues the Christmas tree industry and its consumers. Water loss from cut trees reduces cosmetic appeal and eventually creates a fire hazard. Inquiry into the problem is generally lacking. It could be desirable to maintain a high water content throughout the commercial handling, storage, and home use of cut Christmas trees. Use of antitranspirants has generally been ineffective, while some storage techniques have been beneficial. During home use, it is thought that trees with high initial moisture content will remain hydrated if the stem base is recut, immediately immersed in water, and kept continuously immersed. Effective storage methods provide protection from sun and wind, temperatures below 2.0°C and high atmospheric humidity.

Although it is known that these storage and handling practices will keep the trees fresh, little is known about the effects of environmental variables on rates of moisture loss from cut trees or about anatomical and morphological manifestations of dehydration. Desiccation levels and rates that result in damage have not been established. Dependable visual methods to recognize damaged trees are lacking. Flammability (201), needle loss (8) and percent mois-

ture content (154) are commonly measured, but techniques to measure the latter two factors lack precision. Anatomical events that occur during water stress-induced abscission in Douglas-fir are not known. Knowledge of such events could be useful in understanding and perhaps controlling water loss and reducing needle abscission. Antitranspirant effectiveness in reducing moisture loss is poorly documented. The objectives of this study were to develop methods for studying the postharvest physiology of Douglas-fir Christmas trees; to assess the major factors affecting postharvest performance and to develop techniques for improving and prolonging postharvest quality.

Chapter 2

REVIEW OF LITERATURE

Introduction

Pacific Northwest races of Douglas-fir indigenous to the coastal range west of the Cascades are climatically adapted to high winter rainfall (199, 220). Morphological and physiological features for water conservation are poorly developed in this species. Coastal ecotypes possess a thinner needle cuticle (105) than inland ecotypes and with stomata closed, may still transpire at a rate 50% that of the open value (98). Mesophyll resistance plays a greater role than stomatal resistance in regulation of water loss (51, 70, 115). Resistance to water loss from coastal ecotypes is low when compared to inland ecotypes (145, 220) or to several species of pine and true fir (115). When foliage moisture content is high (winter), stomata remain open at night (51, 159) and Douglas-fir stomatal conductance can exceed 4 mm/sec (135), compared to 1.4 mm/sec for red pine (208) and 1.9 mm/sec for grand fir (159). Under increasing water stress, coastal ecotypes continue to transpire, whereas corresponding transpiration rate of inland ecotypes declines (220).

Stomatal Physiology

Stomatal closure in coastal Douglas-fir typically begins at -2.0 MPa (85, 86, 87, 88, 159, 160) as leaf-air vapor pressure difference increases and needle water content decreases (51, 115). Closure may be gradual (70, 115) or may not occur at all (51) as

water deficits develop. The critical water potential for stomatal closure may vary with time (12), or with environmental factors that modify stomatal response (95) such as water stress history (94, 199) as well as differences in clonal or cultivar response to water stress (98).

Environmental Effects

Environmental factors affecting stomatal conductance are irradiance (171), leaf water status (159, 172), ambient humidity (103, 104, 166), leaf temperature (65, 77, 151) and carbon dioxide concentration (67, 150, 194). Each of the factors may act independently (95), or simultaneously (80) to affect stomatal movement. Several treatises on stomatal physiology have been published (46, 73, 110, 127, 150, 195, 222, 223) and an in depth view will not be given here.

Temperature

Douglas-fir stomatal responses to temperature are important from the standpoint of storage treatments for cut trees. Temperature effects are difficult to quantify because changes in temperature will simultaneously result in changed leaf-air vapor pressure difference (99, 161). The temperature response curve is similar to a normal distribution (77), with closure occurring at both extremes and an optimal midrange for opening. At the cold end of the scale (-2.0°C), ponderosa pine (41) and Douglas-fir stomata (151) have been shown to close. As temperature increases, stomatal opening

increases (40, 71). Optimal range values for stomatal conductance are species variable (64, 167, 172), as are decreases in conductance with increasing temperature above the optimum range (39, 68, 152).

Atmospheric Moisture Content

Stomatal response to atmospheric humidity is thought to occur by feedback and/or by feedforward modes. The feedback process involves stomatal response to leaf water status (150), although internal leaf carbon dioxide concentration may also have an influence (194). In contrast, during feedforward mode, stomata respond to changes in the water relations of epidermal or guard cells (65, 79, 166, 167). Douglas-fir stomata close at a threshold water deficit (159) which may be associated with a leaf turgor pressure of zero (93, 197). This indicates that substantial water losses occur before stomata respond to changes in leaf water status. This is important in cut trees because water storage capacity in needles and stem tissue is limited (3.2 liters for a 2 m Douglas-fir compared to 66,000 liters for an 80 m Douglas-fir, 160). Waring et al. (209) concluded that a maximum of 50% of the total water transpired by mature Douglas-fir trees may come from the sapwood water reservoir. For juvenile trees, root water storage is of greater importance during high atmospheric demand (78, 82, 106).

Abscisic Acid

The hormone most associated with water stress and stomatal closure is abscisic acid (ABA). Increase in plant moisture stress has been associated with an increase in ABA concentration (13, 116, 217, 219). An abrupt increase in ABA concentration and a distinct decline in stomatal conductance at a critical water potential (zero turgor pressure) have been demonstrated in Douglas-fir (16, 87, 138) as well as with other species (1, 114, 146). There is general agreement that ABA content increases as water potential decreases but ABA concentration may remain high (7, 37, 138), decrease rapidly after drought recovery (13, 69, 116), or decrease during prolonged water stress (88). During water stress, ABA appears to directly or indirectly affect stomatal movement (29, 101, 221). This may be accomplished by ABA's ability to inhibit potassium ion uptake and hydrogen ion extrusion from guard cells (205). Application of ABA to the foliage has in some cases resulted in stomatal closure (29, 33, 90, 101). Aspinal (10) concluded that ABA's role in severe or prolonged water stress may be minimal, and Murphy (135) found no relationship between ABA, stomatal conductance and water potential in coastal Douglas-fir during the winter months.

ABA's endogenous role in stomatal closure is not yet clear. Rapid induction of water stress results in stomatal closure (119, 173) but there is a distinct lag in ABA accumulation after the stomata begin to close (13, 68, 206). Stomata from epidermal strips will close or open rapidly when exposed to streams of either dry or humid air (126). This may be due to rapid removal of guard cell

water (173) and loss of turgor. In contrast ABA seems to affect stomatal closure during slow development of water stress.

Foliar Water Absorption

Cuticular water absorption will occur if there is a gradient of decreasing water potential from the atmosphere to the leaf. A wet cuticle swells and becomes permeable to water (163, 164), whereas stomatal water uptake appears to be negligible (30, 198). Survival of water stressed conifer seedlings has been increased by absorption of water from leaf surfaces (77, 89, 177, 184, 185, 186, 187, 188, 189, 204). Cremer & Svensson (28) reported that it took 22 hr of misting to replace water that was lost by cut shoots of Pinus radiata during 2 hr of rapid transpiration. Michaelis & Michaelis (129) and Gates (57) found that detached conifer branches absorbed enough water during a winter night to compensate for that lost during the day. Water uptake can occur through specific areas on a leaf or through the whole leaf. Leyton & Juniper (112) showed that water uptake by Pinus sylvestris needles was mainly through the adaxial needle surfaces normally enclosed in the needle bundle sheath, whereas in Pinus radiata, water was absorbed by the entire needle surface (111).

Cell Shrinkage During Water Stress

The volume of living cells and tissues change considerably with changes in water content. During rapid induction of water stress, cells tend to shrink (98). Water stress develops first in

the leaves and is then transmitted through increased tension in the xylem sap to the cambial sheath (99, 100). Chaney & Kozlowski (23) found that leaf thickness of water stressed "Calamondin" orange trees was negatively correlated with leaf-air vapor pressure difference. Stem diameters of intact large trees contracted during the day and recovered their original volume at night (19, 165). Shrinkage has been measured in young bark tissue when water was extracted from the 100-cell thick band of living tissue that lines the branches and stem (78, 81, 182). This shrinkage may lead to damage if water stress is not relieved (98). Water usage from these tissues for transpiration resulted in stem drying of Douglas-fir trees that had been laterally root pruned (24).

Cell and Organelle Response to Water Stress

Effects of water deficits at the cell and organelle level may be either reversible or irreversible, depending on the level and the duration of the stress. Chloroplasts appear to be the most sensitive organelle to desiccation (58). They lose ribosomes (62, 74, 124) and decrease in RNA content (121). Abnormal decreases in respiration during severe water stress may be due to mitochondrial breakdown (96). Stocker (183) attributed death of plants from desiccation to disorganization of the fine structure of the protoplasm.

There appears to be a threshold water content which, when passed, results in the destruction of leaf cell compartments and release of hydrolytic enzymes (202). Release of hydrolytic enzymes increases protein degradation and amino acids accumulate (11), espe-

cially proline (102). Incipient plasmolysis resulted in loss of organelles in cells of sunflower leaves stressed to -1.5 MPa (49). Plasmolysis was irreversible below -2.0 MPa (48).

Water Relations of Cut Trees

Water Loss from Cut Trees

Drying eventually results in loss of quality and is a serious marketing problem. Postharvest handling procedures are generally not standardized and provisions to reduce water loss are generally not made. Excessive water loss of cut trees does not generally occur while trees are in the Northwest due to the cool and wet conditions that prevail during harvest. Cool air lowers the leaf-air vapor pressure difference (100) and losses of water from wet foliage are minimal (213). Trees are generally transported to markets via closed vans, which protect them from wind exposure. Desiccation problems appear most frequently at the retail lot. The bulk of Northwest Douglas-fir Christmas trees are shipped to California, where the winter climate is radically different from the Pacific Northwest. Due to the warm and dry environment, small trees with very limited water storage capacity may dry to a critical moisture content within 3 to 4 days. A recent survey of water potential from trees displayed for sale on retail lots indicated that 68% of the retail lots had trees that were drier than -3.5 MPa (Chastagner, personal communication).

Stomatal Behavior After Excision

Stomatal response to environmental stimuli appears to change when a branch or a stem is detached. Foliage from excised branches transpires faster than attached foliage of equivalent water content (191). This apparently universal phenomenon is termed the "Ivanov Effect" (75), and may be attributed to the release of tension in the cohesive water columns in the water conducting elements (179). Detached branches of Pinus sylvestris (139) and Abies amabilis (192) lost their sensitivity to leaf air vapor pressure difference. Roberts (157) found that a decrease of about -0.3 MPa in water potential occurred in Pinus sylvestris within 30 min after severing and that similar but smaller changes in water potential occurred in Picea abies within the same period after cutting (156). Detached branches of Abies amabilis (192) which were allowed to dry decreased in water potential from -0.5 to -2.8 MPa within 4 hr. When cut trees were rehydrated, they absorbed water at a rate 51% faster than intact trees, presumably because stomata remained open and root resistance was eliminated (157). Halevy (63) reported that detached citrus leaves transpired at a rate 25 to 50% higher than attached leaves during hot, dry days. During mild days, rates of transpiration for both detached and intact leaves were similar.

Critical Water Content

Trees allowed to dry to or beyond a critical moisture range may be damaged (Chastagner, personal communication, 8, 201). Chastagner's critical moisture range was based on water potential and it

occurred between -3.0 to -4.0 MPa for Douglas-fir. Damaged trees appeared healthy, but rehydration resulted in needle abscission. Further drying caused the attached needles to lose their deep green color, become brittle and reduced their capacity to rehydrate. The critical moisture range reported by Van Wagner (201) and Ahrens & Stephens (8) was based on percent moisture content and it appeared to be species dependent. They found that drying below the critical moisture range resulted in decreased ability to rehydrate, increased needle loss and increased capacity to ignite and support combustion.

Abscission

Needle abscission on cut trees appears to be a consequence of water loss. Campbell & Vines (22) and Facey (45) attributed needle abscission in cut conifer branches to rapid water loss. Differential drying of separation layer cells and protective layer cells caused the former to contract and tear, resulting in abscission.

Rehydration

The failure of cut trees to rehydrate may be due to decreased stem conductivity (43). During rapid water loss, xylem elements cavitate (130). Cavitation of water conducting elements reduces the stem cross sectional area available for water movement. Byrne et al. (21) showed that a high flow resistance in cotton roots was directly related to cavitation. Puritch (149) and Gregory (61) found that insect and disease attack in Abies grandis and Pinus sylvestris resulted in decreased conductivity due to increases in air-filled

conducting wood. Cavitation can also occur in leaf xylem elements (212). Once cavitation occurs in water stressed trees, rehydration occurs slowly (210).

Storage Methods

Christmas trees, like almost all harvested horticultural products require handling that avoids water loss and maintains high quality. Use of commercial cold storage (123, 128), ice application during transit (26), outdoor storage in plastic sleeves (196) or under opaque polyethylene (9) have been successful in maintaining a water content above the damage level. The success of these methods hinges on maintaining a low temperature and a high humidity atmosphere. As successful as the methods appear to be, Christmas tree industry acceptance and usage is limited.

Antitranspirants

An attractive solution to reducing moisture loss in retail lots could be application of effective antitranspirants (ATs) before or after harvest. The beneficial effects of ATs in reducing transpiration have been reported in a diversity of crops (4, 5, 6, 31, 32, 33, 34, 52, 55, 108, 120, 122, 148, 178). Antitranspirants used on cut trees to reduce moisture loss have been less successful (34, 36). Antitranspirant effectiveness appears questionable because they are more permeable to water vapor than to carbon dioxide by a factor of at least 4 (55, 207, 216). An AT that is successful in reducing moisture loss could also "suffocate" a plant due to decreased res-

piratory gas exchange (33).

Antitranspirants are classified into two broad categories: (1) film types that form a surface film on foliage, and (2) metabolic types that act chemically to close the stomata. This discussion will focus mainly on film types of ATs.

Antitranspirant effectiveness is strongly influenced by environmental factors such as insolation, temperature, and wind, as well as by species differences in leaf morphology, stomatal anatomy, and cuticular structure (33, 54). Under low water stress conditions, ATs were effective in reducing water loss in Fraxinus americana and Acer saccharum (33). Under high temperature and high insolation, AT effectiveness on the same species was greatly reduced, perhaps due to film cracking (32). Increased leaf temperature as a result of reduced transpiration by AT application, has been reported (33, 39, 178, 193, 215). Heat energy absorbed by leaves is generally dissipated and the temperature of untreated leaves remains relatively close to ambient temperature (53, 55). Stomatal closure during midday water deficits apparently does not cause significant injury (152).

Antitranspirant toxicity has been reported in a number of different crops (55, 108, 193, 208). Waggoner (208) stressed the advantages of incomplete coverage with ATs and Davies & Kozlowski (33) noted that film cracking directly over the stomatal pores could prevent phytoxicity. Uneven AT coverage was found to provide a favorable transpiration-photosynthesis ratio (33, 35). A film forming AT is needed that can selectively retain water vapor within

plant tissues while allowing diffusion of respiratory gases. There is currently no such product commercially available.

Of the metabolic inhibitors that act to close stomata by direct control, phenylmercuric acetate (PMA) has been most widely used in experiments to control water loss. PMA inhibits electron transport, NADP reduction, photophosphorylation, reduces chlorophyll content (203), and influences the protein component of guard cell membranes and, hence, potassium ion movement (180). PMA's mode of action on stomatal closure is not completely understood, but may it be related to accumulation of respiratory carbon dioxide (19, 144). The most effective exogenously applied chemical for stomatal control has been abscisic acid (113, 133), but as noted earlier, its effects are inconsistent and poorly understood.

Conclusions

The need for specialized handling of Christmas trees to reduce water loss is obvious. Most attempts to maintain high quality in cut trees do not involve a unified approach that starts with the producers and ends with the consumers. Such an approach has been successful in the fresh fruit, vegetable and commercial cut flower industries (27, 117).

Douglas-fir's adaptation to a mild and wet winter environment make it suceptible to rapid water loss when cut and removed from its natural environment. Douglas-fir's tendency to maintain open stomata at night, decreased capacity to sense and adjust to lower leaf-

air vapor pressure differences and thin cuticle that still allows significant moisture loss when stomata are closed are serious disadvantages when cut trees are exposed to desiccating conditions. In addition, the limited water storage capacity in stems and needles results in a short drying time before the critical moisture range is reached when trees are exposed to desiccating conditions. This thesis examines the effects of environmental variables on desiccation, establishes the critical moisture range at which damage occurs, describes the desiccation damage and evaluates methods to reduce water loss in cut Douglas-fir Christmas trees.

Chapter 3

EFFECTS OF DRYING ON CUT DOUGLAS-FIR

Abstract

Cut coastal Douglas-fir trees (1.0 to 1.5 m) were allowed to dry to various water potentials in a greenhouse. About one-half of the tree population dried to -3.5 MPa lost significant quantities of needles, but were otherwise comparable in appearance to the control. Drying to -4.0 MPa or below resulted in significantly lower water uptake rates after rehydration and also resulted in irreversible damage. Changes in percent moisture content and stomatal conductance generally paralleled changes in ψ_L but were less useful indices of the damage threshold ψ_L. Bark wrinkling and % broken needles were useful visual indices of the damage threshold ψ_L. Subjective ratings of quality were unacceptable indices of trees' moisture status because damage may occur while the trees appear to be fresh. Water potential was the most useful single measurement of Christmas tree water status, and provided an accurate index of the damage threshold ψ_L.

Introduction

Water content is a critical component of Christmas tree quality affecting appearance (12), needle retention (1), and safety (3, 10). The determination of tissue hydration levels at which tree damage occurs and evaluation of factors regulating moisture loss are needed to develop improved quality of storage, shipment and display procedures that maintain tree acceptability. Van Wagner (18) described a "critical moisture content" for white spruce, balsam fir, and Scots pine as the maximum amount of water lost before trees lose the capacity to rehydrate. In Douglas-fir, Chastagner (personal communication) identified a threshold range (-3.0 to -4.0 MPa) at which about 40% of the trees were damaged, although trees at the threshold appeared fresh. Damage became most apparent after rehydration, and was characterized by severe needle abscission. Water potential below -4.0 MPa caused progressively more severe and visible damage.

The objectives of this study were: (1) to define the damage threshold ψ_L of cut Douglas-fir trees, (2) to study the relationship of the threshold ψ_L to various measurements of plant water status and, (3) to identify morphological changes associated with the damage threshold ψ_L.

Materials and Methods

Douglas-fir trees (1 to 1.5 m tall) were cut and allowed to dry in a greenhouse (day/night temperature 16/10°± 5°C) to four levels of ψ_L before rehydration (-2.5, -3.0, -3.5, and -4.0 MPa). Control trees were rehydrated immediately after harvest. Trees were rehydrated by sawing 2 cm from the stem base and immediately immersing the recut end in water (13).

Water potential (ψ_L), moisture content (percent dry weight), osmotic potential (ψ_L) and stomatal conductance (g_S) were measured immediately after harvest and at regular intervals thereafter. Water uptake after rehydration, bark wrinkling, percent broken needles, percent needle loss and visual ratings of quality were also evaluated periodically.

Water potential was measured with a pressure chamber (PMS Instruments, Corvallis, OR) (14) on 1 randomly selected 3 cm twig from each of 10 trees per treatment on each sampling date. Pressure was introduced into the chamber at 0.07 MPa/sec (19). Percent moisture content was determined by random sampling two, 5 cm twigs from each of 10 trees per treatment on each sampling date. Samples were stored and sealed in plastic bags and weighed within 30 min after detachment. All samples were dried at 70°C for 24 hours and then reweighed. Percent moisture content was calculated by dividing moisture loss by dry weight, and multiplying the quotient by 100. Osmotic potential was measured with a vapor pressure osmometer (Model 5100 C, Wescor Inc., Logan, Utah). Samples were frozen at

-70°C and ψ_π was determined on the sap extracted from the thawed samples. Individual values of ψ_π were adjusted for apoplastic water volume (41.65% from pressure/RWC curve, unpublished data) before calculating turgor pressure (ψ_p). Turgor pressure was calculated from the following relationship:

$$\psi_L = \psi_\pi + \psi_p \qquad (15)$$

where ψ_L = Total water potential

ψ_π = Osmotic potential

ψ_p = Turgor pressure

Stomatal conductance (g_S) was measured with a steady state porometer (Model LI-1600, Licor Inc., Lincoln, Nebraska) (2). Measurements were taken on 2 individual 1 year-old branch tips on each of 5 trees per treatment at each sampling date. The 2 measurements were averaged for g_S calculations. At the end of the experiment, leaf surface area was measured with a leaf surface area meter (Model 3100, Licor Inc.) and g_S was computed on the basis of projected one-sided leaf area (7), and expressed as $mm \cdot sec^{-1}$.

Water uptake rates were determined by periodically refilling the reservoir to a predetermined level. To prevent evaporation, the inside of each reservoir was lined with a plastic bag that was tightly wrapped around the stem of each tree.

Bark wrinkling was evaluated subjectively by observing its presence or absence on current year's growth. Bark wrinkling was usually first visible at the point of needle attachment. Wrinkles ran parallel to the twig axis and varied from 1 to 5 mm long. The percent of broken needles was measured by bending 10 needles between

thumb and forefinger and recording whether or not they broke.

Percent needle loss was determined on 2 randomly selected, 8 cm twigs from each of 10 trees per treatment. Individual twig samples were gently rubbed once along the twig axis and the fallen needles were weighed to the nearest 0.1 mg. Percent needle loss was calculated by dividing the weight of the needles that dropped during hand rubbing by the total needle weight and multiplying the quotient by 100.

Visual ratings of quality were evaluated by 2 individuals on the basis of general appearance, needle browning, and needle loss. The rating scale for each category were as follows: general appearance, (1) poor, (2) fair, (3) good, (4) excellent; % needle necrosis, (1) 75 - 100, (2) 50 - 75, (3) 25 - 50, (4) 0 - 25; % needle loss, (1) 75 - 100, (2) 50 - 75, (3) 25 - 50, (4) 0 - 25; bark wrinkling, + = present, - = absent; % broken needles, + = bent without breaking, - = broke when bent. The experimental design was a randomized block design with 5 replications and 2 trees per replication.

Results

In the greenhouse, cut Douglas-fir trees dried to about -2.5 MPa in 3 days and to -3.0 MPa in 4 days (Fig. 3.1). The drying rate below -3.0 MPa was slower; -3.5 MPa was reached in 12 days and -4.0 MPa in 21 days. In each treatment, trees that were not irreversibly injured rehydrated to a ψ_L above -0.9 MPa. After rehydration, ψ_L declined slowly and remained above -2.5 MPa for at least 3 weeks when trees were kept supplied with water. Percent moisture content (Fig. 3.2) was initially about 55% and changes in percent moisture content paralleled changes in ψ_L in the -2.5 and -3.0 MPa treatments. Percent moisture content in the -3.5 and -4.0 MPa treatments declined only slightly from day 4 to 12 when ψ_L declined from -2.6 to -3.9 MPa. Upon rehydration, percent moisture content increased to a range of 54 to 58% for all treatments then decreased slowly and usually remained above 53%.

Turgor pressure (Fig. 3.3) closely paralleled ψ_L. Zero ψ_p in the -3.5 and -4.0 MPa treatments was reached on day 12. Trees in the -4.0 MPa treatment had zero ψ_p for 9 days. Trees in the -3.5 MPa treatment were rehydrated on day 12, and 2 days later, both ψ_p and ψ_L were at comparable levels as measured at the start of the experiment. Upon rehydration on day 21, ψ_L and ψ_p in the -4.0 MPa treatment followed nearly the same pattern as the -3.5 MPa treatment. In each treatment, trees rehydrated to a ψ_p above 2.2 MPa. After rehydration, ψ_p declined slowly with no significant differences among treatments.

Stomatal conductance (Fig. 3.4) declined with decreasing ψ_L,

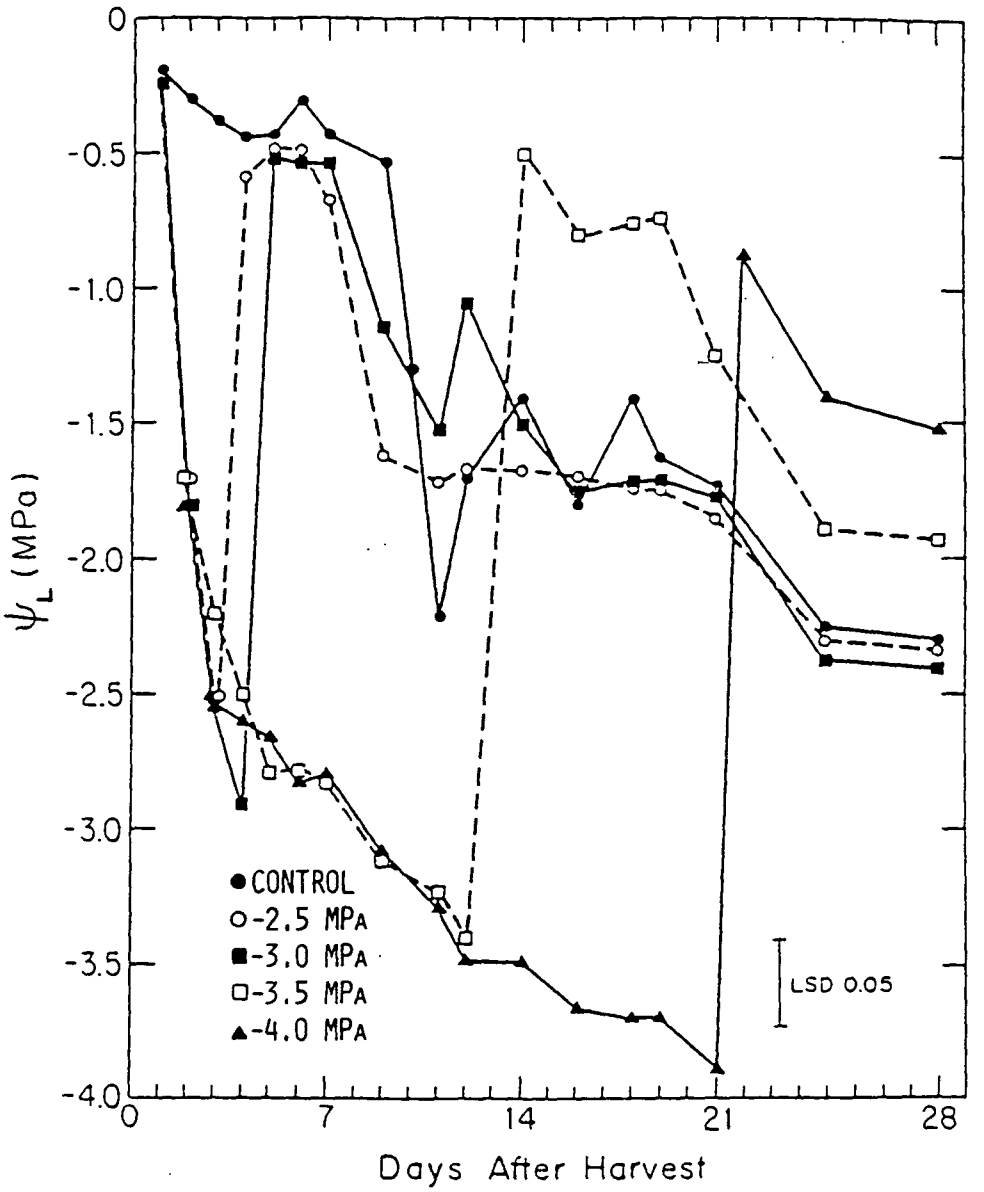

Fig. 3.1. Drying and rehydration rates of cut Douglas-fir trees stressed to various water potentials. Each data point is the mean of samples measured from 10 trees.

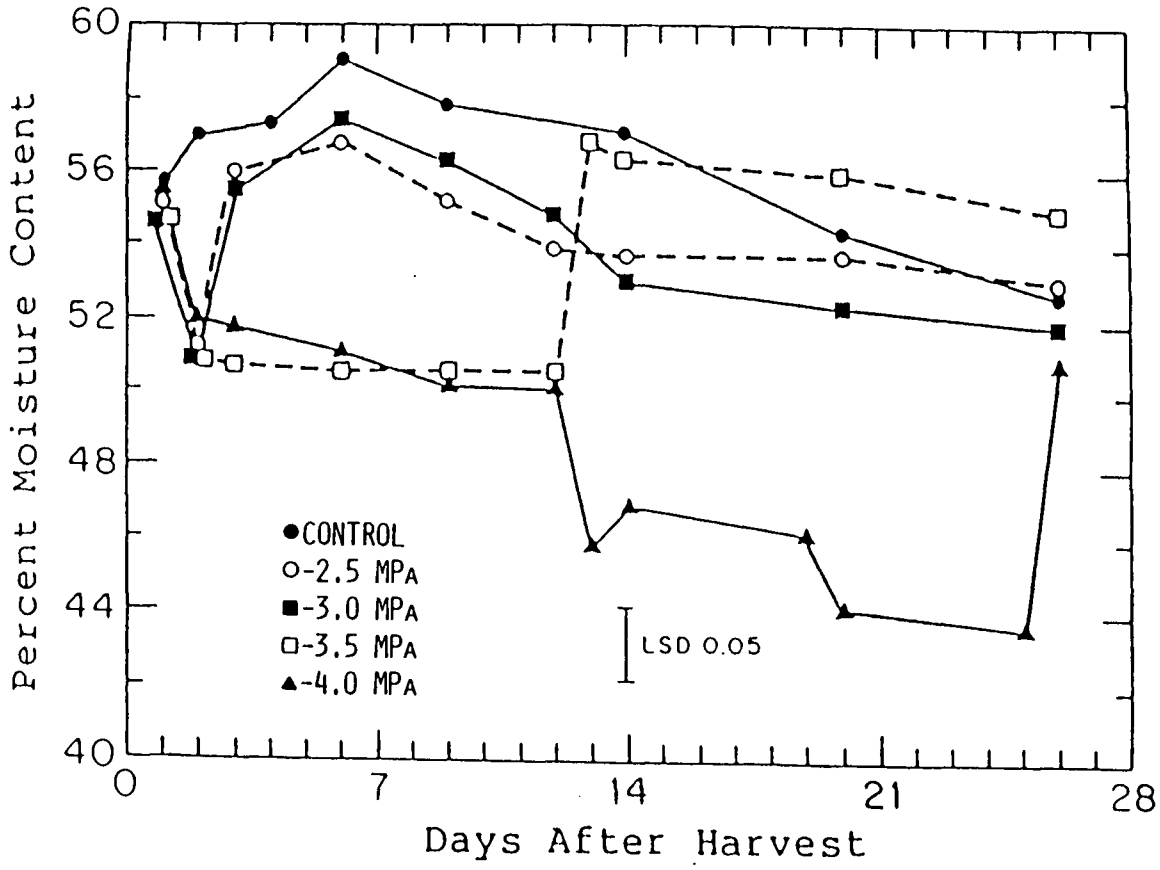

Fig. 3.2. Influence of water stress on percent moisture content of cut Douglas-fir. Each data point is the mean of samples measured from 10 trees.

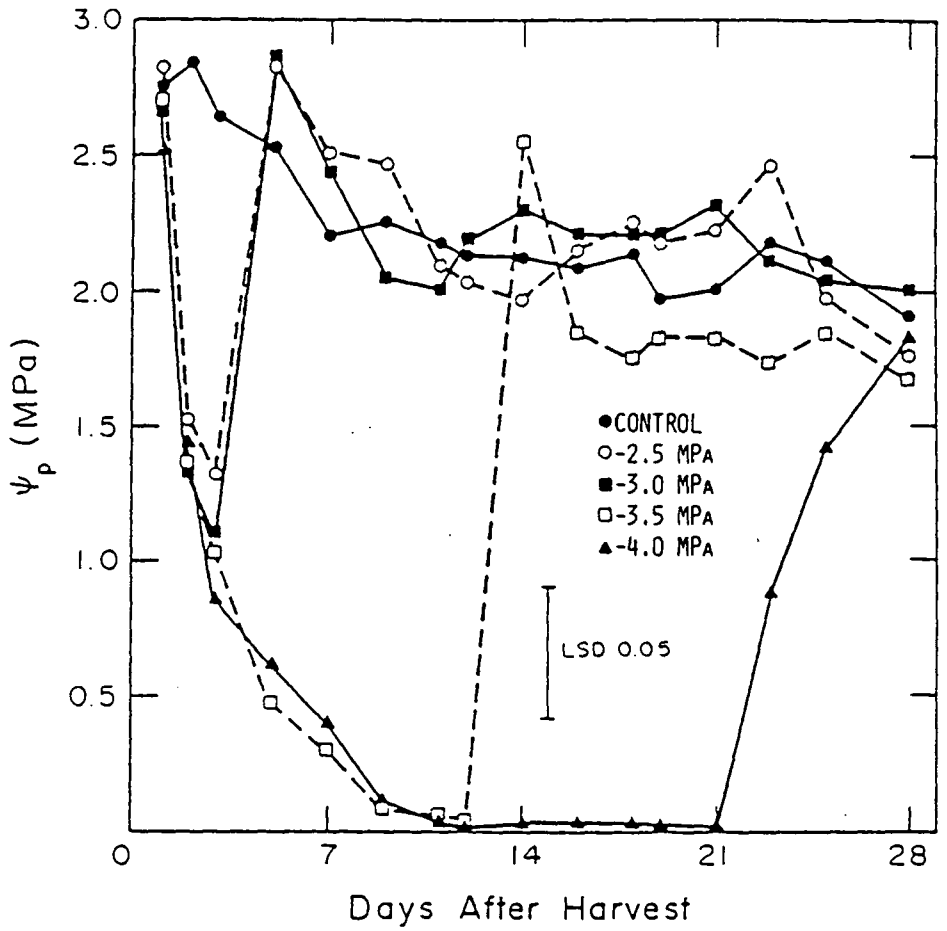

Fig. 3.3. Influence of water stress on turgor pressure of greenhouse stored trees. Each data point is the mean of samples measured from 10 trees.

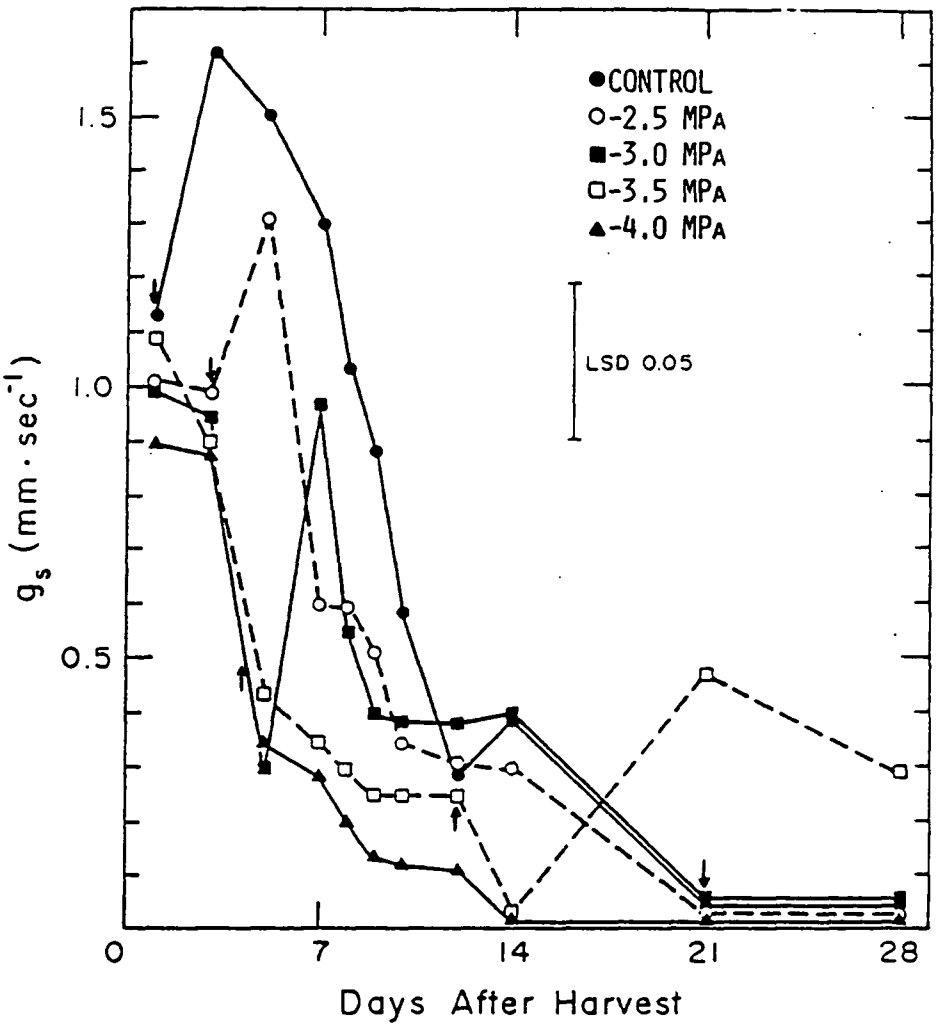

Fig. 3.4. Influence of water stress on stomatal conductance of cut Douglas-fir trees. Each data point is the mean of samples measured from 10 trees. Arrows indicate rehydration date.

but did not reach zero until ψ_L was below -3.5 MPa. Rehydration caused a transient increase in g_s, but g_s did not respond to rehydration in trees dried to -4.0 MPa.

Water uptake rate (Fig. 3.5) for the first 12 days after rehydration was not significantly different among the treatments, except in the -4.0 MPa treatment. Rates of daily water uptake for the first 12 days after rehydration were: control, 279 ml; -2.5 MPa, 183 ml; -3.0 MPa, 198 ml; -3.5 MPa, 241 ml and -4.0 MPA, 33 ml.

Morphological indicators of moisture status could be useful to consumers of Christmas trees. The highly sclerified and lignified needles of Douglas-fir prevents the appearance of wilt symptoms, but bark wrinkling was observed to increase with decreasing ψ_L (Fig. 3.6). Wrinkling was apparent on less than 1% of the sampled twigs at -2.3 MPa. Below about -2.8 MPa, wrinkling was easily detected on at least 50% of the twigs. Percent broken needles (Fig. 3.7) declined rapidly below -3.3 MPa. Between -3.3 MPa and -3.5 MPa, the percentage of broken needles dropped from 75% to 15%. Below -4.0 MPa, needles dried severely and could be broken easily by rubbing the twigs (data not shown).

Needle abscission (Table 3.1) was stimulated by a ψ_L below -3.5 MPa, but was severe in only about one-half of the trees. Varying percentages of trees shed needles, but about one-half of the trees in either treatment population shed considerably more needles than the other one-half. Abscission was apparent within 12 days after harvest and began before rehydration, although abscission was intensified by rehydration.

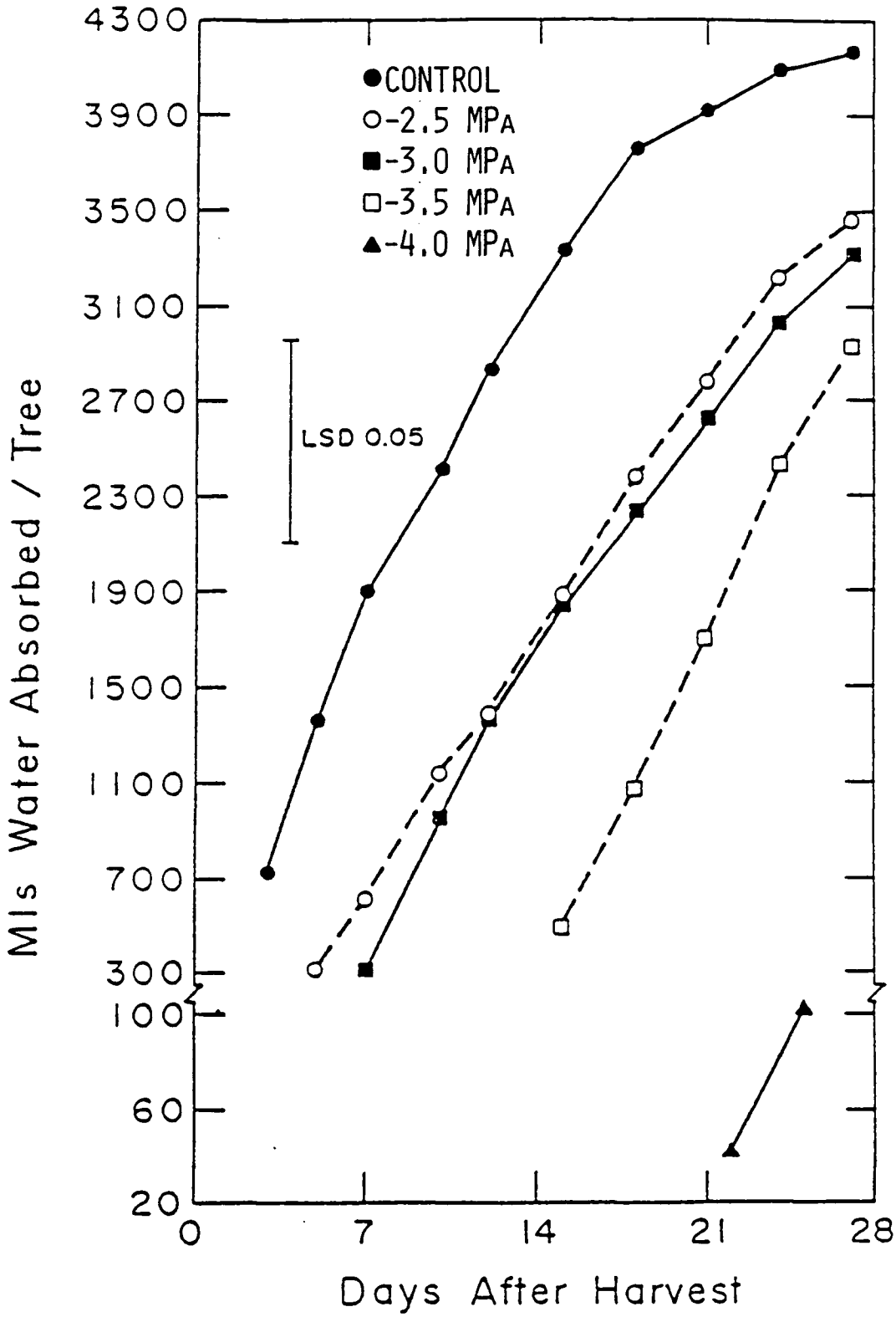

Fig. 3.5. Influence of water stress on cumulative water absorption of greenhouse stored cut Douglas-fir. Each data point represents the mean of 10 trees per treatment.

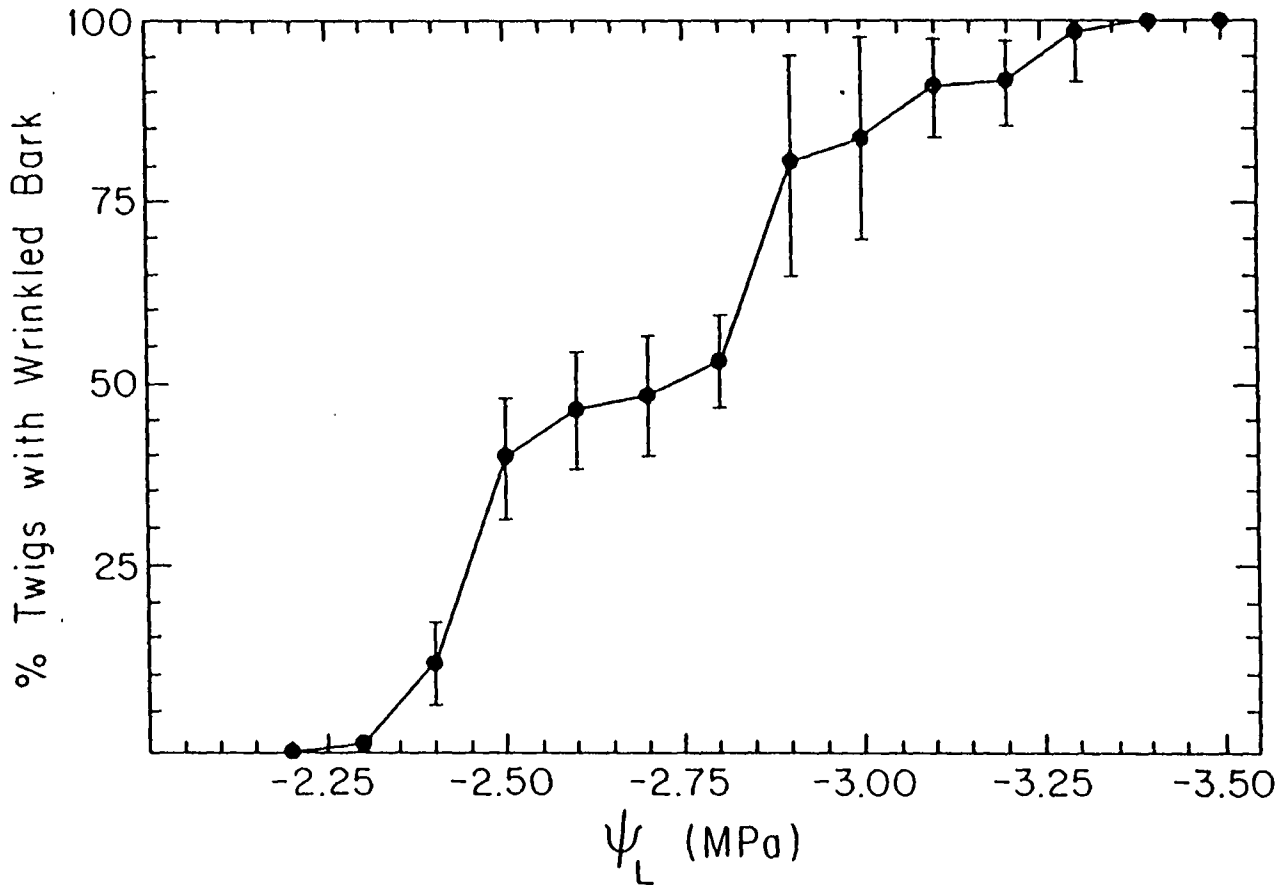

Fig. 3.6. The relationship of % of twigs with wrinkled bark to increasing water stress on cut Douglas-fir trees. Data points are the means of at least 50 samples. Vertical bars indicate standard error.

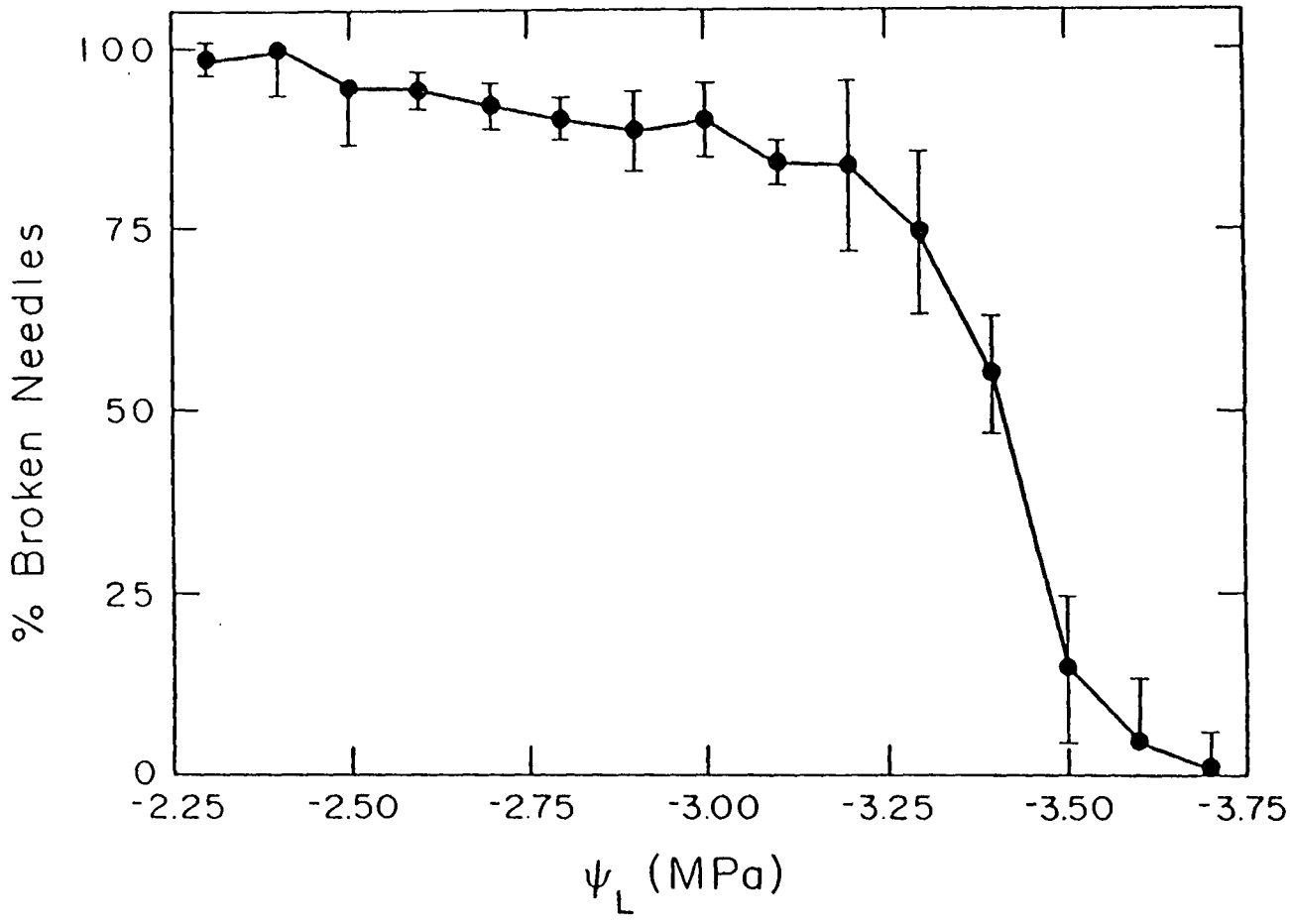

Fig. 3.7. The relationship of % broken needles with wrinkled bark to increasing water stress on cut Douglas-fir trees. Data points are the means of at least 50 samples. Vertical bars indicate standard errors.

Treatments		Days After Harvest								
Rehydration		12			21			28		
MPa	Day	MPa[z]	abscising	non-abscising	MPa	abscising	non-abscising	MPa	abscising	non-abscising
check	1	-1.7	0.0 a	0.0 a	-1.7	0.0 a	0.0 a	-2.3	4.2 a	0.0 a
-2.5	3	-1.7	0.0 a	0.0 a	-1.9	0.0 a	0.0 a	-2.3	6.6 a	0.0 a
-3.0	4	-1.1	0.0 a	0.0 a	-1.9	0.0 a	0.0 a	-2.4	7.0 a	0.0 a
-3.5	12	-3.5	15.8 b	0.0 a	-1.3	15.4 b	0.0 a	-1.9	13.4 b	5.0 b
-4.0	21	-3.5	14.4 b	1.4 a	-3.9	14.0 b	0.4 a	-1.5	26.6 c	5.4 b

Table 3.1. Percent needle loss from greenhouse-stored, cut Douglas-fir at various water potentials. [z] ψ_L at sampling for needle loss. [y] mean separation within columns by Duncan's multiple range test, 5% level.

Visual ratings of quality (Table 3.2) did not indicate any differences among treatments 10 days after exposure to drying conditions. The -3.5 and -4.0 MPa treatments were distinguished from the other treatments by the presence of wrinkled bark. Bark wrinkling on the -3.5 MPa treatment disappeared after rehydration on day 12. Needle loss was more severe on -3.5 and -4.0 MPa treatments. The general appearance of trees in the -4.0 MPa treatment deteriorated markedly and needle necrosis, needle loss and bark wrinkling were severe by day 16. After day 16, tree appearance did not change significantly. Rehydration of trees in the -4.0 MPa treatment on day 21 did not improve general appearance.

DAYS AFTER HARVEST

	16				23				30			
Treatment	MPa	General Appearance [z]	Needle Necrosis	Needle Loss	MPa	General Appearance	Needle Necrosis	Needle Loss	MPa	General Appearance	Needle Necrosis	Needle Loss
check	-1.8	4.0 a	4.0 a	4.0	-2.0	3.4 a	3.4 a	4.0	-2.6	3.2 a	3.2 a	4.0
-2.5 MPa	-1.7	3.4 a	3.8 a	4.0	-2.1	3.2 a	3.2 a	4.0	-2.7	3.0 a	3.0 a	4.0
-3.0 MPa	-1.9	3.2 a	3.8 a	4.0	-2.1	3.4 a	3.6 a	4.0	-2.8	3.2 a	3.4 a	4.0
-3.5 MPa	-0.8	3.2 a	3.4 a	3.6	-1.6	3.4 a	3.8 a	3.8	-1.9	3.1 a	3.4 a	3.6
-4.0 MPa	-3.7	2.4 b	2.6 b	3.8	-0.9	2.6 b	2.6 b	3.8	-1.5	2.2 b	2.4 b	3.6
		*	*	N.S.		*	*	N.S.		*	*	N.S.

Table 3.2. Effect of drying on ratings of general appearance, needle necrosis and needle loss of cut Douglas-fir. [z]Mean separation within columns by Duncan's multiple range test, 5% level.

Discussion

Desiccation damage to cut Douglas-fir trees occurred at a threshold ψ_L. Trees dried to -2.5 or -3.0 MPa showed no visible signs of injury, but one-half of the trees dried to -3.5 MPa exhibited needle loss. These damaged trees looked fresh and comparable to the control trees in general appearance. Drying to -4.0 MPa resulted in progressively greater damage; a marked decline in general appearance, increased needle loss, bark wrinkling, needle necrosis, and reduced water uptake. A similar threshold ψ_L was associated with increased needle abscission (on 40% of the sampled trees) in Douglas-fir by Chastagner (personal communication). In contrast, Conklin (4) observed that some severely dehydrated cut Douglas-fir trees lost very small quantities of needles, becoming shriveled, brittle and remaining firmly attached to the twigs. Rink and Thor's data (12) on needle fall show a large variance among replicates, the differences within species and treatments being well over 50% in many cases. Factors other than drying apparently affect needle loss. Stevens (16) observed that needle loss varied among selected Douglas-fir progenies. He concluded "that the female parent may exert a strong influence on needle retention of open pollinated offspring". Regardless of its effect on needle loss, water stress below -3.5 MPa progressively reduces quality. Below -4.0 MPa, needles lose their natural color and become brittle.

Measurements of percent moisture content, ψ_π, and g_s were generally parallel to ψ_L, but were less useful indices of the damage

threshold than ψ_L . Percent moisture content remained unchanged during an 8 day period prior to the onset of the damage threshold, and did not accurately reflect changes in ψ_L during a critical period of a tree's drying cycle.

Turgor pressure was a good index of the damage threshold ψ_L , but was difficult to measure. The procedure to measure ψ_π is time consuming and calculations require a correction factor obtained from a pressure/RWC curve.

Stomatal conductance decreased with decreasing ψ_L but increased after rehydration. These results are similar to those of Untershuetz et al., (17) and Murphy (11) who reported high transpiration rates in unstressed and intact seedlings and a gradual decline in g_s with increasing water stress. A lack of g_s response after rehydration of trees stressed to -4.0 MPa was probably due to the small quantities of water absorbed in these irreversibly damaged trees.

Water absorption rates did not appear to be related to water stress over the desiccation range from above -0.5 MPa to -3.5 MPa. However, trees dried to -4.0 MPa absorbed 94% less water than control trees. This dramatic decline in water absorption rate may be due to pit pair closure caused by cavitation of the water columnm (8). As water content decreased, a progressively larger percentage of pit pairs may have closed, resulting in decreased permeability. Edwards and Jarvis (5) found that a 10% reduction in relative water content of Pinus contorta stems resulted in a 10% reduction in stem permeability. A reduction in stem permeability would have a direct effect on water uptake. In this experiment, percent moisture content

of trees in the -4.0 MPa treatment was reduced by 9% before rehydration.

To a consumer of harvested horticulutral products, appearance is a major criterion of quality. Wilting and shriveling are useful indices of moisture status in fruit, vegetable and floral crops (9). Mature conifer needles are incapable of showing visible wilt symptoms due to the large proportion of lignified elements in needle tissue (6). In Douglas-fir, bark wrinkling appeared to be closely associated with ψ_L prior to the occurrence of the damage threshold (Fig. 3.6 and 3.1), but bark wrinkling gave no indication of a tree's water status above -2.0 MPa.

Percent broken needles was a less sensitive measure of the damage threshold because the change occurred closer to the damage threshold. More than 75% of the sampled needles could be broken at -3.3 MPa versus 15% at -3.5 MPa due possibly to loss of turgor pressure. Bark wrinkling and percent broken needles combined could be useful indices of moisture status to Christmas tree consumers. If one-half the sampled twigs on a tree show bark wrinkling and over 90% of the sampled needles can still be broken, it would still be safe to buy the tree, provided it was rehydrated immediately. Conversely, if all the twigs on a tree have wrinkled bark, and more than 75% of the needles cannot be broken by bending, the probability is high that the tree has already passed the damage threshold.

Subjective ratings of quality were unacceptable indices of trees' moisture status because damage may occur while the trees appear to be fresh. Ratings done on trees with ψ_L below -3.5 MPa

show a distinct decline in quality, but no damage was detected on trees with ψ_L above -3.5 MPa.

A damage threshold ψ_L that resulted in needle abscission on nearly 50% of a sampled tree population occurred at -3.5 MPa. Trees at this stage of water stress appeared fresh and if rehydrated did not exhibit further damage. Drying to -4.0 MPa resulted in progressively severe and irreversible damage.

Water potential was an appropriate single measurement of Christmas tree water status, and a suitable index of the damage threshold ψ_L. Measurements of ψ_π and g_S show close agreement with ψ_L. Percent moisture content did not appear to be a reliable index of the damage threshold. Bark wrinkling and percent broken needles may be useful morphological indicators of the damage threshold ψ_L in Douglas-fir.

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Chapter 4

EFFECTS OF STORAGE CONDITIONS AND FOLIAGE WATERING ON DRYING OF CUT DOUGLAS-FIR TREES

Abstract

Cut Douglas-fir trees were stored in either a polyethylene-covered lath house or in a greenhouse. The foliage of trees in each storage environment was watered regularly or allowed to remain dry. Trees stored in the lath house and watered twice daily maintained a water potential (ψ_L) above the damage threshold (-3.5 MPa) for 33 days. Unwatered trees dried to -2.8 MPa within 14 days. When watered trees were brought into the greenhouse, ψ_L declined from -1.3 to -3.2 MPa within 12 days and turgor pressure (ψ_p) declined from 2.2 to 0.0 MPa within 8 days. Greenhouse stored watered and non-watered trees dried from -0.8 to about -3.2 MPa within 3 days. Watered trees remained at an average -3.3 MPa for 12 days while unwatered trees dried to -5.8 MPa. Watered trees maintained a healthy appearance throughout the experiment. Unwatered tree foliage lost its natural green color and became dry and brittle. Rehydration did not improve the appearance of these damaged trees.

Introduction

Pacific Northwest Christmas trees are frequently cut about 1 month prior to shipment. Postharvest storage methods are not standardized and in most cases, special precautions are not employed to protect cut trees from desiccation. Within 3 to 4 days, exposure of cut trees to warm and dry conditions can result in drying to a ψ_L (-3.0 to -4.0 MPa) that results in damage (Chastagner, personal communication). Damaged trees desiccated to -3.0 to -4.0 MPa appeared fresh and healthy in most cases. Drying to a ψ_L below -4.0 MPa, however, resulted in foliage abscission, needle necrosis, and shriveling. A recent survey (Chastagner, personal communication) indicated that 68% of the sampled retail lots in California had Douglas-fir trees with a ψ_L lower than -3.5 MPa.

Douglas-fir accounts for about 74% of the nearly 5.5 million Christmas trees produced annually in Oregon and Washington. Compared to other Christmas tree species such as pine and true fir, coastal Douglas-fir has a low resistance to water loss (15), perhaps due to climatic adaptation (35). Compared to inland ecotypes, coastal Douglas-fir transpire at higher rates (31), and possess a thinner needle cuticle, allowing loss of water through avenues other than stomata (14). Needles of coastal Douglas-fir with closed stomata transpired at a rate 50% that of needles with open stomata, while inland ecotypes with stomata closed transpired at a slower rate; 2 to 20% that of open stomata (12).

Moisture loss of Christmas trees has been observed to be re-

duced by ice application during transit (3), and storage in shaded, moist (19) and wind protected areas (5, 9). Bulk storage at -6°C (16) and refrigerated van shipment (17) have also been effective, but are expensive.

The objective of this work was to study the effects of various storage conditions and foliar watering on maintenance of a moisture content above the damage threshold ψ_L of cut Douglas-fir.

Materials and Methods

Douglas-fir trees (1 to 1.5 m tall) were cut and stored in either an unheated polyethylene-lined lath house (day/night temperature 7/2°±10°C), or in a greenhouse (day/night temperature 16/10° ±5°C). Temperatures during harvest ranged from 2° to 6°C and relative humidity from 70 to 95%. In the lath house, trees were watered twice daily and in the greenhouse, the trees were watered at least 6 times per day. Trees were rehydrated by cutting 2 to 4 cm of stem base and immediately immersing the cut end in water (21).

Xylem water potential (ψ_L) (24) was measured with a pressure chamber (PMS Instruments Co., Corvallis, OR). A 3 cm twig was randomly sampled from each tree on alternate days. Pressure was introduced into the chamber at 0.07 MPa/sec (33). Osmotic potential (ψ_π) was measured with a vapor pressure osmometer (Model 5100 C Wescor Inc., Logan, Utah) starting on day 34, when the trees were moved into the greenhouse. Samples measured for ψ_π were frozen at -70°C and ψ_π determined on the sap extracted from the thawed tissue. Osmotic potential was adjusted for apoplastic water volume (41.65% from pressure/relative water content curve, unpublished data) before calculating turgor pressure (ψ_p). Turgor pressure was calculated from the equation:

$$\psi_L = \psi_\pi + \psi_p \qquad (25)$$

where: ψ_L = total water potential

ψ_π = osmotic potential

ψ_p = turgor pressure

Air water potential (ψ_a) (20, 22) was calculated by using the formula: $$\psi_a = \frac{RT}{V} \ln\left(\frac{\%RH}{100}\right)$$

where: RH = Relative Humidity

R = 8.317×10^7 erg/mole degree (ideal gas constant)

T = Temperature in degree Kelvin

V = 18 cm^3/mole (partial molal volume of water)

To convert to Megapascals (MPa), the product of the equation was divided by 1.0×10^7 dyne/cm^2. Temperature and relative humidity were recorded with a hygrothermograph and global radiation with a Robitzsch bimetallic pyranograph. The experimental designs were randomized blocks with 2 trees per block and each experiment replicated 5 times.

Results

Lath house watered and unwatered trees dried to nearly the same ψ_L (-2.8 MPa) within 14 days after harvest, but watered trees recovered to a maximum ψ_L of -1.3 MPa (Fig. 4.1). Unwatered trees were brought into the greenhouse and were rehydrated on day 14. Environmental data recorded for the duration of outdoor storage was not related to ψ_L if the entire storage period was considered at one time. However, when the outdoor storage period was divided into 3 discrete units of the single curve representing ψ_L of watered trees, some environmental factors and ψ_L were significantly related. From day 1 to 4, radiation (Table 4.1b) and day ψ_a (Table 4.1a) had the most significant impact on foliar water uptake (r^2= 0.59 and 0.66). From day 4 to 14, there was a ψ_L decline from -0.3 to -2.8 MPa, day ψ_a decreased from -21 to -60 MPa, night ψ_a remained nearly the same, and radiation (Table 4.1b) increased from 112 to 152 langleys (ly). Radiation and day ψ_a (r^2= 0.75 and 0.70) had the most significant impact on ψ_L decline. Collectively, radiation, day and night ψ_a accounted for 94% of the variability associated with the ψ_L decline. From day 14 to 26, ψ_L increased from -2.7 to -1.3 MPa, day ψ_a increased from -60 to -21 MPa, night ψ_a remained nearly steady between -6 and -9 MPa and radiation decreased from 152 to 48 ly. During this period, day ψ_a had the most significant effect on ψ_a (r^2= 0.87), and radiation, day and night ψ_a accounted for 87.4% of the total variability.

After the watered trees were brought into the greenhouse, (no foliar watering once inside), ψ_L declined to -3.2 MPa in 12 days and

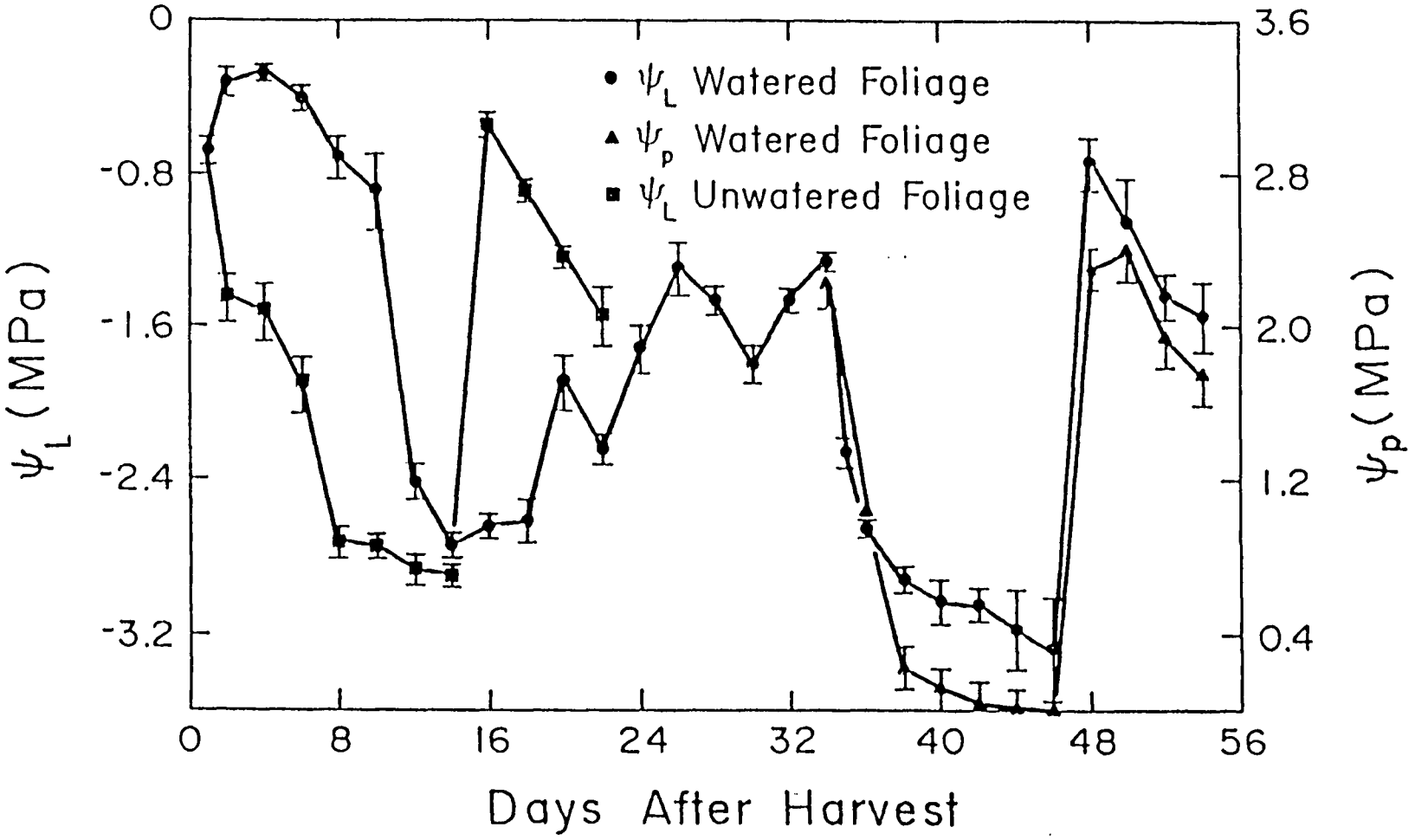

Fig. 4.1. The effect of foiliage watering on water potential of lath house stored trees. Turgor pressure was calculated during indoor storage, starting on day 34. Vertical bars represent standard errors.

Table 4.1a. Day and night ψ_a during lath house storage

Day	Day/Night ψ_a	Day	Day/Night ψ_a
1	-87/-16	18	-64/-8
2	-66/-10	19	-58/-7
3	-58/-8	20	-64/-9
4	-36/-6	21	-47/-9
5	-21/-11	22	-58/-16
6	-58/-9	23	-35/-8
7	-16/-11	24	-38/-12
8	-27/-11	25	-37/-8
9	-39/-8	26	-23/-7
10	-39/-8	27	-62/-6
11	-48/-7	28	-46/-7
12	-29/-16	29	-48/-13
13	-57/-11	30	-66/-7
14	-68/-9	31	-72/-16
15	-59/-11	32	-80/-17
16	-68/-8	33	-16/-3
17	-63/-8	34	-18/-5

Table 4.1b. Insolation during lath house storage

Day	Langley · 24 hr.$^{-1}$	Day	Langley · 24 hr.$^{-1}$
1	117	18	216
2	135	19	168
3	78	20	192
4	87	21	85
5	114	22	147
6	108	23	115
7	54	24	174
8	90	25	63
9	96	26	49
10	68	27	144
11	105	28	120
12	183	29	134
13	228	30	198
14	156	31	225
15	201	32	260
16	183	33	63
17	177	34	84

ψ_p declined from 2.2 to zero MPa in 8 days. The rates of drying and rehydration were similar to fresh cut trees (Fig. 3.1).

Indoor storage (Fig. 4.2) resulted in drying to about -3.3 MPa within 3 days regardless of treatment. Watered trees remained at an average -3.3 MPa for nearly 2 weeks however, unwatered trees dried to -5.8 MPa. Fourteen days after starting the experiment, watered trees appeared healthy, whereas unwatered tree foliage had lost its natural green color and felt dry and brittle. Rehydration did not improve the visual appearance of unwatered trees.

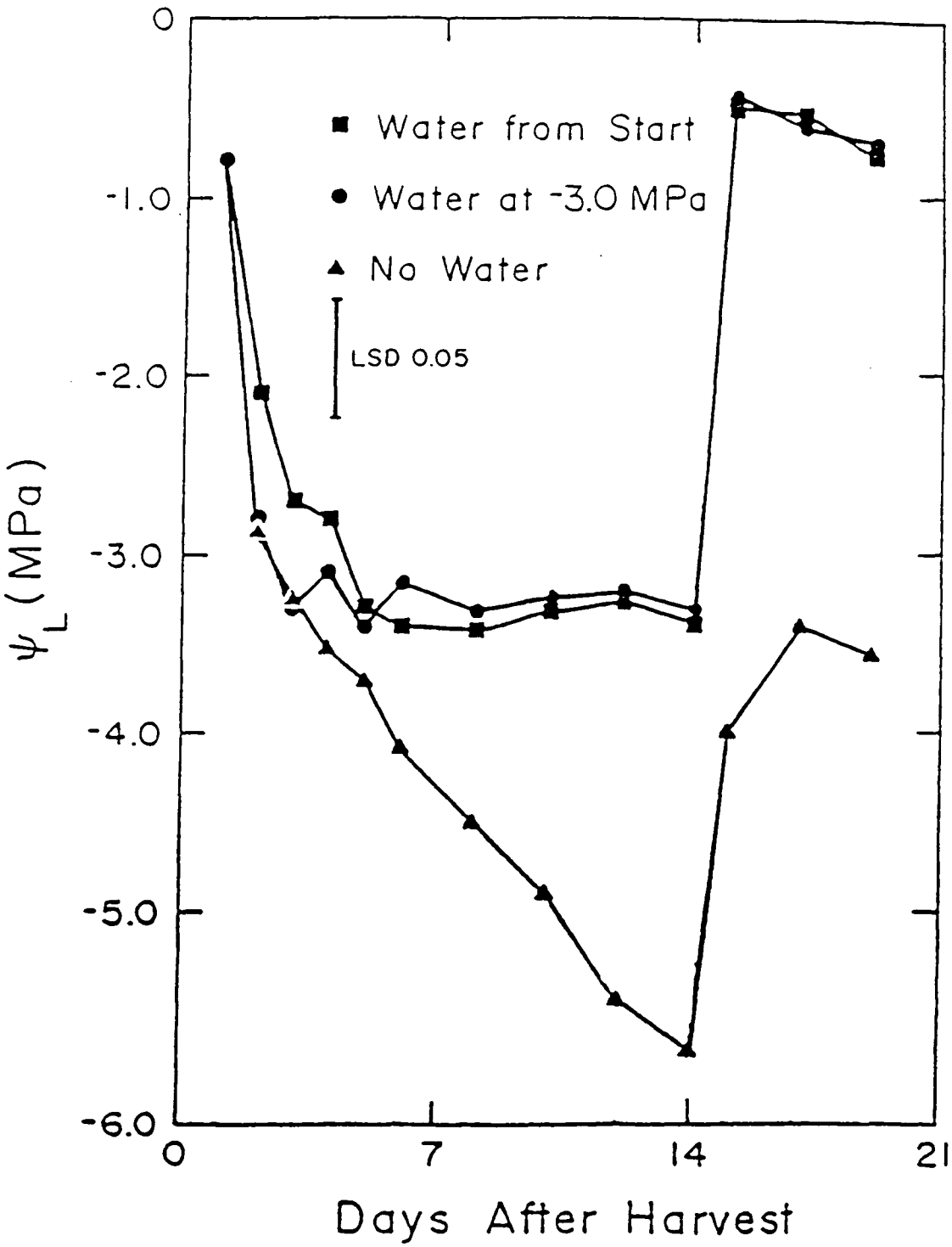

Fig. 4.2. The effect of foliage watering on water potential of greenhouse stored trees.

Discussion

Foliar watering of either lath house- or greenhouse-stored trees maintained a ψ_L above the damage threshold. Enough water was absorbed under lath house storage to cause rehydration and under greenhouse storage to replace water lost through transpiration. Foliar water absorption has been shown to occur in several conifers (4, 18, 25, 28), provided there is a favorable ψ gradient from air to leaf. Gates (8) found that during a winter night, severed conifer branches took up moisture that was 3 to 4 times the amount lost by transpiration from the same branches on a cold day. The primary requisites for foliar water absorption appear to be low temperature and high humidity. Decreasing temperature decreases the atmosphere's ability to hold water vapor and as a consequence, relative humidity increases and the leaf-air vapor pressure difference decreases. Under low temperature (less than 10°C) and high humidity (greater than 90%), evaporation of water may have occurred first from wet needles followed by evaporation of water from mesophyll cell walls (34). On the other hand, when temperature increases from 10° to 20°C and the absolute humidity remains at 60%, the leaf-air vapor pressure difference increases 2-fold (13). Cremer & Svensson (4) reported that foliar water absorption of cut and misted Pinus radiata could not equal transpiration during sunny days (greenhouse conditions).

For watered trees stored in the lath house, ψ_L was significantly correlated to day ψ_a and to radiation, but not to night ψ_a. The correlations were significant when the curve for watered trees was

divided into 3 distinct periods of time. During the first period (day 1 to 4), increases in ψ_L were related to increases in day ψ_a (r^2 = 0.66). During this period, day ψ_a increased from -87 to -36 MPa. Given that the foliage was constantly wet, the leaf-air vapor pressure difference across stomata was likely to be very small (21) and flow of water from mesophyll cells to the atmosphere was restricted (11, 34). Water movement into conifer needles was likely to occur under such conditions (24, 25, 26, 27, 28, 30, 31).

During the second period (day 4 to 14), decreases in ψ_L were related to decreases in day ψ_a (r^2 = 0.70) and to increases in radiation (r^2= 0.75). An increase in radiation resulted in decreased day ψ_a (r^2= 0.74). Day ψ_a decreases because the moisture carrying capacity of the air increases with increase in temperature (2, 4). If temperature increases from 10° to 20°C, the leaf-air vapor pressure difference will increase 2-fold (14). Therefore an increase in radiation will likely result in increased day temperatue, lower ψ_a and decreased ψ_L. Collectively, radiation, day and night ψ_a accounted for 94% of the total variability associated with the ψ_L decline.

During the third period (day 14 to 26), increases in ψ_L were related to increased day ψ_a (r^2= 0.87). Foliar water uptake occurred within a range of 2.2° to 10.0°C and 91 to 95% relative humidity. Since the decline in day ψ_a leveled off on day 14, it is difficult to predict how much more ψ_L would have decreased if day ψ_a had continued to decrease. This is because the rate of decline of ψ_L normally decreases after passing -2.6 MPa. Collectively, radiation, day and night ψ_a accounted for 87% of the variability associated with in-

creased ψ_L. This suggests that there may have been physiological adjustment. If cell solutes became concentrated as water was lost during days 8 to 14, a ψ gradient favorable for water movement into the foliage could have been established (28). With stomata closed, (ψ_L was less than -2.0 MPa) and the foliage continually wet, transpiration was probably drastically reduced (10) due to maintenance of a saturated microclimate. With the foliage constantly wet, the cuticle probably became water permeable (23). For lath house watered trees, the cuticle apparently did not present an effective barrier against water movement into the foliage from day 14 to 34. Although radiation was high during the start of rehydration, at least one-half of it was blocked by the lath structure and it did not appear to be related to ψ_L (r^2= 0.005).

Turgor pressure of watered lathouse stored trees during greenhouse storage paralleled ψ_L until day 42 when ψ_p approached zero, but ψ_L declined an additional -0.2 MPa before the trees were rehydrated. The approach to zero ψ_p at a ψ_L of -3.1 MPa may be an indication of the approach to the damage threshold (Fig. 3.1 and Table 3.1), if water deficits continue. If desiccation induced damage is related to cell plasmolysis, then the stage is set for such to occur. Needles from trees allowed to dry below -3.5 MPa (Fig. 4.3) became shriveled and light colored and eventually brittle. These irreversible symptoms of damage are indicative of cell plasmolysis (8, 32).

Watered, greenhouse stored trees dried rapidly during the first 3 days of storage (Fig. 4.2). The rapid ψ_L decline for watered trees may have been due to insufficient watering frequency and or to

reduced capacity to absorb water as well as to the low ψ_a (normally lower than -70 MPa). Seedlings were watered every 2 hours and in many instances, the foliage appeared dry when it was time to rewater.

Equilibration of watered trees occurred at -3.3 MPa. Apparently, under existing environmental conditions, a ψ gradient favorable for water movement into the trees existed. Stone (28) reported that nightly foliar watering caused a similar water movement into ponderosa pine seedlings stored in a greenhouse. This occurred after soil water had been depleted to the permanent wilting point. The seedlings' water requirements were met by foliar watering for 2 to 3 months.

Equilibration or a point in the ψ_L curve where further decreases are severely restricted, appears to be affected by environmental conditions. Under warm and dry greenhouse conditions, ψ_L declines, but at a diminished rate after -3.0 $\pm$ 0.3 MPa is passed (Fig. 3.1, 4.1, 4.3, 5.2). Under cool (Fig. 5.3) or cool and wet (Fig. 4.1, 5.1) conditions, decreases in ψ_L are arrested after passing -2.8 $\pm$ 0.2 MPa. These distinct changes in the drying rate could be important when considering the storage needs of Douglas-fir. As long as trees are kept in a cool and wet environment, a sufficiently high moisture content can be maintained to prevent the deterioration of quality. Moisture content will apparently decrease, depending upon the degree of deviation away from the ideal cool and wet conditions.

Unwatered trees stored in the lath house or greenhouse were 59 and 86%, respectively, less effective than watered trees when compa-

ring the number of days ψ_L was maintained above the damage threshold in each storage environment. Under lath house conditions moisture condensed on the foliage. Dew formation on needle surfaces probably created a microclimate with nearly 100% relative humidity, which probably decreased transpiration for as long as the foliage was wet (34). Bhasker (1) observed that winter mist at night maintained viability of newly planted Pinus kesiya seedlings, while seedlings deprived of the mist died within 20 days. Unwatered greenhouse stored trees were exposed to a warm atmosphere with an ψ_a in excess of -70 MPa (Fig 4.3) for over 14 days. Water potential of unwatered greenhouse trees declined at nearly -0.4 MPa/day. After -3.5 MPa was reached, there was some needle abscission, but progressive water deficits were associated with loss of bright green color, and needle shriveling followed by needle embrittlement. This type of damage, as emphasized earlier, may be due to cell plasmolysis.

The large difference between rehydration rate of watered versus unwatered treatments stored in the greenhouse could be due to decreased stem permeability and or cell death due to plasmolysis. Edwards and Jarvis (6) showed that in Pinus contorta, a 10% decrease in relative water content resulted in a 10% decrease in stem permeability. In a previous experiment (Fig. 3.1), trees stressed to -4.0 MPa with resultant 9% reduction in percent moisture content (Fig. 3.2), absorbed water at a rate 94% lower than the control (Fig. 3.5). In this experiment, watered trees (-3.3 MPa) rehydrated to an 85 % higher ψ_L than unwatered trees (-5.8 MPa). The water column in the latter was probably cavitated beyond repair (13) and stomata

incapable of opening due to severe plasmolysis (32).

A ψ_L above the damage threshold can be maintained for at least 1 month under lath house conditions, provided the foliage is kept wet. Environmental conditions such as radiation and ψ_a had a significant impact on water uptake or loss. Maximum water uptake (with wet foliage) occurred when air temperature was below 10°C and relative humidity above 91%. Trees stored under warm and dry conditions resulted in rapid desiccation but maintained a ψ_L above the damage threshold ψ_L for at least 2 weeks without measurable signs of injury if the foliage was kept wet. This could be important during retail lot display, since during that time trees are similarly exposed.

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Chapter 5

EFFECTS OF ANTITRANSPIRANTS AND STORAGE CONDITIONS ON DRYING OF CUT DOUGLAS-FIR TREES

Abstract

Antitranspirant effectiveness in reducing water loss in cut Douglas-fir was studied under various environmental conditions. Cut trees were dipped in various commercial antitranspirant solutions before exposure to the storage environment. Vapor Gard treated trees stored outdoors maintained an average water potential (ψ_t) of -0.4 MPa for 31 days. Water potential of control trees declined from -0.4 to -2.6 MPa within 12 days and maintained that average ψ_t for an additional 20 days. Once brought indoors, Vapor Gard treated trees dried to -3.3 MPa within 14 days and the control within 2 days. Vapor Gard treated tree ψ_t remained above -1.4 MPa for 18 days during lath house storage, while control ψ_t declined to -2.5 MPa within 5 days. Approximately 35 to 50% of Vapor Gard treated trees became necrotic about 5 days after exposure to greenhouse conditions. Control and Vapor Gard-treated trees dried at the same rate during refrigerated storage at 5°C. Under greenhouse conditions, trees treated with Vapor Gard, Wilt-Pruf, and Cloudcover dried at nearly the same rate as the control to -3.3 MPa within 4 to 6 days.

Introduction

Douglas-fir is the major Christmas tree species produced in the coastal regions of the Pacific Northwest. Its resistance to moisture loss is low compared to several species of pine and fir (19) or to inland sources of Douglas-fir (33). Cut Douglas-fir exposed to warm and dry conditions (20°C and less than 50% relative humidity) reached a damage ψ_L (-3.0 to -4.0 MPa) within 3 to 4 days (Chastagner, personal communication). Trees at the damage ψ_L may look fresh, but progressive water deficits resulted in needle browning, shriveling, abscission and inability to rehydrate. Currently, Christmas tree postharvest handling and storage methods are not standardized or regulated, and precautions to reduce moisture loss generally are not made.

To circumvent poor handling practices, antitranspirants could be used to reduce moisture loss. Several reports indicate that use of film-forming antitranspirants are effective in reducing moisture stress (2, 6, 8, 11, 21) in a variety of crop species. Conversely, antitranspirant-treated cut trees have been shown to lose moisture during exposure to warm and dry conditions. Davis & Fretz (7) and De Roo (9) reported that antitranspirant application to cut trees exposed to room temperature was effective in reducing moisture loss only after rehydration. Palpant (22) concluded that the storage environment has a more significant impact on cut tree moisture content than antitranspirants. Davies and Kozlowski (8) similarly noted that antitranspirant effects were in many cases directly

related to the testing environment. Specific antitranspirant-storage environment effectiveness however has not been reported.

The objective of this study was to reduce moisture loss of cut Douglas-fir trees by combining antitranspirant application with various storage conditions.

Materials and Methods

Douglas-fir trees (1 to 1.5 m tall) were cut and stored outdoors (day/night temperature 6/2°±9°C), in an unheated polyethylene lined lath house (day/night temperature 7/2°±8°C), in a walk-in refrigerator (5°C) or in greenhouse (day/night temperature 16/10° ±5°C).

Xylem water potential (ψ_L) (25) was measured periodically with a pressure chamber (PMS Instruments, Corvallis, OR). Water potential measurements were made on 1 randomly selected, 3 cm twig per tree from each of 10 trees per treatment on each sampling date. Pressure was introduced into the chamber at a rate of 0.07 MPa/sec (30). Stomatal conductance (g_S) was measured with a steady state porometer (Model LI-1600, Licor Inc., Lincoln, Nebraska) (4, 16). Measurements were taken on 1 year old twig tips at 2 different sites on each of 5 trees per treatment. At least 1 cm of needles was stripped on the proximal side of the measured tip to separate the measured leaf area from the rest of the needles on the twig. The 2 measurements were averaged for g_S calculations. At the end of the experiment, leaf surface area was measured with a surface area meter (Model 3100, Licor, Inc.) (10). Stomatal conductance was computed on the basis of projected one-sided leaf area (15).

Visual ratings of quality were conducted by 2 individuals. The rating categories and scale were as follows: general appearance, 1 = poor, 2 = fair, 3 = good 4 = excellent; % needle necrosis, 1 = 75 - 100, 2 = 50 - 75, 3 = 25 - 50, 4 = 0 - 25; % needle loss, 1 = above

75, 2 = 50 - 75, 3 = 25 - 50, 4 = 0 -25.

The following antitranspirants were screened at their recommended concentration: Wilt-Pruf (Di-1-p-menthene) at 5%, Vapor Gard (Poly-1-p-menthene-8-9-diyl) at 5%, Envy (chemical name unknown) at 14% and Cloud Cover (chemical name unknown) at 6.6%. Antitranspirants were applied as a dip. Treated trees were moved to their respective storage environments after treatment. After reaching a ψ_L of -3.0 to -3.3 MPa, trees were rehydrated by cutting 1 to 2 cm of stem base and immediately immersing the cut end in water (23). Rehydrated trees were stored in the greenhouse. The experimental design for all experiments were randomized blocks with 5 replications and 2 trees per replication.

Results

Outdoor storage and Vapor Gard treatment was the most effective combination for maintaining a ψ_L above the damage threshold (Fig. 5.1). During the first 4 days (rainy period Table 5.1b, and saturated night air Table 5.1c), ψ_L of all trees increased from -1.7 to -0.3 MPa. The wet period persisted until day 8, and ψ_L of all trees remained above -1.5 MPa. A ψ_L decline of -1.5 MPa for the control and Envy-treated trees from day 10 to 12 was associated with a preceding sunny day (r^2= 0.64). The control and Envy-treated trees remained at an average -2.6 MPa for 20 days. A 70% increase in sunshine from day 10 to a period from day 19 to 22 (Table 5.1a) did not affect ψ_L of the control and Envy treatments (r^2= 0.05), but ψ_L of Vapor Gard-treated trees decreased from -0.2 to -0.8 (Fig. 5.1). Day ψ_a declined sharply between day 18 and 24. After day 24, radiation decreased, precipitation, day and night ψ_a increased and ψ_L of Vapor Gard-treated trees increased to -0.3 MPa, but none of the measured environmental factors either singly or collectively were correlated to the increased ψ_L. After 32 days of outdoor storage, all trees were brought into the greenhouse. The control and Envy-treated trees dried to -3.3 MPa within 2 days and Vapor Gard-treated trees within 14 days. Two days after rehydration, ψ_L of the control and Envy treated-trees increased to that measured at the beginning of the experiment, followed by a gradual decline to a low of -1.4 MPa in 18 days. Rehydration of the Vapor Gard treatment required 8 days: ψ_L increased to -1.7 MPa, -0.3 MPa lower than that measured before

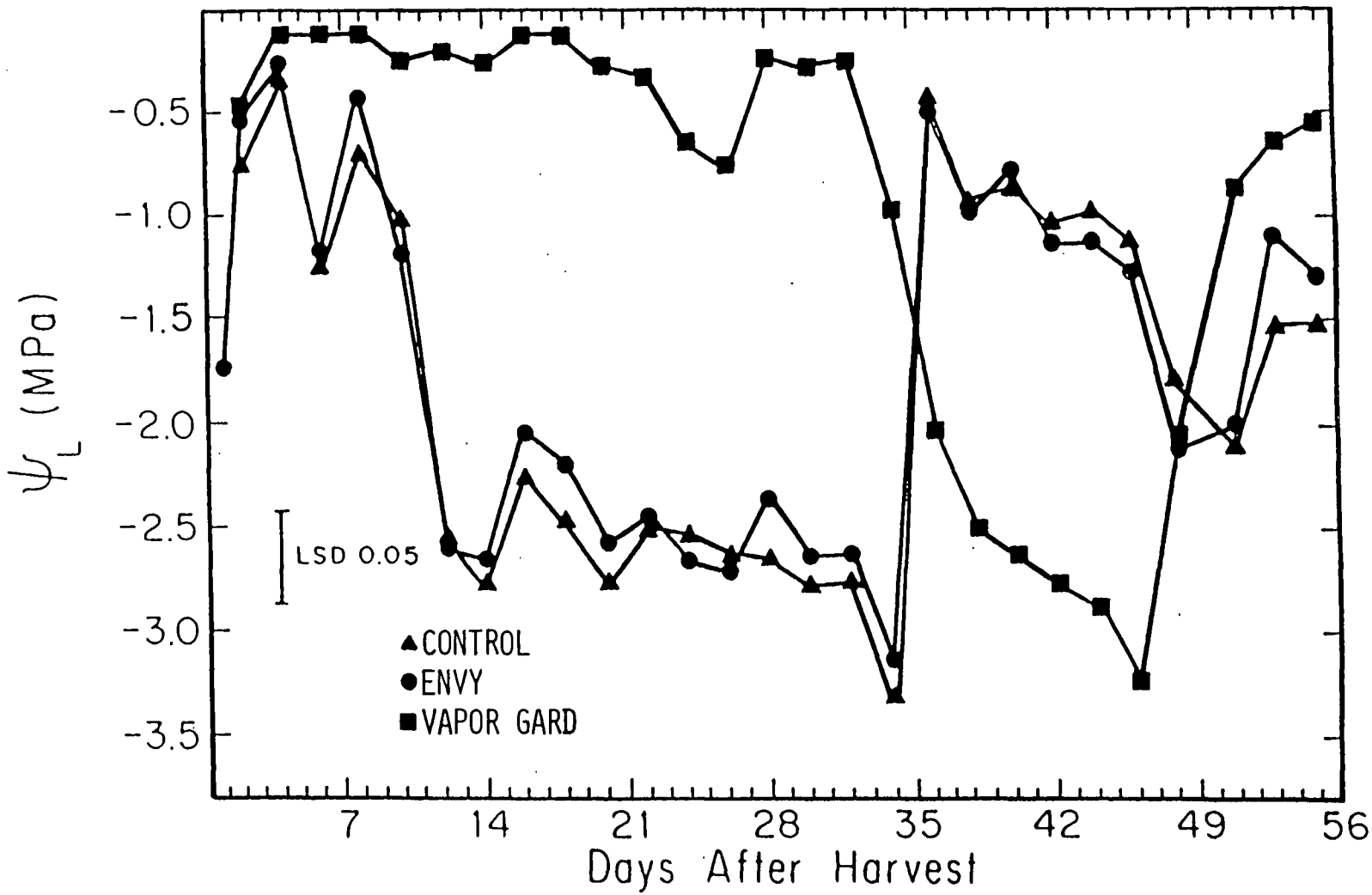

Fig. 5.1. Water potential of antitranspirant treated cut trees during outdoor storage (days 1 to 31) and indoor storage (days 32 to 55).

Table 5.1a. Insolation during outdoor storage

Day	Langley · 24 hr.$^{-1}$	Day	Langley · 24 hr.$^{-1}$
1	70	17	24
2	90	18	78
3	102	19	24
4	78	20	176
5	44	21	175
6	63	22	177
7	54	23	144
8	58	24	156
9	54	25	117
10	60	26	135
11	132	27	78
12	54	28	87
13	30	29	114
14	60	30	108
15	72	31	54
16	36		

Table 5.1b. Daily precipitation during outdoor storage

Day	mm · 24 hr.$^{-1}$	Day	mm · 24 hr.$^{-1}$
1	4.57	17	0.0
2	0.0	18	0.0
3	9.65	19	0.0
4	30.23	20	0.0
5	11.68	21	0.0
6	0.51	22	0.0
7	2.03	23	0.0
8	13.21	24	0.25
9	0.25	25	0.0
10	0.0	26	6.60
11	0.0	27	11.94
12	0.0	28	1.02
13	0.25	29	5.59
14	0.0	30	13.21
15	9.65	31	0.25
16	17.53		

Table 5.1c. Day and night air water potential during outdoor storage

Day	Day/Night ψ_a	Day	Day/Night ψ_a
1	-9.2/-7.8	17	-19.8/-6.6
2	0.0/0.0	18	-21.0/-7.8
3	-13.6/0.0	19	-62.3/-13.3
4	-10.7/0.0	20	-44.8/-6.5
5	-2.6/0.0	21	-32.7/-6.5
6	-8.1/0.0	22	-66.3/-7.7
7	-20.0/0.0	23	-86.6/-10.4
8	-1.1/0.0	24	-66.0/-16.3
9	-14.0/-6.7	25	-58.1/-9.2
10	-14.0/-8.1	26	-36.8/-7.9
11	-23.4/-10.9	27	-21.2/-13.6
12	-20.0/-10.9	28	-57.2/-10.8
13	-16.8/-6.7	29	-15.2/-8.1
14	-20.0/-7.9	30	-27.9/-10.9
15	-29.8/-5.1	31	-41.4/-10.7
16	-16.7/-6.7		

exposure to greenhouse conditions.

After 18 days of lath house storage, ψ_L of Vapor Gard-treated trees was above -1.4 MPa; of Wilt-Pruf-treated trees it was -2.5 MPa; and of control trees it was -2.9 MPa. All trees were brought into the greenhouse on day 18. Control trees were rehydrated on the same day and required 10 days to reach maximum ψ_L. Vapor Gard and Wilt-Pruf-treated trees were rehydrated 4 days after exposure to greenhouse conditions and rehydration was complete within 2 days. Vapor Gard-treated trees reached a maximum ψ_L above -0.5 MPa and it remained above that level for an additional 8 days. Control and Wilt-Pruf-treated tree ψ_L declined an average -0.6 MPa after reaching maximum ψ_L.

Stomatal conductance of lath house-stored, Vapor Gard treated-trees was less than 1 mm/sec on all sampling dates, except on the day the trees were brought into the greenhouse (Fig. 5.2a). Stomatal conductance of the control and Wilt-Pruf increased slowly after rehydration.

Some Vapor Gard treated-trees originally stored in the lath house developed severe needle necrosis (Table 5.2) about 23 days after harvest. Necrotic needles appeared water-soaked and were distributed along entire lengths of branches. This type of damage was followed by needle abscission. Necrotic needles on the control were dry, hard, sporadically distributed throughout the branches and did not abscise. Wilt-Pruf-treated trees had no visible needle damage throughout the length of the experiment.

Storage at 5°C did not significantly improve Vapor Gard treat-

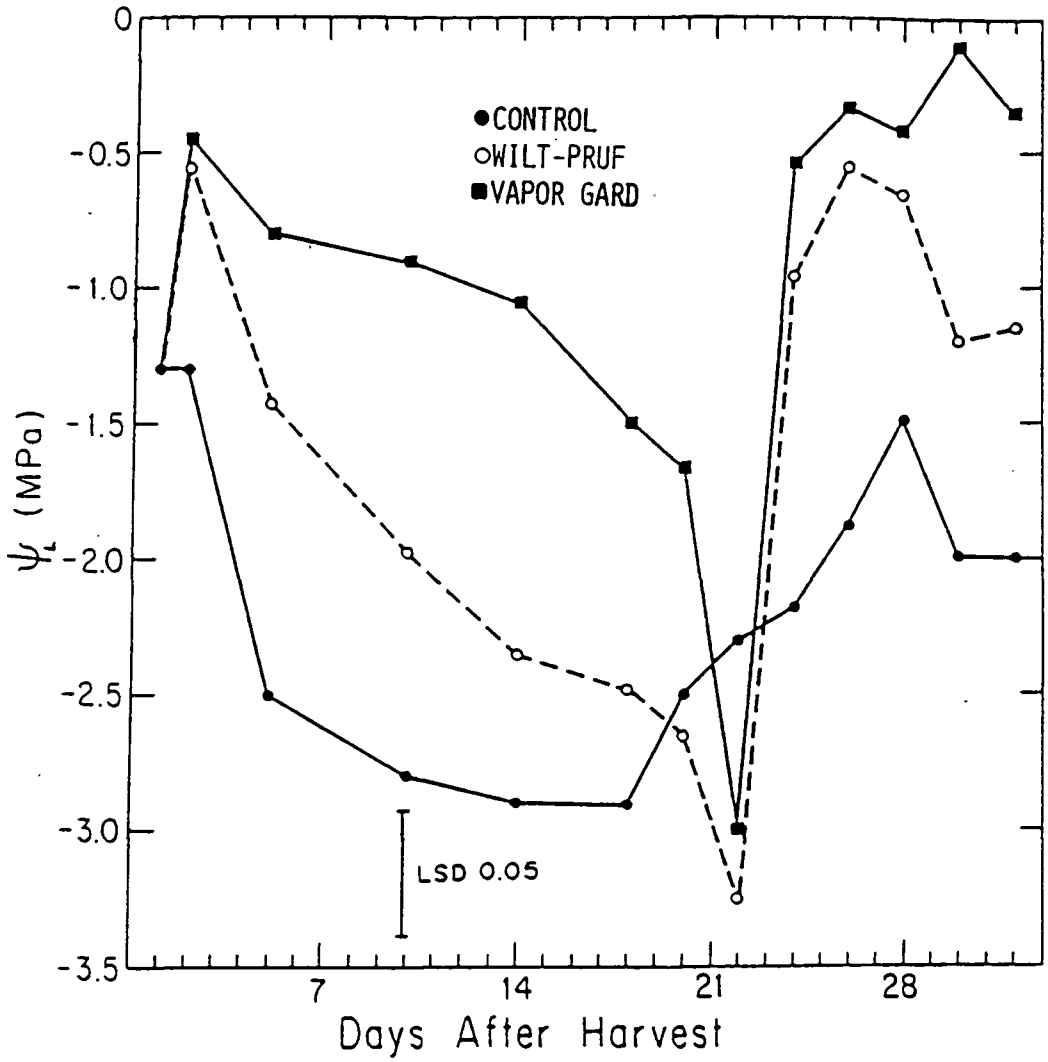

Fig. 5.2. Influence of drying on water potential of antitranspirant treated trees during lath house storage (days 1 to 17) and indoor storage (days 18 to 32).

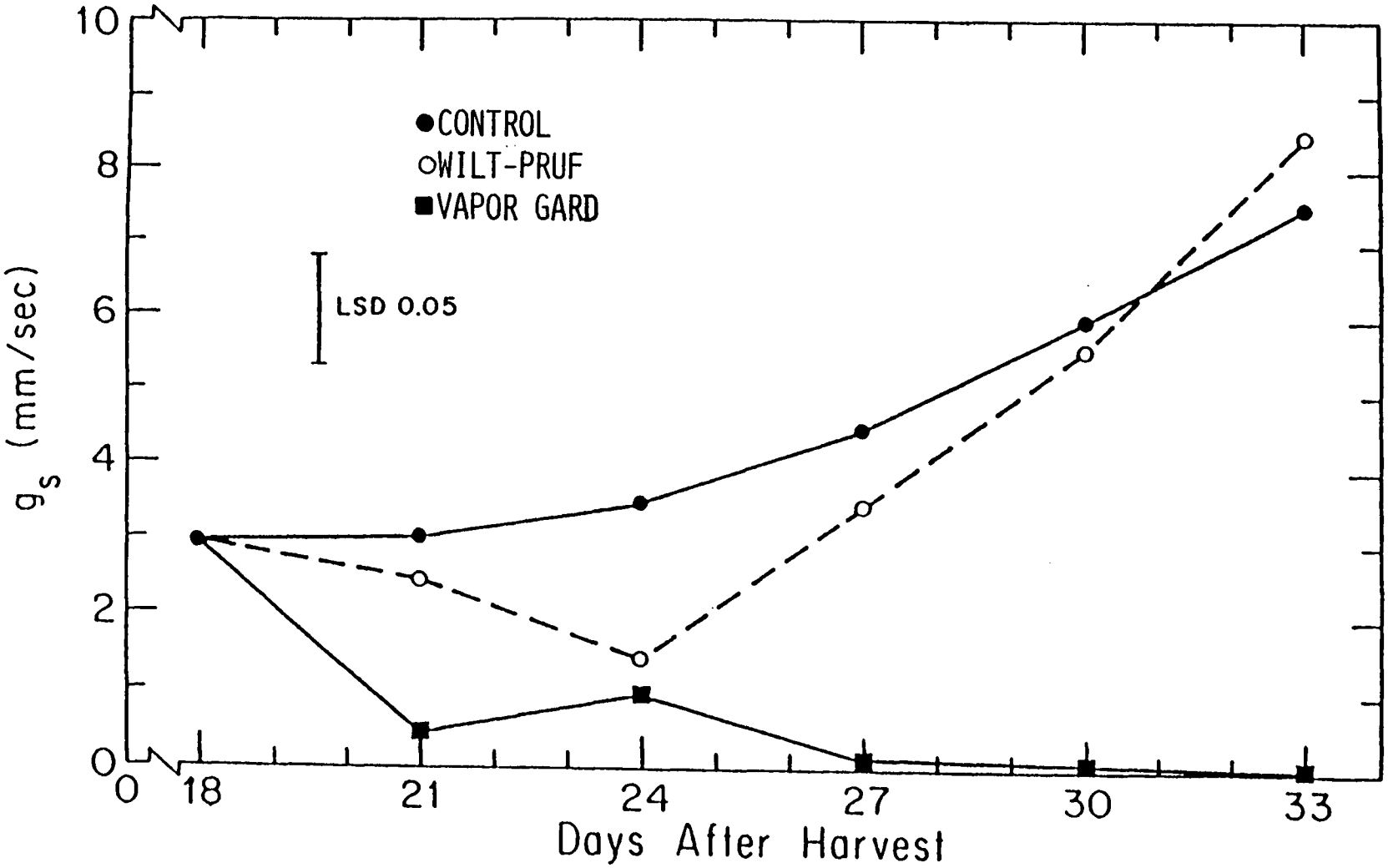

Fig. 5.2a. Stomatal conductance of lath house stored antitranspirant treated trees during greenhouse storage (days 18 to 32).

Treatments	WEEKS AFTER HARVEST							
	3				4			
	MPa	General Appearance	Needle Necrosis	Needle Loss	MPa	General Appearance	Needle Necrosis	Needle Loss
Vapor Gard	-1.0	4.0	4.0	4.0	-0.4	2.9	2.8	3.2
Wilt-Purf	-2.3	4.0	4.0	4.0	-0.6	4.0	4.0	4.0
check	-2.9	4.0	4.0	4.0	-1.5	3.0	3.5	4.0
		N.S.	N.S.	N.S.		N.S.	N.S.	N.S.

Table 5.2. Effect of drying on ratings of general appearance, needle necrosis, and needle loss in cut Douglas-fir trees. N.S. = Not significant at the 5% level of analysis of variance.

ment effectiveness (Fig. 5.3). Both the control and Vapor Gard treated-trees dried at the same rate for 22 days. On day 26, ψ_L of Vapor Gard-treated trees was nearly -4.0 MPa compared to -3.0 MPa for the control. On that same day, trees were taken to the greenhouse and rehydrated. Trees from both treatments reached a ψ_L maximum similar to that measured at the beginning of the experiment. Severe bark shriveling observed on Vapor Gard treatment on days 23 to 26 disappeared upon rehydration.

Greenhouse storage immediately after treatment with antitranspirants was the least desirable storage method (Fig. 5.4). Trees from all treatments dried to -3.3 MPa within 4 to 6 days. After rehydration, Vapor Gard-treated tree ψ_L remained above -0.5 MPa for 20 days, while the ψ_L of the other treatments declined slowly.

Under all test environments, foliage of Vapor Gard-treated trees looked glossy and felt sticky. Foliage of Wilt-Pruf-treated trees always remained equal to that of fresh cut trees and foliage of the control, Envy and Cloudcover treated trees became dull green within 3 to 6 days, followed by occasional needle necrosis after greenhouse storage.

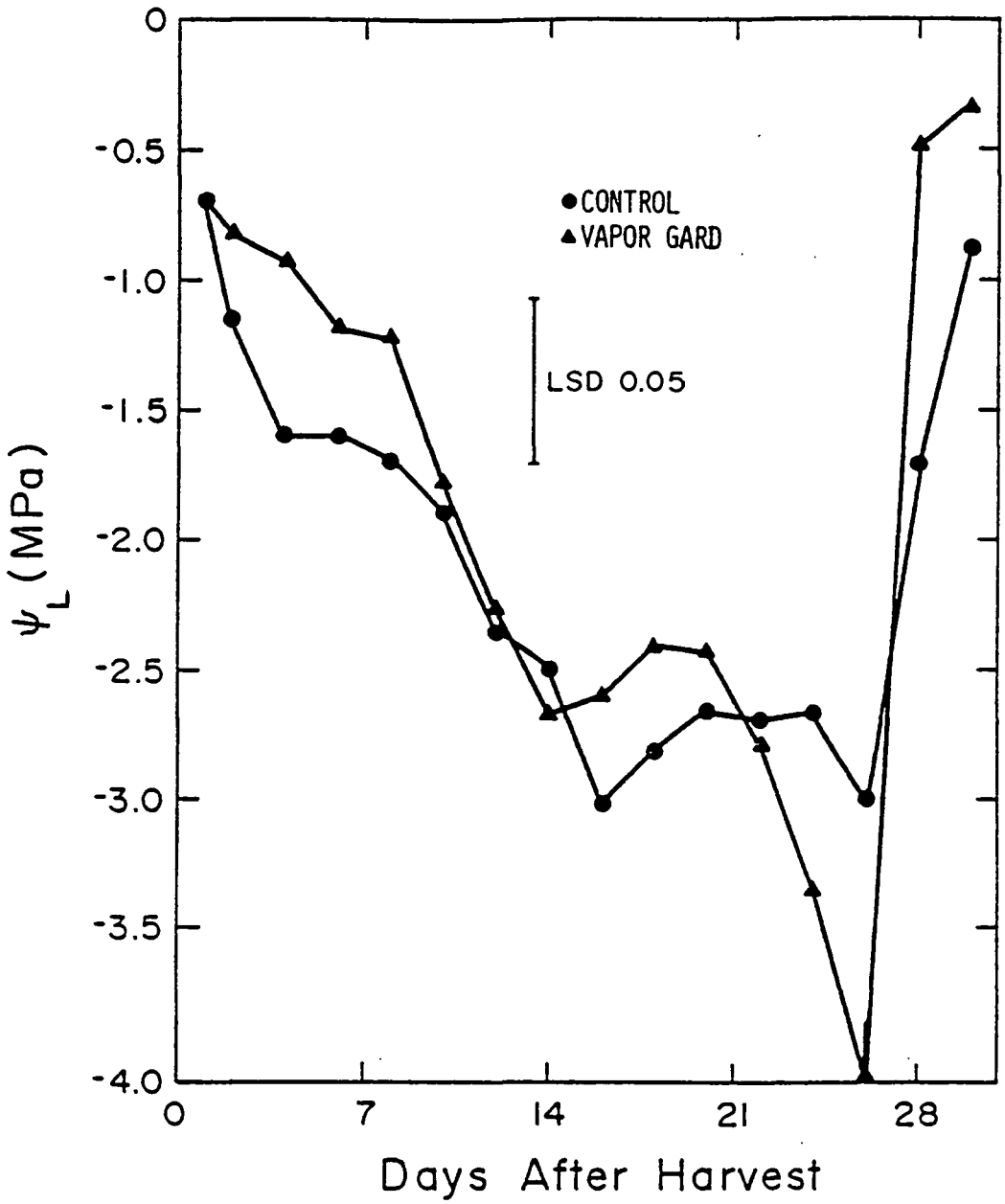

Fig. 5.3. Influence of drying on water potential of anti-transpirant treated trees during refrigerated storage (days 1 to 25) and greenhouse storage (days 26 to 30).

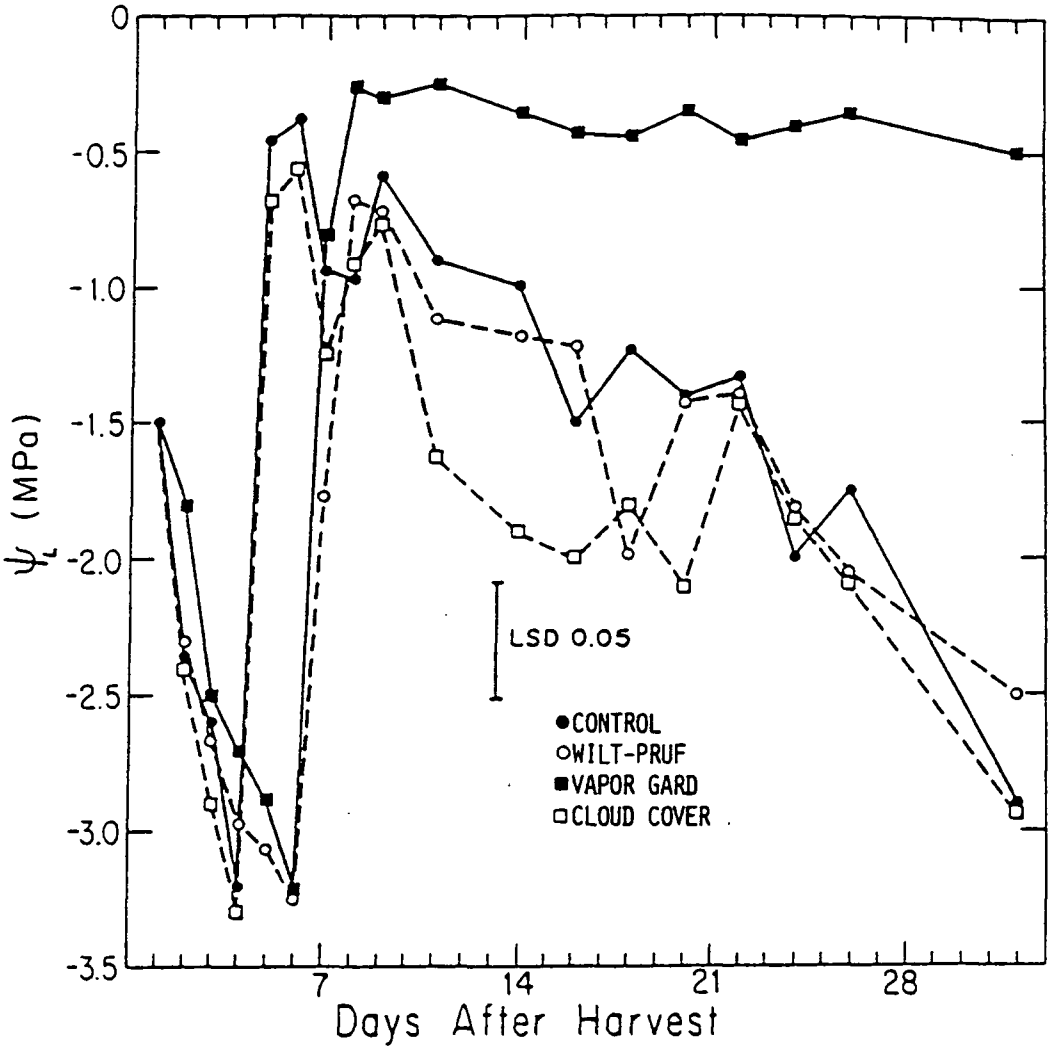

Fig. 5.4. Influence of drying on water potential of anti-transpirant treated trees during greenhouse storage.

Discussion

Antitranspirant effectiveness in maintaining a ψ_L above the damage threshold was closely related to the storage environment (9). Of the antitranspirants tested, Vapor Gard was the most effective in moisture retention. This may have been due in part to its ability to form a long lasting and resilient film on plant surfaces (1, 2). During the first 8 days, outdoor stored trees (Fig. 5.1) had the advantage of high rainfall, day ψ_a generally above -16 MPa and a saturated night ψ_a. These conditions are conducive to foliar water uptake (14, 20, 26, 27) and reduced transpiration (17, 31). When the rains stopped, radiation increased and both day and night ψ_a decreased, ψ_L of the control and Envy-treated trees also decreased (from -1.1 to -2.6 MPa), probably to a point where stomatal closure (24) prevented further losses. On the other hand, Vapor Gard-treated trees maintained their high water status, despite exposure to adverse conditions. Roberts (23) similarly observed that ψ_L decreased for cut Scots pine trees during sunny days, and increased during cloudy or rainy days.

Comparison of outdoor to lath house storage, Vapor Gard treatment effectiveness was reduced in the latter by 88% (Fig. 5.1 & 5.2). Outdoor storage had rain for one-half of the total storage period, 36% higher night ψ_a, 24% lower day ψ_a, and an average of 62% more light (Tables 5.1a & 5.1b). The apparent advantage of decreased radiation in the lath house was probably of small consequence, since Douglas-fir water loss is not related to light intensity but more to

atmospheric moisture (12, 24). Since outdoor and lath house experiments were not run concurrently, environmental data were disimilar and it is difficult to conclude which of the storage environments might have yielded the better results. Despite a general ψ_L decline during lath house storage, visual ratings of quality were excellent 3 weeks after harvest.

In the 5°C storage refrigerator, ψ_L of Vapor Gard-treated trees declined at a rate 96% faster than outdoor storage and 62% faster than lath house storage. Water potential of the control in the 5°C storage refrigerator declined at a rate 83% slower than outdoor storage and 22% faster than lath house storage. Compared to outdoor and lath house storage, trees stored in the 5°C storage refrigerator had no dew condensing on the foliage. This lack of foliage moisture did not seem to affect drying rate of the control trees, but it appeared to substantially affect Vapor Gard's performance in reducing the rate of water loss. In addition, the equilibration ψ_L for the control was -0.4 MPa lower than either outdoors or lath house storage.

Vapor Gard-treated trees dried to -4.0 MPa did not develop injury symptoms typical of drying below -3.5 MPa (needle abscission) before or after rehydration. After removal from 5°C storage, a comparison of the control and Vapor Gard treatments showed the presence of bark wrinkling on all Vapor Gard-treated trees, which disappeared after rehydration. These results pose some interesting questions regarding the damage threshold ψ_L. Under greenhouse conditions, the damage threshold has been shown to be reached in 12 days

(Fig. 3.1). In this experiment, the threshold ψ_L was reached in nearly 25 days. Campbell and Vines (5), showed that in Picea excelsa, the rate of drying appeared to trigger the abscission mechanism. Fast drying to 60 or 70% moisture content resulted in abscission, while slow drying to 65% moisture content did not cause abscission. The effects of rapid versus slow drying could be important when considering the storage needs of cut Douglas-fir trees.

Storage under greenhouse conditions resulted in drying of all treatments close to -3.3 MPa within 4 to 6 days (Fig 5.4). Treatment with Vapor Gard was not effective in reducing water loss until after rehydration (7, 9, 11). Under greenhouse conditions, there was no characteristic equilibration such as was measured for outdoor, lath house and 5°C refrigerator storage. The mechanism to reduce water loss appears to be operable only under moderate environmental water stress. Douglas-fir does not possess well developed morphological or physiological adaptive features to withstand high air vapor pressure deficits (18, 33). Antitranspirant effectiveness appears to be closely related to the trees' natural ability to reduce water loss under moderate air vapor pressure deficit. Under high air vapor pressure deficit, antitranspirants are practically ineffective. This could make antitranspirant application uneconomical because the gains in moisture retention would probably not balance the deficits of application costs.

A disadvantage of using Vapor Gard treatment is the resultant shiny and sticky needles. These characteristics detract from the trees' appearance and would probably complicate handling and bulk

storage of treated trees. Another disadvantage of Vapor Gard treatment was the development of phytotoxicity in some experiments that resulted in needle necrosis and abscission. Injury may have been due either to phytotoxicity or to decreased gas exchange (Fig. 5.2a). Carbon dioxide and oxygen diffusion were probably reduced by a factor equal to or greater than that measured for water vapor (13, 29). Similar toxicity has been reported in other crops (8, 13, 28, 32). Davies and Kozlowski (8) concluded that uneven coating might decrease toxicity due to "suffocation".

Vapor Gard's effectiveness in maintaining a water content above the damage threshold ψ_L appears to be closely related to the presence of external needle moisture. Vapor Gard-treated trees allowed to dry slowly below the -3.5 MPa damage ψ_L did not show visible signs of injury before or after rehydration. Vapor Gard application to reduce moisture loss in cut trees is not recommended due to the formation of shiny and sticky foliage after treatment. Vapor Gard application as a dip may in certain cases result in phytoxicity to Douglas-fir cut trees.

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Chapter 4

ANATOMICAL DEVELOPMENT OF THE ABSCISSION ZONE IN DOUGLAS-FIR

Abstract

Early needle growth occurred from an intercalary meristem formed at the base of the needles. When the needle reached maximum length, the intercalary meristem became inactive and the proximal layer of cells enucleated and developed distinctly modified cell walls. This layer of cells formed the separation layer and was situated between needle and bark tissue. Shortly afterwards, 1 to 2 layers of cells proximal to the separation layer had their lumens filled with a ligno-suberin type of material that had characteristic properties of the stem epidermis. This layer of cells eventually formed the protective layer and it remained attached to the bark surface after abscission. The vascular trace passed intact through both the separation and protective layer. For abscission studies, needles were sampled at specific levels of water stress. Needles from trees that were rehydrated immediately after harvest did not abscise. Needles obtained from trees with abscising needles and stressed to -3.5 MPa showed shrinking and tearing of separation layer cell walls. About one-half of the tree population stressed to -3.5 MPa did not have significant needle abscission. Needle samples taken from this population of trees did not show the characteristic shrinking and tearing

of separation layer cells.

Introduction

Douglas-fir, the principle Christmas tree species produced in the Pacific Northwest, is very suceptible to moisture loss (16). Cut trees undergo rapid dehydration when exposed to warm and dry conditions. As the trees dry, a damage threshold ψ_L (-3.0 to -4.0 MPa, Chastagner, personal communication) is passed that results in moderate to severe needle abscission, which is frequently enhanced by rehydration (1, 2, 8, 13, 19). The reason for the promotion of abscission by rehydration is unknown.

The abscission signal in gymnosperms may be triggered by the rate of drying (4, 5, 32). There is some disagreement about the mechanism of abscission (32), although it seems to be the result of mechanical breakage of cell walls above a protective layer that remains attached to the twig after needle separation (4, 5). An understanding of the anatomical events that lead to and occur during abscission is a first step in indentifying methods to control abscission in Douglas-fir.

The objectives of this study were: (1) to describe the anatomical development of the abscission zone in Douglas-fir seedlings, (2) to search for control points that could be critical to controlling abscission, and (3) to study cell wall changes during abscission induced by water stress in cut trees.

Materials and Methods

Developmental Studies

Samples for developmental studies were collected from 10, 3 year-old potted Douglas-fir seedlings. Samples were collected from bud break through needle maturity. The samples were fixed in formalin-acetic acid-alcohol (FAA), dehydrated by the tertiary butyl alcohol method and embedded in Paraplast (12). Needle samples were sectioned longitudinally at about 12 μm with a steel knife on a rotary microtome. Unfixed samples were sectioned with a freezing microtome at about 25 μm. Stains used are listed in table 6.1.

Abscission

Samples for abscission studies were collected at various levels of water stress from 10 cut trees used to determine the threshold ψ_L (Chapter 3, Fig. 3.1). Xylem water potential (30) was measured with a pressure chamber (PMS Instruments, Corvallis, Oregon). Pressure was introduced into the chamber at 0.07 MPa/sec (36). Two twigs per tree from each respective treatment (Fig. 3.1) were measured each time needles were sampled for microscopic examination.

Samples were fixed in Karnovsky's fluid (14), then dehydrated and embedded using the Ruddell Method (26). The low acid glycol methacrylate was purified 5 times with activated charcoal before use. Sections were cut at 4-6 μm using a rotary microtome and steel knife. All sections were stained with toluidine blue (6).

Table 6.1. List of stains used. *Indicates stain used on unfixed sections.

Cellulose	Cutin/Suberian	Lignin	Nucleus	Pectins	Starch
1 [y] Fast Green	6 Safranin	8 Safranin	11 Safranin	13 Hydroxyl-amine Ferric Chloride*	14 IKI
2 Acid fuchsin	7 Sudan IV*	9 Chlorine-Sulfite	12 Heidenhain's Iron Hematoxylin		
3 Delafield's Hematoxylin		10 Periodic Acid Schiff's			
4 Periodic Acid Schiff's					
5 Toluidin Blue					

[y]Stain Code Number	Reference
1, 2, 3, 6, 7, 8, 11, 12	12
4, 10	9
5	6
9	31
13	25
4	11

Results

Development

Three broadly defined but distinct developmental stages occur during needle ontogeny. During early needle growth (stage 1), procambial cells were elongate and were continuous with the shoot (Fig. 6.1). Prior to bud burst (Stage 2), an intercalary meristem was formed at the base of the needle (Fig. 6.2) (23). Highly protoplasmic cells with large, darkly stained nuclei encircled the procambial strand. Cells in the intercalary meristem divided anticlinally (Fig. 6.3). When the needle reached maximum length (stage 3), growth from the intercalary meristem ceased (Fig. 6.4).

A distinct set of morphological events occurred when needle length approached maximum. First, the intercalary meristem became inactive, followed by safranin staining of cell walls on the proximal layer of the intercalary meristem. This characteristic staining began to show at the needle margins (Fig. 6.5) and it proceeded towards the interior of the needle until the entire needle was isolated from the stem (Fig. 6.6). As these changes occured, nuclei were lost and a secondary wall developed that was several times thicker then cortical parenchyma cell walls distal to it (Fig. 6.7). It was later determined that needle separation occurred at this point. Separation layer cells had small lumens, thin primary walls, and thick secondary walls with numerous simple pits. As the needle continued to age, a layer of cells proximal to the separation layer stained with safranin. The staining pattern was not limited to the

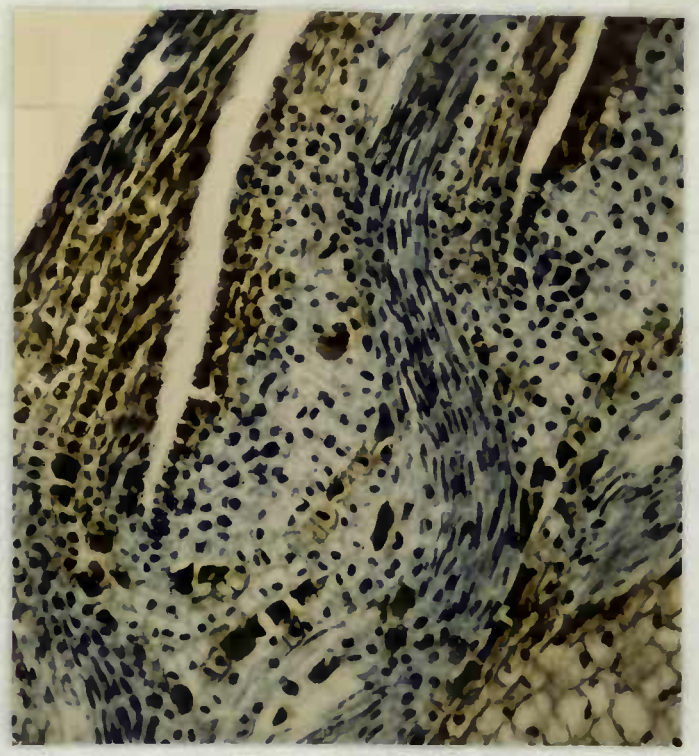

Fig. 6.1. Needle sample from swollen bud. Section stained with Heidenhain's iron haematoxylin and fast green. Rapid developing shoot procambium is continuous with the needle.

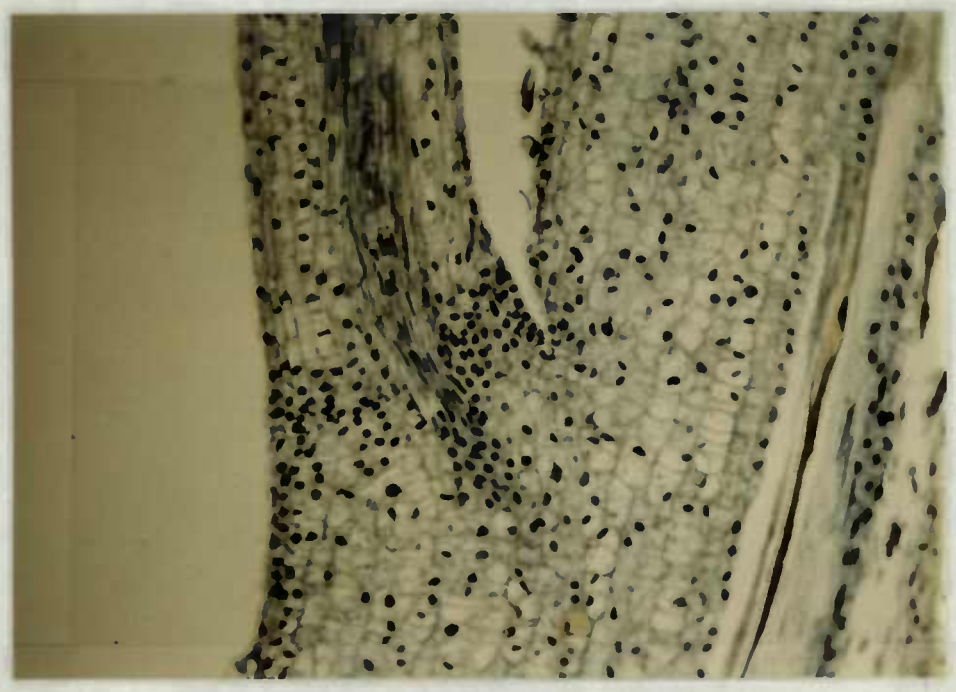

Fig. 6.2. Formation of an intercalary meristem a few days prior to bud break. Section stained with Heidenhain's iron haematoxylin and fast green.

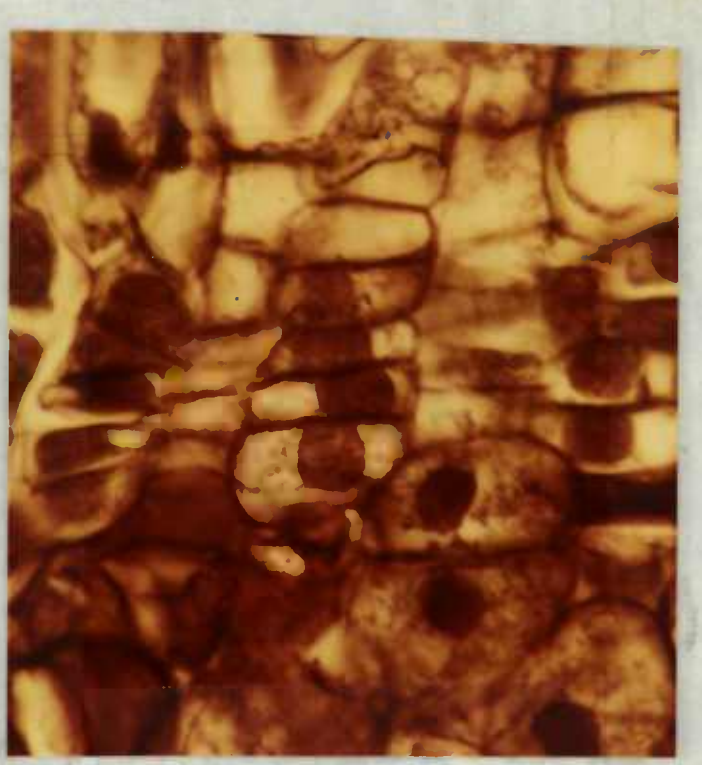

Figure 6.3. Anticlinical cell division at the intercalary meristem. Section stained with safranin and fast green.

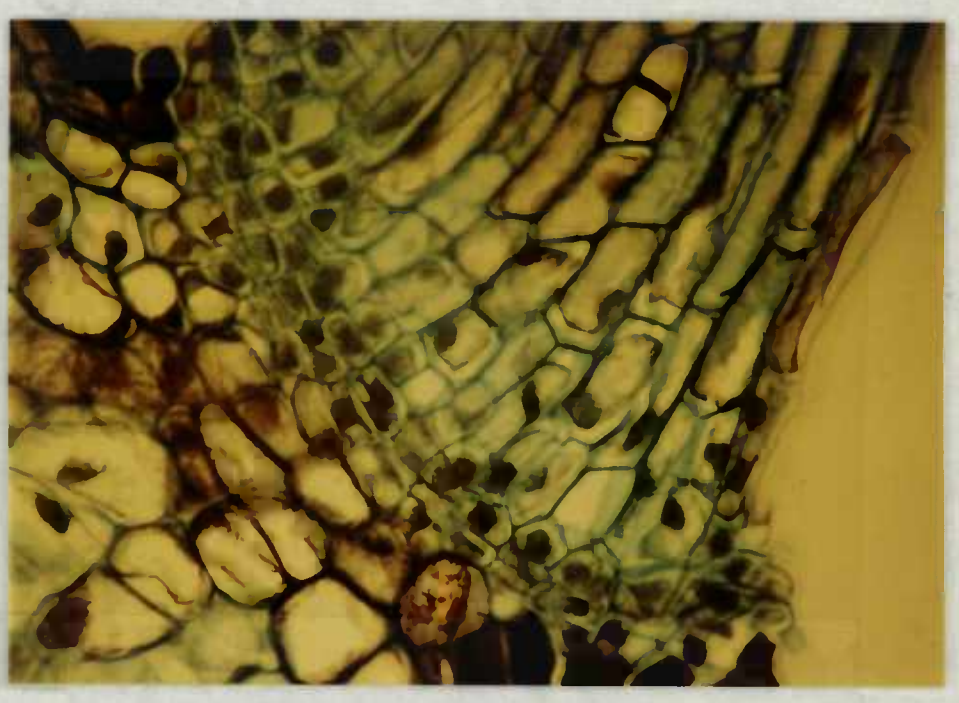

Figure 6.4. Cessation of needle growth from the intercalary meristem. Section stained with safranin and fast green.

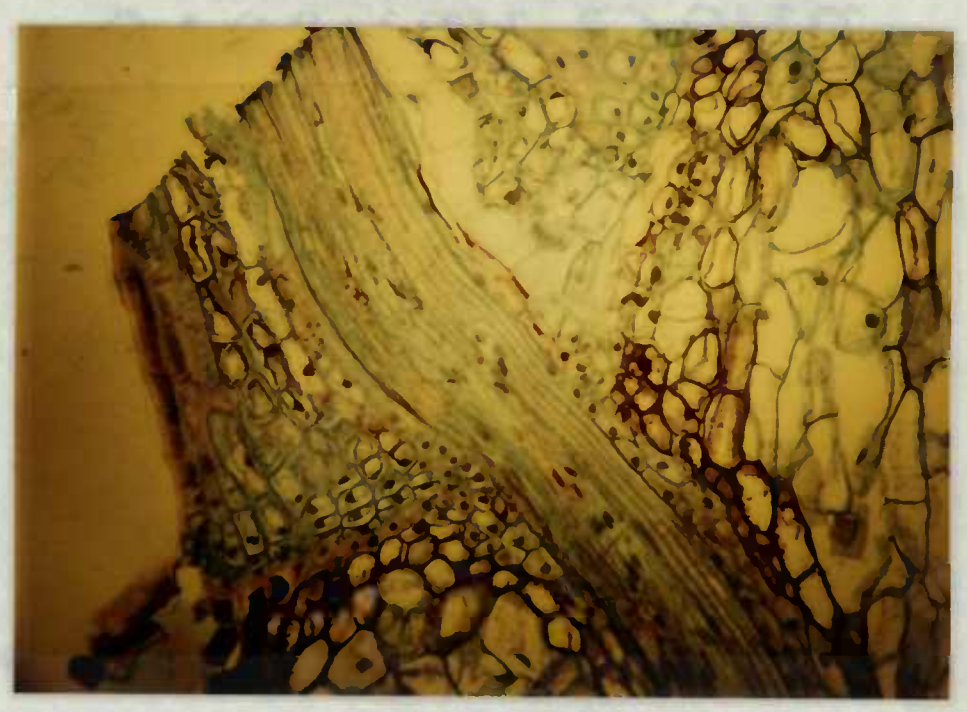

Figure 6.5. Formation of separation layer by impregnation of cells walls in the proximal layer of cells of the intercalary meristem. Section stained with safranin and fast green.

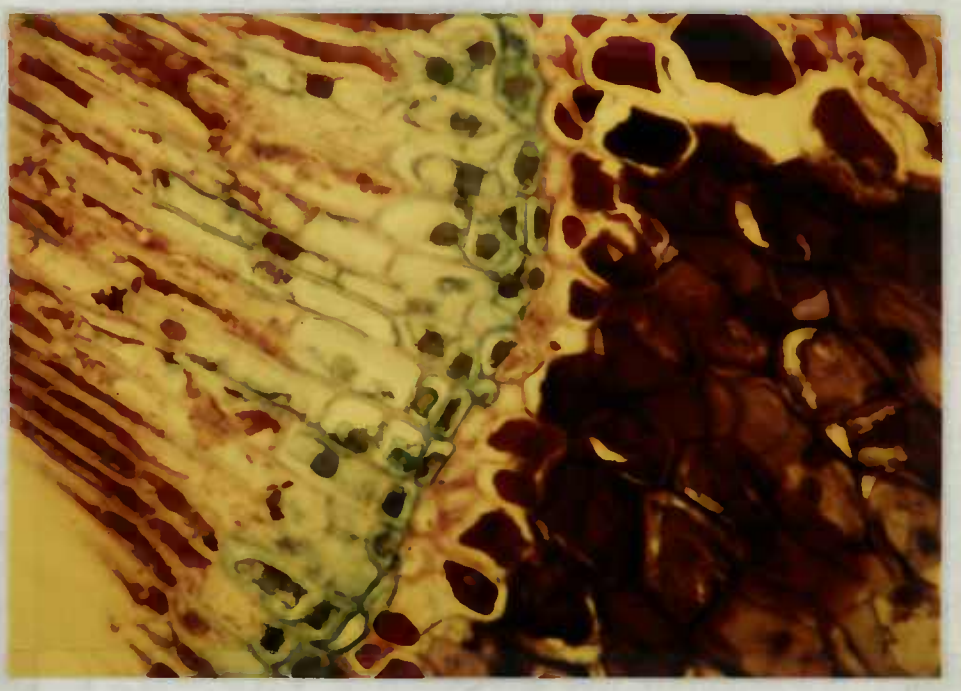

Figure 6.6. Separation layer completely formed. Nuclei and protoplasts lost. Section stained with safranin and fast green.

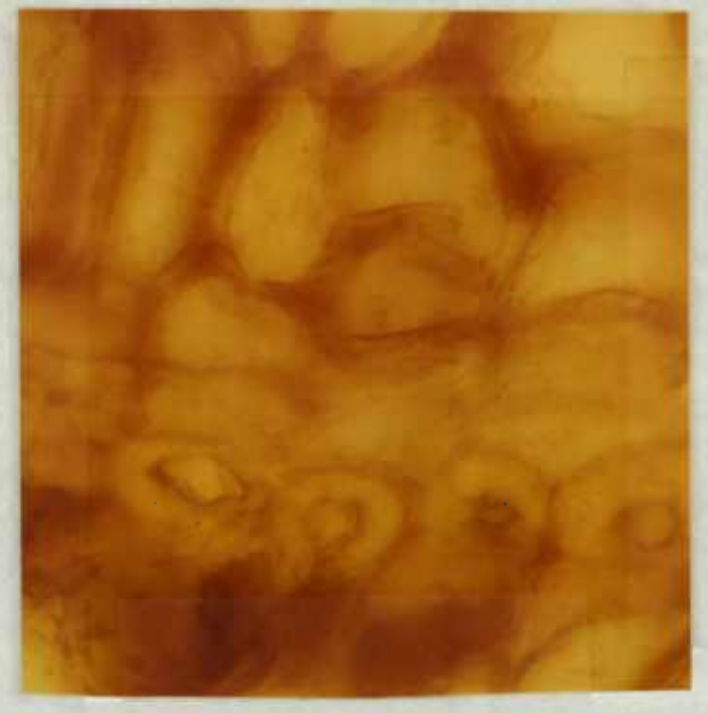

Figure 6.7. Thick and prominantly pitted secondary walls of the separation layer. Unfixed section stained with hydroxylamine ferric chloride.

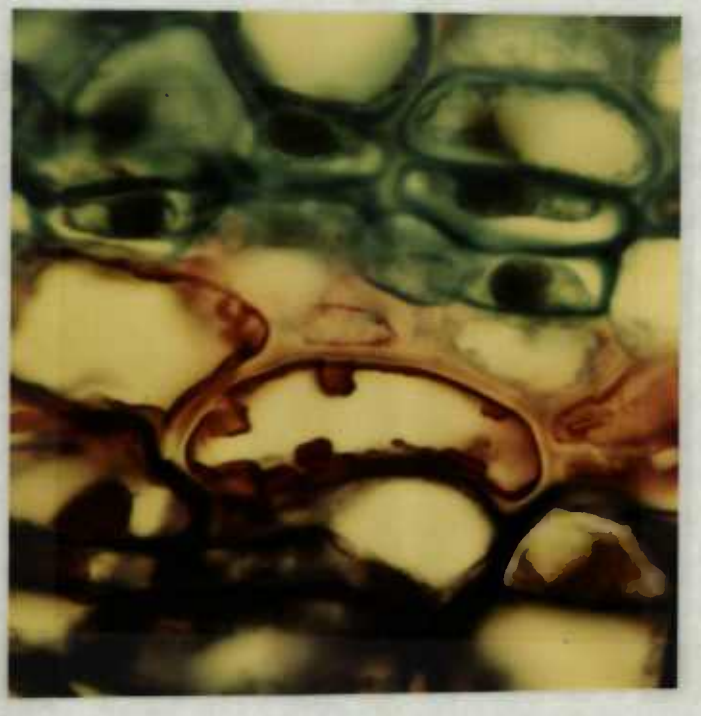

Figure 6.8. Formation of protective layer by filling the lumen of cells with a dense, safranin staining material. Section stained with safranin and fast green.

cell walls, but included the lumen as well. The lumen of these cells were slowly filled with a dense, safranin staining material (Fig. 6.8). This layer of cells is the protective layer, and in 3 years, it may be 3 to 5 cells deep (Fig. 6.9).

Starch was not detected on the separation layer in the mature needle (Fig. 6.10), but cells distal or proximal had high starch content. Separation and protective layer cells did not stain with Delafield's haematoxylin (Fig. 6.11) or with periodic acid-Schiff's reaction (Fig. 6.12). Unfixed sections stained with hydroxylamine ferric chloride for pectic substances indicated traces of pectins between adjacent separation layer cells (Fig. 6.7). Separation layer cells were lightly stained with acid fuchsin (Fig. 6.13). Lignin (chlorine-sulfite test) was present in protective layer cells, but not in separation layer cells (Fig. 6.14). Staining with Sudan IV showed a positive stain reaction on the needle cuticle only.

Abscission

Needles sampled from trees that were rehydrated immediately after harvest did not abscise and did not show separation layer cell wall tearing over a 22 day period (Fig. 6.15). Samples obtained from these trees 22 days after rehydration had some loss of cell wall integrity but no evidence of extensive cell wall breakage (Fig. 6.16). At ψ_L of -3.0 to -3.5 MPa, which stimulated needle abscission, severe shrinkage of the separation layer cell walls was apparent (Fig. 6.17), followed by loss of cell wall integrity (Fig. 6.18) and

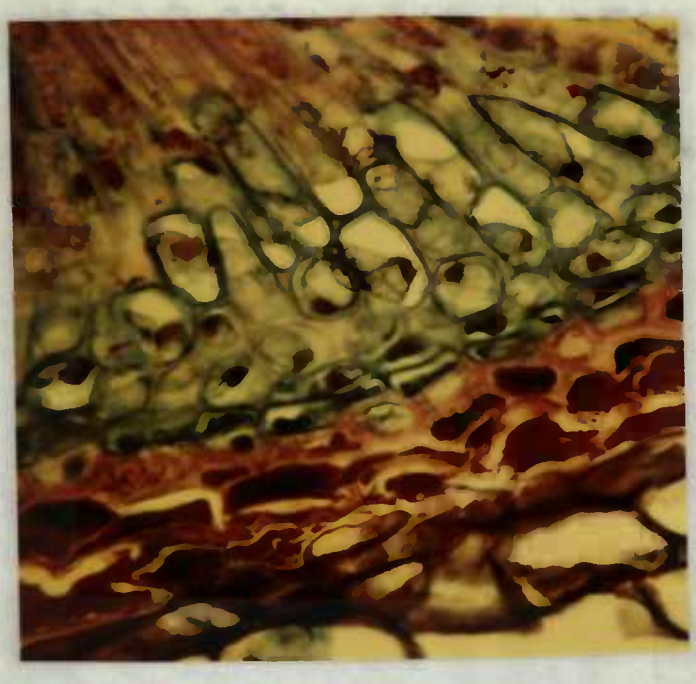

Figure 6.9. Abscission zone completely formed. Section stained with safranin and fast green.

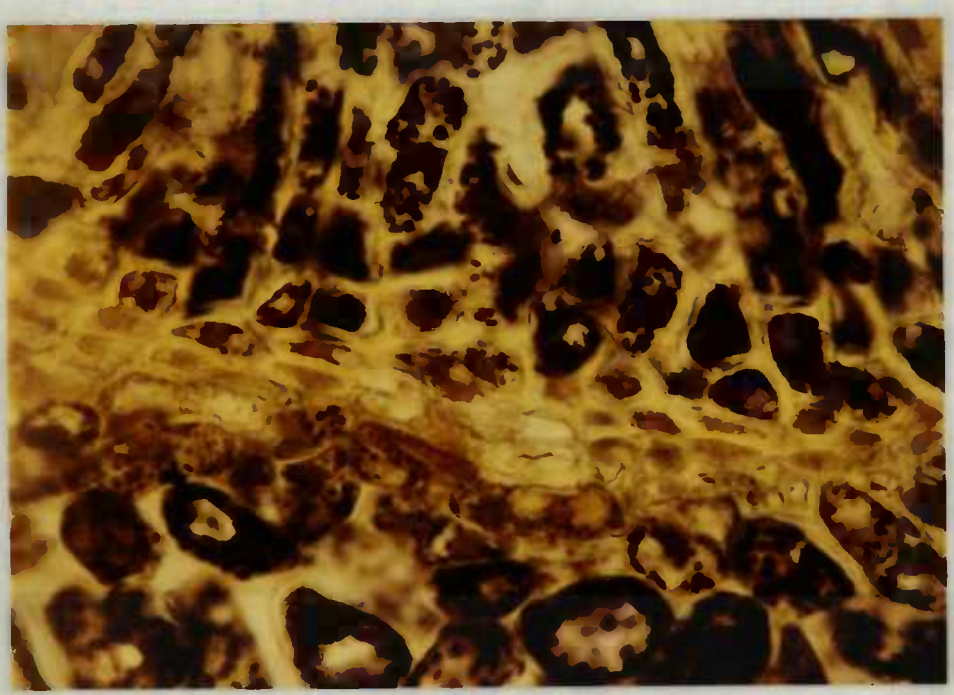

Figure 6.10. Starch localization on a section from a mature needle stained with potassium iodide. Starch presence undetectable on separation and protective layer cells.

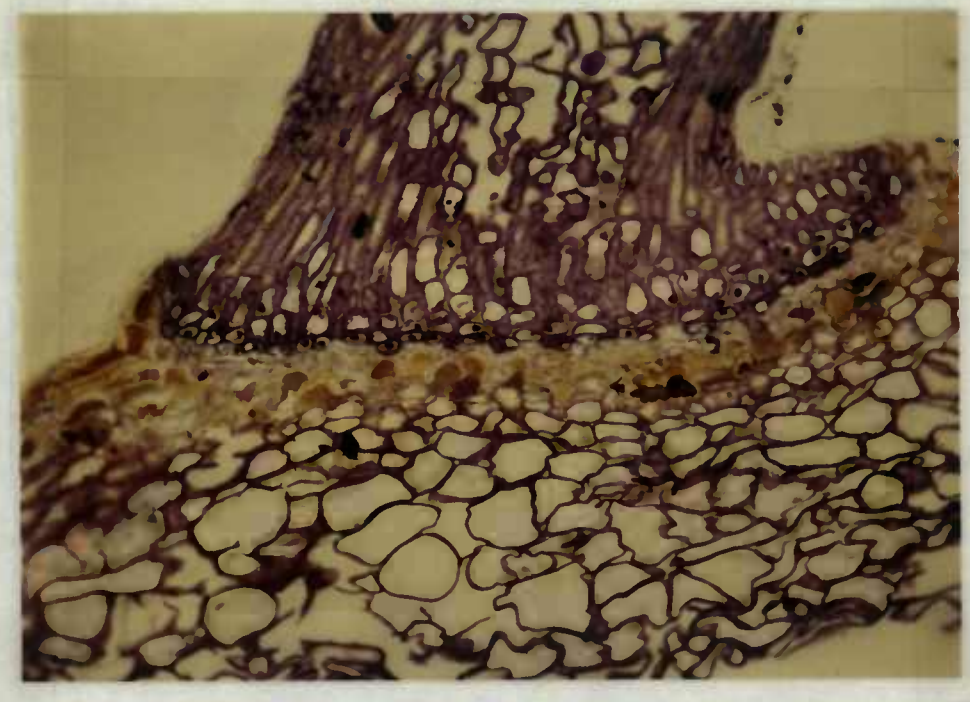

Figure 6.11. Section from mature needle stained with Delafield's haematoxylin. Separation and protective layers not stained.

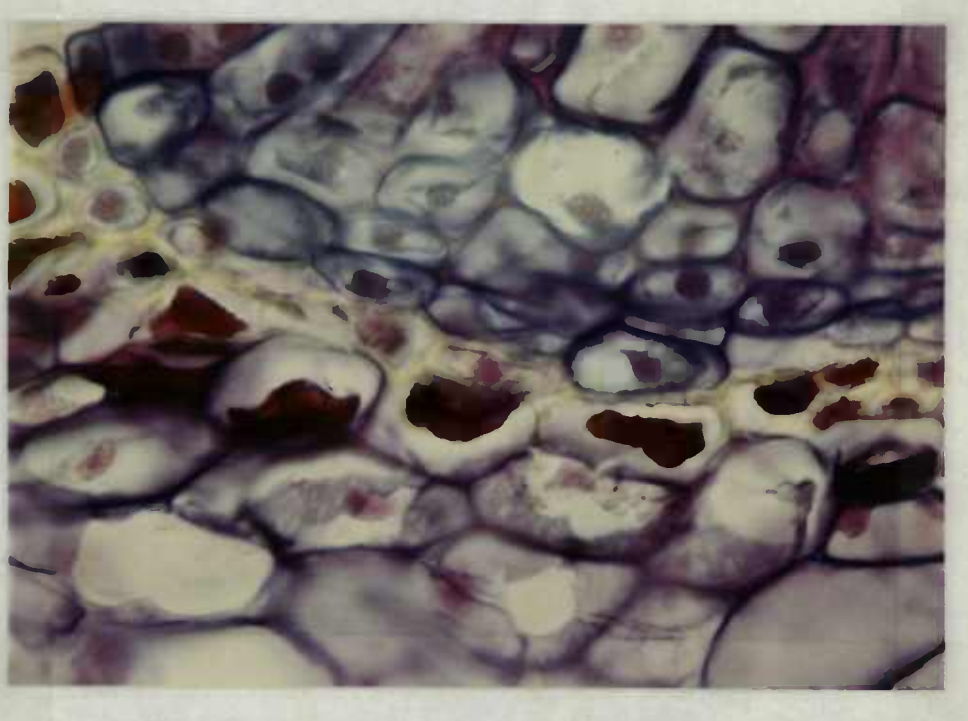

Figure 6.12. Section from a mature needle stained with Periodic acid-Schiff's reaction and fast green. Separation of layer cells not stained.

Figure 6.13. Section from a mature needle stained with acid fuchsin and fast green. Separation layer cell walls not stained.

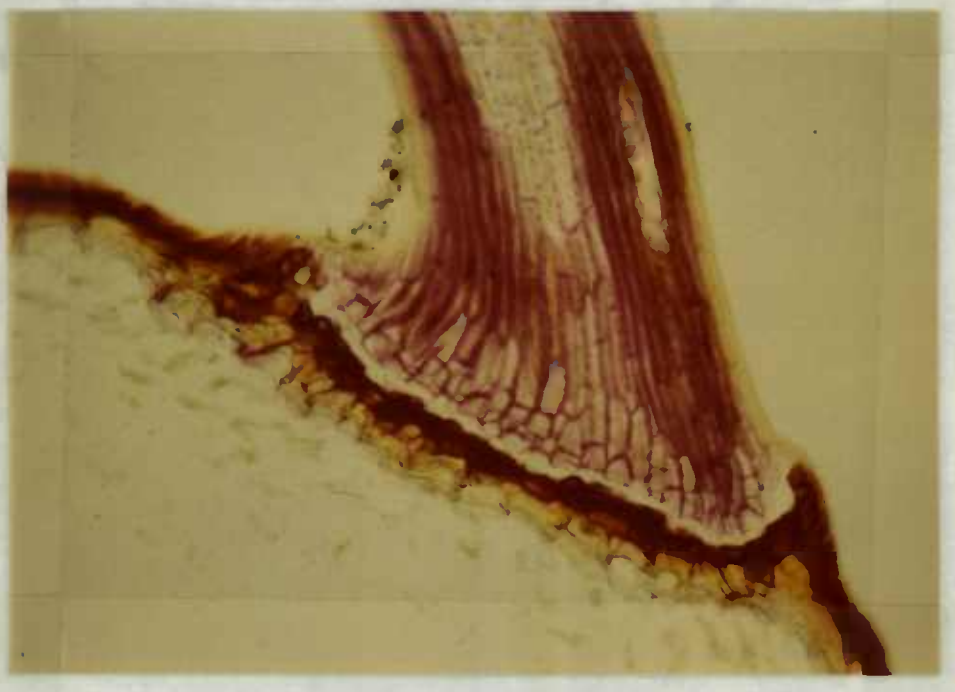

Figure 6.14. Section from a mature needle stained with chlorine-sulfite. Separation layer cell walls not stained.

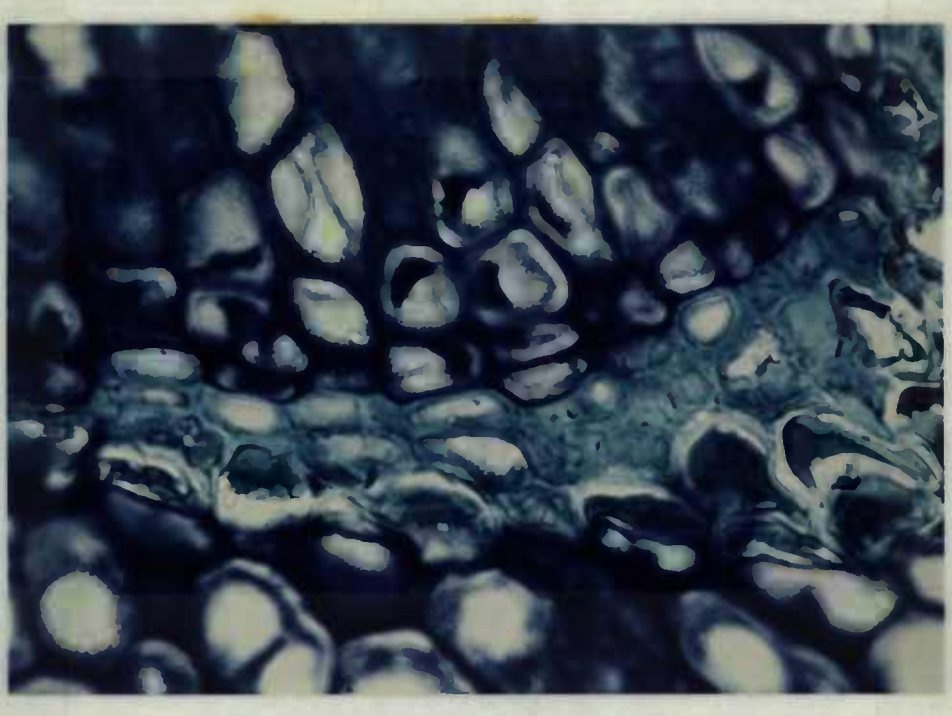

Figure 6.15. Intact separation layer cells from trees at -0.5 MPa. Section stained with toluidine blue.

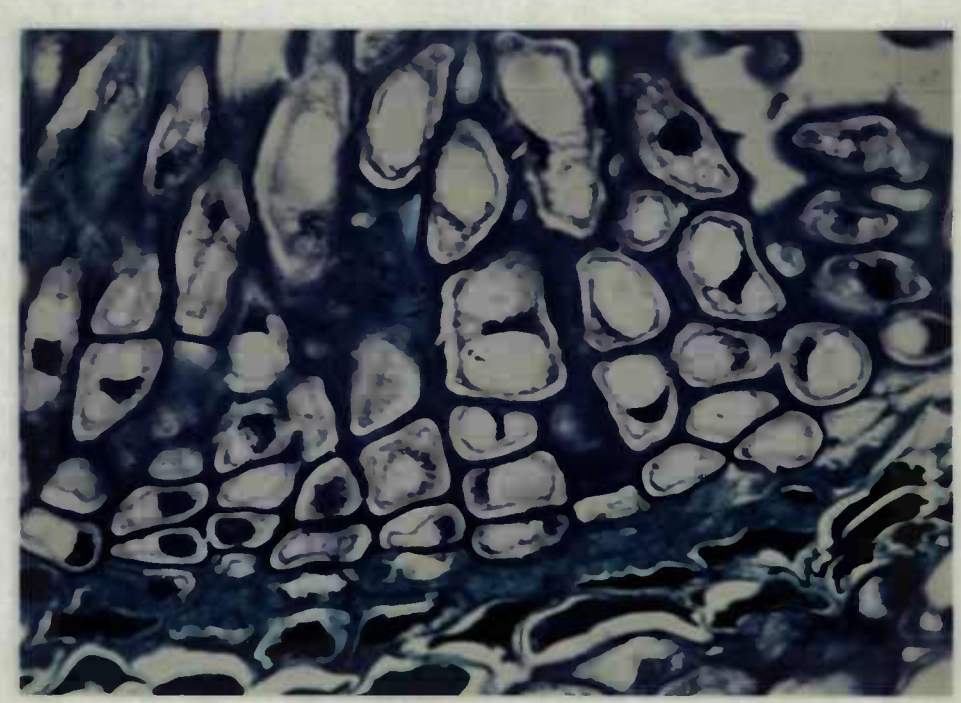

Figure 6.16. Needle section from a tree at -2.4 MPa. Section stained with toluidine blue.

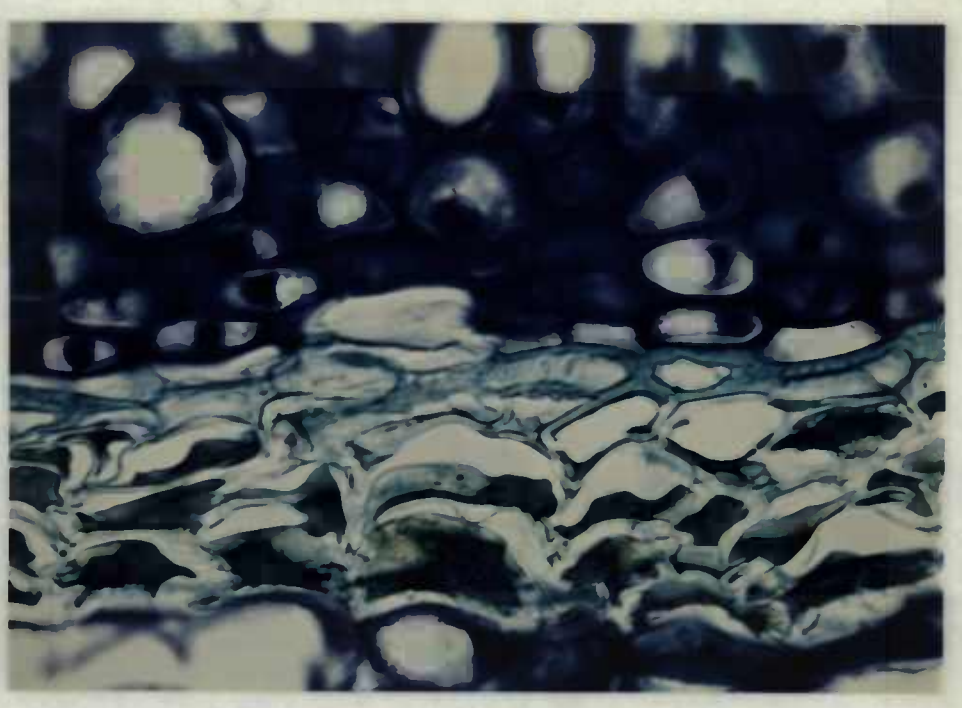

Figure 6.17. Shrinking and tearing of cell walls in the separation layer. Needle section from a tree at -3.5 MPa. Section stained with toluidine blue.

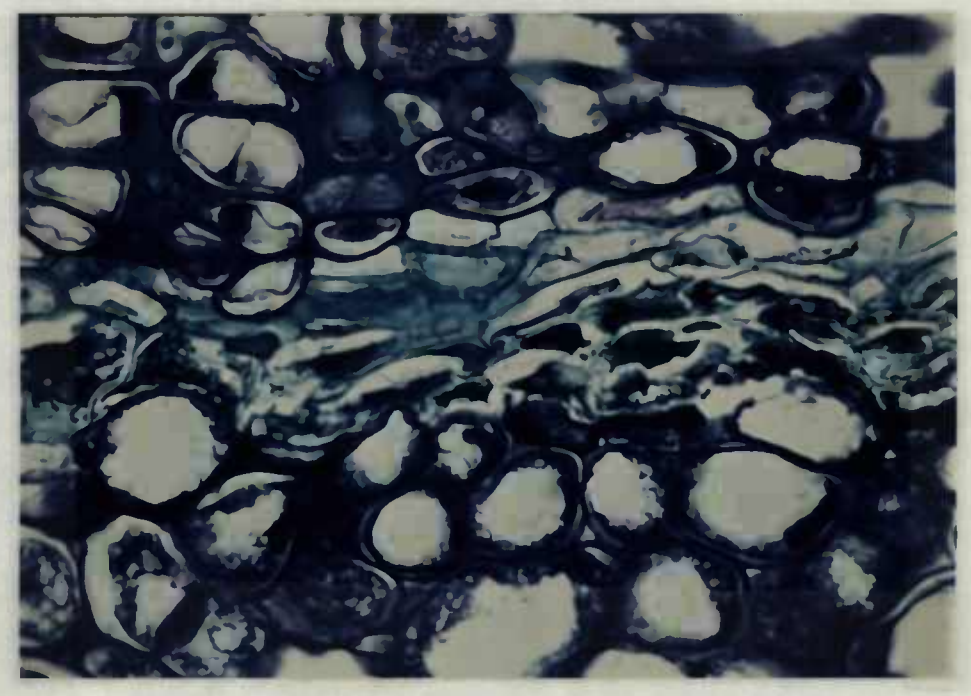

Figure 6.18. Loss of separation layer cell wall integrity. Needle sample from a tree stressed to -3.5 MPa. Section stained with toluidine blue.

separation of needle from stem tissue (Fig. 19). Collapse of the separation layer cell wall typically began at the needle interior and moved towards the periphery. Samples collected from trees that did not have abscising needles, but stressed below -3.5 MPa usually had very little cell wall tearing in the separation layer (Fig. 6.20) even when stressed to -5.0 MPa.

Comparison of chemically fixed (FAA, Fig. 6.8 or Karnovsky's, Fig. 6.15) with unfixed sections (Fig. 6.7) shows considerable shrinkage of separation layer cells that were chemically fixed. Cortical parenchyma cell walls did not seem to be affected during dehydration. Fresh samples cut from trees with abscising needles had shrunken separation layer cell walls and different degrees of tearing. This type of shrinkage was similar to shrinkage seen on samples dehydrated for plastic or paraffin embedding, or unfixed samples bathed for 5 min in 50% ethanol.

Trees water stressed to -3.5 and -4.0 MPa each had two distinct populations, one with abscising and another with non-abscising needles (Table 6.2). In each treatment, the 2 populations composed 50% of the total.

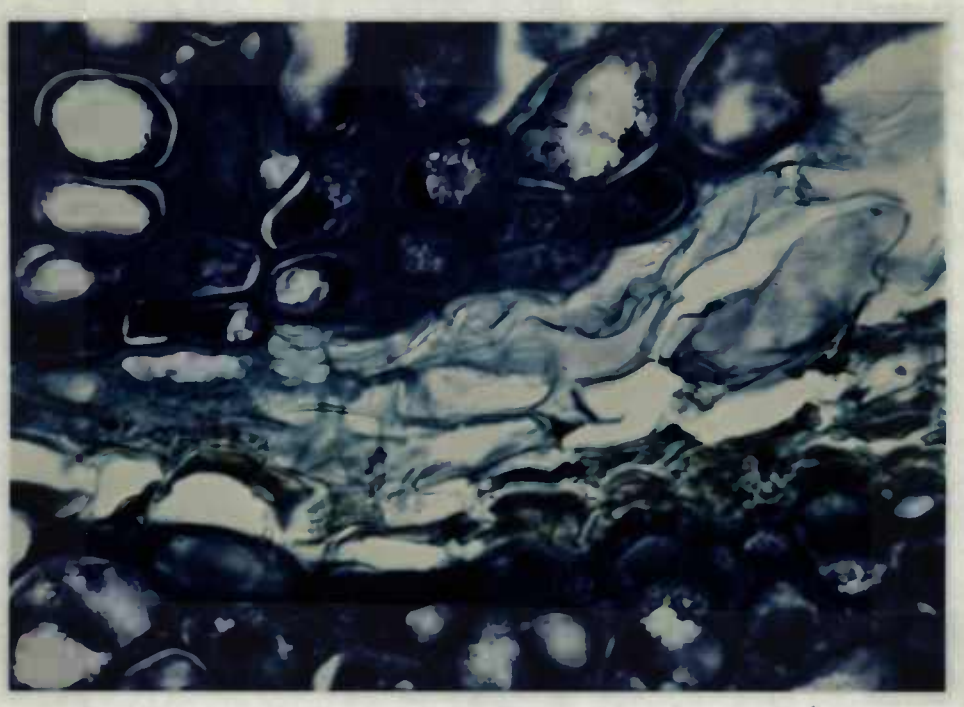

Figure 6.19. Separation of needle from stem tissue at -3.5 MPa. Section stained with toluidine blue.

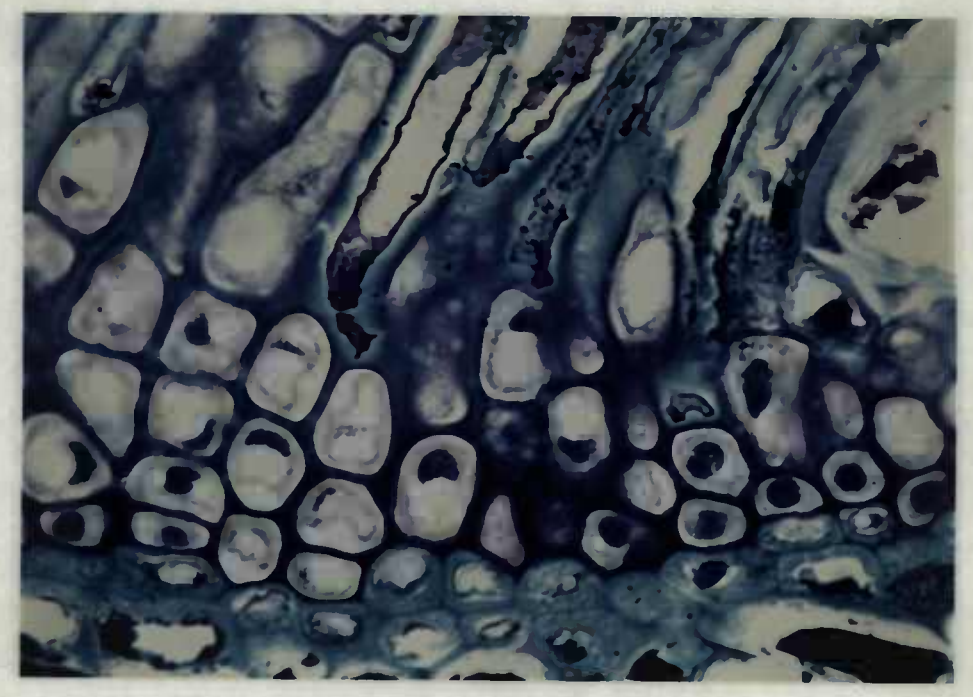

Figure 6.20. Lack of separation layer cell wall shrinkage in a sampled obtained from a tree with non-abscissing needles and stressed to -4.0 MPa. Section stained with toluidine blue.

Table 6.2. Percent abscission from 8 cm twig samples collected from tree populations with and without abscising needles.

Treatments	% Abscission from 8 cm Twig Samples			
	Days after Harvest			
	12	15	21	28
-3.5 MPa	21 + 16 +	33 + 18 +	30 + 16 +	30 + 12 +
-3.5 MPa	0 - 0 -	0 - 6 -	0 - 0 +	7 + 6 +
-4.0 MPa	27 + 19 +	14 + 12 +	0 + 29 +	10 + 18 +
-4.0 MPa	4 - 0 -	0 - 6 -	0 - 0 +	0 + 19 +
check	0 -	0 -	0 -	4 -

+ = tearing of separation

- = no tearing of separation

Discussion

Neger (21) observed that formation of an abscission zone is an integral component of gymnosperm needle ontogeny. In Douglas-fir, the separation layer was formed from the proximal layer of cells in the intercalary meristem a short time after the needle ceased extension growth. In contrast, most angiosperms lack a distinct separation layer (26). The separation layer in angiosperms becomes recognizable because of accumulation of cytoplasm and organelles (2, 3, 35) after receiving the abscission stimulus. Formation of the separation layer in Douglas-fir is first evident both by positive staining with safranin and decreased affinity for Delafield's haematoxylin. Similar results were reported by Stosser et al. (34) in Prunus, and by MacKenzie, (18) in Rubus. The staining pattern with safranin begins at the needle's periphery and proceeds towards the interior of the needle until the vascular trace is reached. Separation layer cells stain with safranin (stains lignin, suberin and cutin) but do not with chlorine-sulfite test (specific for lignin), periodic acid-Schiff's or with Sudan IV. Several workers have detected lipids, but not lignin lining separation layer cells (24, 27, 32). Facey (5) found that the lipid component that lined separation layer cells in Picea glauca and Abies balsamea was not of the same type as that present on the cuticle. The substance that impregnates the walls of the separation layer in Douglas-fir is probably composed of a cutin-like substance. The basis for this assumption are lack of staining with Delafield's haematoxylin, chlorine-sulfite, or

Sudan IV, but positive staining with safranin.

Development of the separation layer cell wall from meristematic tissue at needle bases has been reported in Picea abies (10) and development of thick secondary walls with numerous simple pits and empty lumens have also been observed in Picea pungens (32) and Picea abies (10). Since formation of the abscission zone appears to be an integral component of Douglas-fir needle ontogeny, efforts to prevent its formation with the intent to prevent or retard abscission would be difficult. It therefore appears that knowledge of environmental, physiological, and/or biochemical factors affecting abscission could be useful in controlling needle abscission.

Lee (16) first demonstrated that water loss was a factor activating abscission. Water stress stimulates abscission in a number of species (8, 19). Campbell & Vines (4) proposed that needle abscission in Picea excelsa was due to differential drying rates between 2 distinct classes of cells, namely separation and protective layer cells. They demonstrated that upon rapid water loss, separation layer cells contracted at right angles to the leaf axis, causing cell wall rupture and eventual separation from the protective layer. After conducting several histochemical tests on abscising needles from Picea glauca and Abies balsamea, Facey (5) concluded "that under drying conditions, needle abscission was the result of mechanical tearing of cell walls in a region above the protective layer". In contrast, water loss in angiosperm leaves is not a factor activating abscission, but rather a loss of cohesion among separation layer cells (28).

Sifton (32) concluded that abscission in Picea pungens was related to changes in the pectic components such as occurs in woody deciduous species.

Results of this study indicate that separation layer cell walls are torn during water stress. Excessive shrinkage of separation layer cell walls began showing at -3.0 MPa, which is the ψ_L at which water stress in cut Douglas-fir trees was expressed morphologically (Fig. 3.6). At -3.5 MPa, more severe cell wall shrinkage and tearing was seen. This type of damage was irreversible, since needle samples taken after the trees were rehydrated had the same or worse tearing. Cell wall shrinkage and tearing first occurred close to the vascular trace and spread towards the needle's periphery. When the separation layer cells were torn, the needle remained attached by the cuticle surrounding the needle and by the vascular trace. A slight touch caused the needles to fall off. Immediately after abscission, jagged portions of the separation layer cells were seen attached to the protective layer.

Mechanical tearing of separation layer cells during water stress does not explain why only approximately one-half of the water stressed tree population lost significant quantities of needles, while the other one-half did not (Table 6.2). Samples taken from those trees that were water stressed (-3.5 to -4.0 MPa) but did not have abscising needles also did not have damaged cell walls in the separation layer. On twigs from a tree with abscising needles, hand rubbing dislodged from 15 to 30% of the total needles on a given sampling date, but most of the needles remaining attached had vary-

ing degrees of tearing evident in the cell walls of the separation layer (Table 6.2). This was ascertained by sampling for needles for microscopic examination before and after hand rubbing. Campbell & Vines (4) observed that the rate of water loss from Picea excelsa was more important than the actual amount of water lost in stimulating abscission. They demonstrated that needle loss from twigs stored in a chamber with desiccant occurred when moisture content ranged from 30 to 40%. Needle abscission was arrested when twigs were stored in a moist chamber for 5 months, although moisture loss was 35%. In this experiment, individual trees or parts of them may have dried at different rates, which may account for the lack of cell wall tearing in needles of some trees when dried below -3.5 MPa. In addition, genetic variability may play a significant role in needle holding capacity of water stressed cut Douglas-fir trees (33).

If cell wall shrinkage results in tearing of the separation layer, cell walls should have torn during dehydration for plastic or paraffin embedding. Shrinkage but no tearing was observed in embedded and unstressed needles. In the same manner, shrinkage of separation layer cells from unstressed and unfixed needles was observed in sections bathed in 50% ethyl alcohol for 5 min, but no shrinkage was observed in sections that were bathed in water. Perhaps in these cases dehydration did not produce the differential shrinkage of cell types thought to result in tearing and abscission. During water stress-induced abscission, differential drying of separation and protective layers is believed to cause abscission (4, 5). Results obtained in this study support this view.

Some basic and fundamental differences between gymnosperm and angiosperm species abscission zones and modes of abscission are apparent (Table 6.3). "Gymnosperm" will refer collectively to Abies balsamea, Picea exelsa, Picea glauca, and Pseudotsuga menziesii since it appears that abscission occurs as a result of the same inductive stimulus and the same separation mechanism (4, 5). The existence of preformed separation layer cells and separation occurring by cell wall tearing are distinctive features of abscission in gymnosperms.

The formation of the abscission zone during needle development precludes the use of treatments to retard its formation. The formation of a protective layer that separates the abscising organ from the rest of the plant body is a fundamental feature of abscission in both gymnosperms and angiosperms. The process of abscission by tearing of separation layer cells during water stress appears to be energetically inexpensive. Needle abscission in this manner probably allows rapid response to adverse environmental conditions by reducing some degree of evaporative surface area, while maintaining photosynthetic area.

Table 6.3. Comparison of gymnosperm and angiosperm abscission zones and abscission.

Observation	Gymnosperm/Reference		Angiosperm/Reference	
Differentiated separation layer cells before abscission stimulus	+	4, 5, 20, 32	-	22, 29
Abscission occurs by dissolution of middle lamella	-	5	+	22, 29
Abscission occurs by tearing of separation layer cells	+	4, 5	-	
Lignin presence in separation layer cells	-	5, 32	-	34, 27
Protective layer left on the epidermis after abscission	+	4, 5, 10	+	15, 38
Abscission zone cells densely protoplasmic	+	5, 39	+	7, 29, 35

+ = true

- = not true

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Chapter 7

CONCLUSIONS

Water loss has a profound effect on Christmas tree postharvest performance. As trees lose water, they become progressively less attractive, lose more needles and become fire hazards. This represents an economic loss to growers, wholesalers, retailers and consumers. The Christmas tree industry has not made a united effort to standardize storage methods to increase postharvest performance. This study dealt with the comparison of various methods to study water stress in cut trees, defined some of the consequences of water stress, such as needle abscission, and also defined optimum storage conditions. The inability of antitranspirants to reduce water loss under high leaf-air vapor pressure difference show that there are no easy answers to the water loss problem. The storage environment appears to be the single most important factor in determining postharvest performance.

Coastal Douglas-fir is ecologically adapted to cool and wet winters. Storage conditions that approached the natural cool and wet environment at the time of harvest were best for maintaining tree longevity and quality. Cut trees cannot adapt to warm and dry conditions typical of retail lots and consumer homes. As a result, trees dry rapidly and are physiologically damaged.

Since storage is the main factor determining water loss, a united effort by growers, wholesalers, retailers and consumers to

handle their trees in a manner that reduces water loss is needed. Such practices are commonly followed with other horticultural products, such as fruits, vegetables, and cut flowers. In each of these industries, everyone concerned with handling of the product makes a conscientious effort to reduce water loss upon which quality depends. Christmas trees are no different in their requirement for extra care to maintain a high water content. The consequences of a failure to provide conditions that reduce moisture loss have resulted in many financial losses and disappointment. In the long run, survival of the Christmas tree industry may hinge on the maintenance of moisture content above a critical level.

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