The fate of canopy water in the Findley Lake Basin, WA

John Sumner Rombold

A dissertation submitted in partial fulfillment of the
requirements for the degree of

Doctor of Philosophy

University of Washington

2003

Program Authorized to Offer Degree: Forest Resources

UMI Number: 3091061

UMI Microform 3091061

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Chair of Supervisory Committee:

Thomas M. Hinckley
Thomas Morris Hinckley

Reading Committee:

Thomas M. Hinckley
Thomas Morris Hinckley

Linda Beck Brubaker
Linda Beck Brubaker

Douglas G. Sprugel

Date: June 13th 2003

University of Washington

Abstract

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John Sumner Rombold

Chair of the Supervisory Committee:
Professor Thomas M. Hinckley
Ecosystem Science

Abstract: This dissertation examined the fate of canopy water in the Findley Lake Basin, WA (47° 04' N, 121° 25'W) during the growing seasons of 1994 and 1995. Field measurements were taken at three sites: Lake (1170 m), Midslope (1230 m) and Ridge (1320 m). Field data included stand and canopy structure, canopy wetness, fog, and throughfall. Water uptake by *Abies amabilis* and *Tsuga heterophylla* shoots was measured in the laboratory. In 1995, the forest canopy was wet one third of all hours. During 1994 and 1995, throughfall was 31.3 and 27.9% less than apparent above-canopy precipitation at the Lake, 14.5 and 8.6% less at the Midslope, and 16.8 and 10.0% less at the Ridge. 1995 fog interception at the Ridge was three times greater than at the Lake ($n = 6$). Fog was related to the percent interception loss of precipitation ($r^2 = 0.64$, $p < 0.01$), but not to the quantity of throughfall. Within-plot throughfall distribution was related to profiles of "shielding" and "collecting" trees (Ridge, $r^2 = 0.34$), and to indices of near-collector leaf area (Lake, $r^2 = 0.39$). Foliar absorption was assessed with a new technique based on shoot drying curves. The method indicated that shoots absorb water ($p<0.01$). Uptake was apparently constant for at least 6 hours. Suprisingly, cuticular conductance to water influx ($g_{c_{in}}$, $3.5 - 7.2$ m s^{-1}) was an order of magnitude greater than cuticular conductance to water efflux

($g_{c_{out}}$, 3.0 – 3.9 x 10^{-5} m s^{-1}). Irrespective of the direction of water flux, shade-adapted shoots were more conductive than sun-adapted shoots, and *Tsuga heterophylla* was more conductive than *Abies amabilis.* A transcuticular flux model was proposed, where water influx occurs through hydrated micropores, while efflux is by diffusion. Estimated 1995 canopy uptake was 2.2 (Ridge and Midslope) to 2.6 (Lake) mm of precipitation, or 2 – 3% of transpiration. Fog sedimentation onto the canopy was the best explanation for the difference in interception between the Lake and the two upper sites. Canopy uptake appears too small to have hydrological significance, but may have physiological importance, especially to plants or plant parts under water stress.

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List of symbols

Quantity	Description
a	effective surface area
a^I	activity of water on side *i*
a^o	activity of water on side *o*
a_{wv}	activity of water vapor
A	photosynthetic flux
AWC	apparent water content
c	concentration of water in the cuticle
c_i	concentration of water at the cell wall side of the cuticle
Δc_I	difference in water concentration on either side of a relevant barrier
c_o	concentration of water at the exterior side of the cuticle
c_p	specific heat of air
c^*_{wv}	saturation concentration of water vapor
D	diffusion coefficient of water in the cuticle
DBH	diameter at breast height
E_I	evaporation
$E_{T_{s\tan d}}$	stand-level transpiration
g_j	conductance of species *j*
g_{aS}	conductance of the shoot boundary layer
g_{aM}	canopy aerodynamic conductance
g^{bl}_{wv}	boundary layer conductance to water vapor
g_c	cuticular conductance
$g_{c_{in}}$	cuticular conductance to water influx
$g_{c_{out}}$	cuticular conductance to water efflux
g_{canopy}	sum of canopy stomatal conductance
$g^{\min}_{shoot}$	$\approx g_c$, the conductance of a shoot after stomatal closure
g_s	stomatal conductance
J_j	flux density of species *j*
J_{shoot}	flux of water loss from the tissues of a shoot
$J_{surface}$	flux of water loss from the surface of a shoot
k	rate constant
K	partition coefficient; a measure of how easily a substance enters a membrane matrix

List of Symbols (continued)

Quantity	Description
K^B	Boltzmann constant (1.3806 x 10^{-23} J K^{-1})
$L^{cuticle}$	hydraulic conductivity of a cuticle
L^{pore}	hydraulic conductivity of a micropore
LAI	leaf area index
m_d	oven-dry mass
N^{*}_{wv}	saturation mole fraction of water vapor in air
N^{bl}_{wv}	mole fraction of water vapor in the leaf boundary layer
N^{pore}_{wv}	mole fraction of water vapor in micropores
p_{atm}	atmospheric pressure
P	permeability coefficient
r_j	radius of species *j*
R	real gas constant
R_n	net radiation
rH	relative humidity
RWC	relative water content
s	slope of the saturation specific humidity versus temperature relationship at mean surface temperature
SLA	specific leaf area
$SPAR_{max}$	maximum silhouette-to-projected area ratio
t	time
T	temperature (°K or °C)
$\overline{V_w}$	partial molal volume of water vapor
VPD	water vapor pressure deficit
w	wind velocity
W_{shoot}	water held by the tissues of a shoot
$W_{surface}$	water present on the surface of a wet shoot
W_{total}	water held by a shoot: $W_{surface} + W_{shoot}$
Δx	cuticle thickness
α	contact angle
γ	psychometric constant
η	viscosity of water
η_{cm}	viscosity of the cuticular membrane
ϑ	the tortuosity coefficient; the extra distance that a diffusant must travel per unit of membrane thickness due to deflection of the diffusion path

List of Symbols (continued)

Quantity	Description
λ	latent heat of vaporization of water
ρ	density of air
σ	surface tension of water
Ψ_{leaf}	leaf water potential
Ψ_{xylem}	xylem water potential
Ω	canopy coupling coefficient

Acknowledgements

Suzanne Herald, Megan Maddox and Danika Gilbert helped to collect some of the data that appears in this dissertation. Richard Walker, Todd Dawson, Bob Teskey, Fred Meinzer and Nate McDowell generously provided ideas that helped to overcome difficult passages. Mark Redlin designed the Findley Lake tower and the moisture sensors used to quantify canopy wetness. The tree physiology lab at UW relied upon his technical prowess. My lab mates Kim Brown and Tim Martin taught me much of what I know about tree physiology. Tim provided the essential meteorological data used throughout this study, and was the first person I would contact for help when I was mired in a chapter. Marty Loefflad, Jack and Cynthia McGrath, Pat Evans, George Grader, Mark Solomon, Gordon Smith, Marty Cavalluzzii, Roberto Gonzalez-Plaza and Rich Doenges reminded me, often, that I needed to finish this sucker. They were right. Paul Allen deserves mention for donating the funds to construct the Allen natural sciences library, an indispensable resource on campus, and Carol Greene for her able management of the Forest Resources library. Lynn Catlett has always been helpful with anything administrative. The students, staff, and faculty of the Northwest Indian College have been tremendously supportive while I have been working on this dissertation. To them I owe a huge debt of thanks. To get this far, I have been inspired by teachers: Shirley Gaddis, John Anderson, Edward Stone, Jeffrey Romm, Garth Voigt, Herb Borman, Brian Boom, Scot Mori, Anthony Anderson, Chris Uhl, Daniel Nepstadt and David Ford. These teachers include my committee, who has stoically seen this ten-year project through to the end: Gordon Bradley, Linda Brubaker, Robert Cleland, Sue Ferguson, Tom Hinckley and Douglas Sprugel. Bob Cleland gave me the idea to use drying curves to quantify water uptake by shoots. Doug Sprugel and Linda Brubaker did a great job reviewing drafts of this dissertation. Doug also provided important data on *Abies amabilis*. My chair, Tom Hinckley, is a true mentor. This dissertation would not have been completed were it not for his encouragement, suggestions, and sometimes cryptic editing. I see tree physiology through his eyes. The staff of the Cedar River Watershed generously made the Findley Lake Research Area available for our use. It is the mother of all *Abies amabilis* field sites. Funding for this study was provided by the United States Forest Service, the Washington State Department of Water Resources, and the University of Washington Department of Ecosystem Science. My wife Sue also provided funding. Every ten-year doctoral student needs some good amigos, and I have four of the best: brothers Peter, Rob, Rich and Joe. Mom and Dad constructed and helped to install the fog collectors. They would have tried to write this dissertation, too, had I let them. Their support for their sons knows no limit. Sue, Cooper and Anna have filled my life with love, and put academia into perspective. The past years have been light, the load has not been heavy. I have been blessed beyond all measure to be so supported by these wonderful people and family.

Dedication

To Mom and Dad
Thank you for everything

Introduction.

The predominant direction of airflow in the upper latitudes of the Northern Hemisphere is from west to east. The prevailing airflow sweeps water-charged low-pressure systems out of the northern Pacific, and brings them inland over the coast of the Pacific Northwest. When these moisture-laden air masses encounter the western slopes of the Cascades, they are forced to rise, cool and discharge their cargo of water. As a consequence, precipitation increases sharply with altitude on the west side of the Cascade Range. From Seattle to Snoqualmie Pass, the orographic lift from 950 meters of vertical displacement enhances precipitation nearly three-fold, from an average of 911mm per year at Seattle, to 2661mm per year at Snoqualmie Pass (Western Regional Climate Center 2000). The copious precipitation in the high Cascades feeds watersheds such as the Tolt, Cedar and Green, which have become critical sources of water for fast-growing communities in the Puget Trough.

Precipitation falling on the west slopes of the Cascades first encounters a lush canopy of evergreen conifers. At low elevations, tree species important in the forest canopy include Douglas-fir (*Pseudotsuga menziesii* (Mirbel) Franco), western redcedar (*Thuja plicata* Donn), and western hemlock (*Tsuga heterophylla* (Raf.) Sarg.). *Pseudotsuga menziesii* and *Thuja plicata* are less common in forests of higher elevations, and are replaced by Pacific silver fir (*Abies amabilis* (Dougl.) Forbes) and mountain hemlock (*Tsuga mertensiana* (Bong.) Carr.). The moderate maritime climate enables the coniferous forests of the Cascades to develop and maintain extensive needle displays, with typical leaf area indices for mature forests ranging between six and twelve square meters of projected needle area per square meter of ground area (Lassoie *et al.* 1995). This implies that where the forest cover is well developed, a plummeting raindrop will strike many needles before falling to the forest floor. Thus, crowns of trees interact with most of the precipitation that falls on the west slopes of the Cascades.

The canopy, with a tremendous surface area for both transpiration and evaporation, is a potent regulator of water cycles. Interactions between forest canopies and precipitation may also profoundly affect fundamental ecosystem functions such as nutrient cycling, decomposition, and the modification of microclimate. Even seemingly small fluxes of water can assume importance if the stand leaf area index is high, the extent of the canopy across the landscape is large, and precipitation events that moisten the canopy are frequent. The upshot for resource managers is that the movement of water into and out of forested watersheds can be sensitive to changes in the structure or composition of the forest canopy. Important ways in which forest canopies influence influxes of precipitation include interception and storage, redistribution, and absorption.

Interception and storage of precipitation. Fifteen to fifty percent of the precipitation that falls on coniferous forests is captured by the canopy, and does not arrive at the soil as throughfall (Zinke 1967). The gap between precipitation and water inputs to the soil can be largely explained by the combined influences of canopy capacitance and evaporation. Canopy capacitance is defined as the amount of water left on the canopy under zero evaporation conditions after rainfall and throughfall have ceased (Gash *et al.* 1995), and can be viewed as a storage pool that must be filled before water can pass to the soil. Canopy capacitance is mechanistically linked to the leaf area index of the stand (Zinke 1967), and ranges from roughly 0.25 mm of precipitation by open stands of intolerant species such as *Pinus pinaster*, to 0.5mm for species of intermediate shade tolerance such as *Pinus strobus* (Voigt and Zwolinski 1964), to more than 1.0 mm for canopies of shade tolerant genera such as *Abies* or *Picea* (Teklehaimanot and Jarvis 1991).

Canopy capacitance alone cannot explain the large difference between precipitation and throughfall. Evaporation is the other component of interception loss, and is typically greater than canopy capacitance. Evaporation continuously depletes the water films that coat canopy surfaces during precipitation events, thereby renewing the ability of tree crowns to hold water. The bulk of evaporation loss from tree crowns occurs during rainstorms, when solar radiation, the atmospheric vapor pressure deficit, and ambient temperature are low. Although it may seem counter-intuitive to have rapid rates of evaporation from wet canopies during rains, the resistance of the boundary layers surrounding conifer shoots is low (Martin *et al.* 1999), and the conductance of structurally heterogeneous conifer canopies is high (McNaughton and Jarvis 1983, Martin *et al.* 1998). Thus, a wet canopy has little resistance to the transfer of water vapor to an unsaturated atmosphere. The required element for evaporation—heat—is supplied by advection from moving air masses as they pass over wet canopies (Calder 1998). Thus, even as canopy capacitance is satiated by precipitation, it is continuously restored by evaporation. The net difference between precipitation above the canopy and the quantity of throughfall underneath is much greater than can be attributed to canopy capacitance alone.

Streamflows typically increase for many years after the forest cover of a watershed has been reduced (Hibbert 1967). The increased water yields are not, as might be expected, attributable to diminished transpiration. Instead, the effect is due to the long-term abatement of interception losses in the forest canopy. Interception loss increases linearly with stand leaf area (Teklehaimanot *et al.* 1991). In contrast, the maximum transpirational fluxes from canopies are similar for vegetation types of diverse structure and composition, and are suprisingly insensitive to leaf area (McNaughton and Jarvis 1983). Forest canopies destroyed by disturbance are replaced within a few years by a canopy of successional vegetation that has a similar evapotranspiration potential per unit area as the original forest, even though its leaf area index may be several times less than that of the old forest canopy. Since the magnitude of interception losses are a function of stand leaf area, interception tracks the rate at which leaf area is developed on the site, and may require thirty years or more to recover to values that approximate those of a mature forest canopy.

The increase in watershed yield when forests are cleared can be substantial. At the H.J.Andrews Experimental forest in the Oregon Cascades, clearcutting augmented stream flows by thirty percent during the first four years after logging (Harr *et al.* 1982). At Hubbard Brook in New Hampshire, a complete deforestation treatment enhanced water yield by 40, 28 and 26% in the first three years after treatment; after seven years of re-growth, the increase in flow was still significantly higher than expected (Borman and Likens 1979). Water yields after clear-cutting at Coweeta Experimental Forest in North Carolina were more than 35% greater in the first year after harvest. Twenty-three years later, runoff from the treated watershed was still significantly greater than if the forest cover had been left intact (Hibbert 1967).

The boost in water yield that results from the reduction of forest cover has been used to justify clear-cutting large tracts of forest, and more recently, the conversion of forest land to other cover types in the name of water conservation. This has been particularly true in the western United States where summer precipitation is low, and water is a critical resource that often limits development. However, where site factors are favorable, forests may harvest water from fog, resulting in more precipitation than would be realized without a forest canopy. Isaac (1946) was one of the first to demonstrate the importance of canopy-intercepted precipitation from fog. By comparing the yield of throughfall collectors placed under the canopy of a ridge-top *Pseudotsuga menziesii* stand and in an adjacent opening, Isaac found that fog drip accounted for an extra 520 mm of precipitation per year underneath the trees. Since Isaac's original research, fog drip has been shown to be an significant source of precipitation in many forest ecosystems, including forests in Hawaii (Eckern 1964), Japan (Hori 1953), Mexico (Vogelmann 1973), Panama

(Cavelier *et al.* 1996) and along the Pacific coast of North America from Monterey Bay north (Oberlander 1956; Kerfoot 1968; Azevedo and Morgan 1974; Harr 1982; Dawson 1998).

From an ecological perspective, fog interception is most important during periods of drought, when precipitation from other sources is scarce. The combination of frequent fog and seasonal drought is typical of a narrow zone along the coast of the Pacific Northwest. Most of western North America has a pronounced summer drought, caused by the seasonal shift of the jet stream to more northern latitudes. Proceeding from north to south along the Pacific Coast, the percentage of annual rainfall that occurs during the months July through September is: Quinalt, Washington, 7.2%; Astoria, Oregon, 7.1%; Otis, Oregon, 6.5%; and Port Orford, Oregon, 4.6% (Franklin and Dyrness 1988). Aestival inflows of fog in this zone are associated with the advection of warm air masses over cold water. Fog formation in the subsiding air is induced by supercooling through contact of the air mass with cold surface water, and the admixture of salt nuclei that provide sites for droplet formation (Woodcock 1978, Woodcock *et al.* 1981). The marine cloud is drawn inland by heating to the east of the Cascade-Sierra crest. Orographic lifting causes additional cooling and augments the water content of the fog when the supercooled marine layer encounters the mountainous coastline. The intruding fog reverses the role of the canopy with respect to precipitation. Instead of a sink for in-coming precipitation, fog drip converts the canopy into a water source at a time of moisture scarcity. During the winter, offshore subsidence is infrequent, and the temperature contrast between the ocean surface and air aloft diminishes. For these reasons, summer fog drip along the West Coast is 30-45% greater than wintertime fog drip (Harr 1982; Dawson 1998).

Dawson (1998) explored the ecological importance of dry season fog drip. In a coastal *Sequoia sempervirens* forest near Arcata, California, an average of 33 mm of rain fell from July to September—less than 3.5% of the annual total. However, during this pronounced summer drought, the *Sequoia* stand was shrouded in fog better than one out of every three days. Over a three-year period, water raked from fog accounted for 22-46% of the annual hydrological input to the soil underneath the tall *Sequoia* crowns. Isotopic analysis showed that fog water was the source of 18% of the summertime *Sequoia* transpiration stream, while 66% of the water used by understory vegetation during the summer drought came from fog drip. Dawson's study clearly demonstrated that tree canopies can have a large impact on the hydrology and ecology of forest ecosystems. Under the correct circumstances, the net influx of water can be enhanced by the presence of a canopy, with large effects on the temperature, moisture regime, and ultimately, the biotic community that inhabits the site. Dawson's study also shows that a tree canopy can often be wet—even during periods when rain is scarce.

Redistribution of precipitation

After the storage capacity of the canopy becomes satiated, films of intercepted precipitation begin to flow along inclined needle and stem surfaces. The architecture of stems and branches channels water descending through the canopy. The crowns of young conifers, with branches angled upward, behave as funnels and tend to divert precipitation towards the central stem (Ford and Deans 1978; Kimmins 1987). Mature conifers, especially genera such as *Picea*, *Thuja* and *Tsuga*, have more pendulous branches and behave as umbrellas, redistributing much of the precipitation incident upon the crown towards the canopy edge (Kimmins 1987, Pederson 1992). As a consequence of the redistribution of precipitation by the canopy, throughfall is delivered to high-input zones at the forest floor, which may receive three times more water than low input zones just a few meters away (Rombold unpublished).

Ecologically, patterns of throughfall delivery on the forest floor may affect soil properties, and the abundance of moisture and nutrients. In a young *Picea sitchensis* plantation in

Scotland, Ford and Deans (1978) found that the zones of highest throughfall were also zones of high fine root density. Seiler and Matzner (1995) reported that gradients of element concentrations in the throughfall solution were significantly correlated with the distance from mature *Picea abies* trees, with the highest concentration of elements in throughfall nearest the stems, and the lowest concentrations towards the canopy edge. In the soil, the patterns of throughfall delivery had established a strong gradient of SO_4^-, which was again most concentrated near the tree stems (Seiler and Matzner 1995). Pederson (1992) also concluded that the concentration of nutrients in throughfall decreases with distance from the stems of mature conifers.

Water absorption in the canopy.

On the west facing slopes of the Cascades where fog, rain, dew and snow are common, the canopy may be wet much of the time. During the period 1931 to 1972, Snoqualmie Pass had a yearly average of 173 days with measurable precipitation (Western Regional Climate Center 2000). At a lower elevation site, Wolfe (1988) used moisture sensors to determine that a *Pseudotsuga menziesii* canopy at the Thompson Research Center in the Cedar River Watershed was wet during 21% of the growing season, and 39% of the winter. Leaf wetness can have large impacts on the physiology of a plant. Leaf wetting can either impair or enhance rates of photosynthesis by plants (Smith and McClean 1989; Brewer and Smith 1994; Ishibashi and Terashima 1995); decouple canopies from the atmosphere (Pittacio *et al.* 1992); promote the germination of fungal spores on leaves (Martin and Juniper 1970); refocus light incident on chloroplasts (Brewer *et al.* 1991); leach nutrients from leaves (Tukey 1977), and enable the absorption of nutrients and pollutants from atmospheric sources (Vose and Swank 1990; Boyce *et al.* 1991, Eilers *et al.* 1992, Lovett and Lindberg 1993).

Wet leaves can also absorb water. Qualitative evidence that the above-ground organs of a wide array of plant species can acquire water when wet has been provided by numerous studies (Breazeale *et al.* 1950, 1951; Slatyer 1956; Stone 1957b; Leyton and Juniper 1963; Vaadia and Waisel 1963; Johnston 1964; Leyton and Armitage 1967; Suárez and Gloser 1982; Karman 1986; Bowden *et al.* 1989; Katz *et al.* 1989; Eilers *et al.* 1992; Grammatikaplolous and Manetas 1994; Schreiber 1994; Yates and Hutley 1995; Munné-Bosch *et al.* 1999). Plant uptake of water applied to leaves has been inferred by an increase in the xylem water potential or leaf relative water content, by the infiltration of fluorescent dyes into the xylem, by isotopic assays of treated plant tissues, or by increased vigor relative to an untreated control group.

Water absorbed by above ground plant parts may have physiological significance. Once routed into epidermal cells, water acquired by leaves should be functionally equivalent to water delivered through the root-stem-shoot pathway. The importance of extra water obtained through surface absorption will be determined by the water status of leaves and branchlets: the closer their water potential is to critical thresholds, the greater the significance of small supplements of water that enhance their function or survival. As an example, Magnani and Borghetti (1995) reported a significant negative relationship ($r^2 = -.7$; $p < .001$) between the temporal occurrence of xylem embolism and light rains in *Fagus sylvatica*. They hypothesized that cavitated xylem elements were refilled with water acquired from wet foliage. The upper crowns of tall trees, in which physiological drought is induced by the combined influences of gravity and the extreme length of the conducting pathway (Ryan and Yoder 1997; Hubbard *et al.* 1999), are a system in which the stress-relieving uptake of water by crowns wetted with fog or light rain may be important. The same may be true for seedlings moistened with dew during times of soil drought.

An exploration of the physiological effects of dew on conifer seedlings led to one of the first demonstrations of foliar water absorption of water by species of conifers. Stone (1957b) performed a series of experiments with drought-stressed seedlings of *Pinus ponderosa*, *Pinus jeffreyi*, *Abies concolor*, and *Calocedrus decurrens*, in which he tested the effect of artificial misting on seedling survival. In his experiment, two year-old seedlings were paired with sunflower plants in one-gallon pots. The soil in the pots was allowed to dry until the sunflowers wilted. At this point the sunflowers were removed, and the pots were sealed with a waterproof barrier. Half of the seedlings were then subjected to nighttime misting, to simulate dew. In comparison to the unmisted control group, misting prolonged the number of days that seedlings of *Abies concolor* (20 additional days), *Pinus ponderosa* (30 additional days), and *Calocedrus decurrens* (72 additional days) survived after the soil had been dried to the wilting point of sunflower, but had no effect on the survival of *Pinus jeffreyi*. Although the soil had been dried to the permanent wilting point, Stone found that both misted and unmisted seedlings continued to remove water from the soil. The most desiccated planting medium was that in which the unmisted seedlings were planted. Two mechanisms could explain the observed differences in seedling survival: water absorption by shoots, or decreased cuticular conductance during the twelve-hour nighttime period when the leaves were wet.

Rates of water absorption by wet conifer shoots have not been well quantified. Measuring water uptake by a wet shoot is problematic, since the water moistening the exterior of the shoot and the water held by shoot tissues has to be somehow partitioned into two pools. The few data that are available indicate that the quantity of water absorbed is small relative to the magnitude of daily losses from transpiration. In the literature, a study by Katz *et al.* (1989) provides the only published estimate of foliar uptake by a conifer. Katz *et al.* measured the change in water potential of *Picea abies* shoots moistened for 200 minutes with distilled water, and then inferred the quantity of water absorbed from pressure-volume relationships. They estimated that wet *P. abies* shoots absorb 0.3-0.4 µl water g^{-1} shoot dry weight hr^{-1}, or roughly 2.3

ml of water absorbed per square meter of one-sided projected leaf area per hour. Assuming a leaf area index of 6 m^2 projected leaf area ha^{-1}, the estimate for stand-level water uptake by a wet *Picea abies* canopy scales to approximately 140 liters ha^{-1} hr^{-1}, or .0014 mm of precipitation per hour.

Extrapolating from Katz *et al.*, the total quantity of precipitation that a *P. abies* canopy can absorb would depend on how long the canopy is moistened by a typical precipitation event; ten hours may be a reasonable estimate. If stand-level transpiration is on the order of one to three mm day^{-1}, then the quantity of water gained by a wet canopy would be about 0.5 – 1.4 percent of daily evapotranspiration, which is roughly thirty percent of typical rates of transcuticular transpiration (Larcher 1995). However, while the rate of shoot uptake reported by Katz *et al.* seems minor from a hydrological perspective, from a physiological perspective the absorbed water was more significant: the water potential of the excised shoots increased from -1.20 MPa to -0.20 MPa in 200 minutes (Katz *et al.* 1989).

Structure of the dissertation

This dissertation explores the fate of summertime precipitation intercepted by the forest canopy of the Findley Lake Basin, Washington. The study required four types of information. First, the quantity and distribution of foliage were needed to link shoot-level measurements to the canopy. Chapter I describes these data, which were obtained at three sites in the Findley Lake Basin that will be referred to throughout the dissertation: a site immediately north Findley Lake (elevation = 1170 m); a second site to the west of Findley Lake on the midslope of Findley Ridge (elevation = 1230 m); and a third site on the summit of Findley Ridge (elevation = 1320 m). Chapter I emphasizes measurements of *Tsuga heterophylla*, since Sprugel (unpublished) had intensively examined the crown structure of *Abies amabilis* at Findley Lake.

The second element of the study was the sources and quantity of precipitation above the canopy, and the pattern and amount of throughfall underneath it. These data are presented in Chapter II. Chapter II also presents critical information on the occurrence of canopy wetness in the Findley Lake Basin during the 1995 growing season.

The third component of the study was water uptake by needles and shoots. Accordingly, after Chapter II, the emphasis of the dissertation shifts from stand- and canopy-level field measurements, to laboratory-based experiments with wet, excised shoots of *Abies amabilis* and *Tsuga heterophylla.* This section is the majority of the dissertation. Chapter III reviews much of what is known in the literature about the cuticles of conifers: their synthesis, structure, and properties with respect to water transport. Chapter IV describes experiments with the Scholander pressure bomb intended to quantify water uptake by *Abies* and *Tsuga* shoots. This first approach proved unsatisfactory. Accordingly, Chapter V introduces a new technique to measure water absorption by wet, excised shoots, and describes the resulting estimates of water uptake by *Tsuga* and *Abies*. Chapter VI develops a model of water movement through *Abies amabilis* and *Tsuga heterophylla* cuticles in an attempt to explain the data of Chapter V.

Transpiration was the fourth and final piece of the study. Fortunately, Martin (1997), Buchmann et al. (1998), and Martin et al. (2001) had studied canopy-level water use and gas exchange in the Findley Basin. Chapter VII integrates their data, together with many of the findings presented in Chapters I-VI, to assess water exchange during the growing season by the Findley Lake Basin forest canopy.

Chapter I. Forest canopy structure in the Findley Lake Basin.

Introduction and objectives

In forests, the canopy modulates the passage of precipitation to the forest floor. This study of water exchange by trees in the Findley Lake Basin will begin with an examination of the structure of the forest canopy. Information on canopy structure will be essential to integrate the measurements and experiments that were carried out at different scales, from stand-level measurements of throughfall, to water absorption by needles.

The purpose of this chapter then, is to establish a foundation for the study of the fate of canopy water in the Findley Lake Basin by describing the quantity and distribution of foliage in the canopy. This task has two components. First, the canopy structures of *Abies amabilis* and *Tsuga heterophylla*—two of the common tree species of the Findley Lake Basin—will be compared. The emphasis will be placed on the canopy structure of *Tsuga heterophylla*, since comprehensive data on the canopy structure of *Abies amabilis* were available from Sprugel (unpublished). It is postulated that the canopy structures of *Abies amabilis* and *Tsuga heterophylla* will be different.

Second, stand-level differences in tree height and diameter, stem density, site index and canopy leaf area will be examined along a gradient of elevation from the basin floor at Findley Lake, to the basin rim on Findley Ridge (Figure I.1). This information will be needed to scale the results of the study to the Findley Lake Basin as a whole. It is anticipated that structure of the stands will vary with elevation.

Materials and Methods

Study site

The Findley Lake Basin (47°04'N, 121°25'W) is located in the Cedar River Watershed at an elevation of 1170 meters. Findley Lake is centered in the horseshoe-shaped basin, draining north to Findley Creek and then into the Cedar River (Figure I.1). Steep headwalls rise up to 300 m (high point of 1451 m) above the lake to the east, south and west. Situated 11.5 kilometers west of the Cascade Crest, Findley Lake has a cool and moist climate, with an average temperature of 5.4°C and mean annual precipitation of 2730 mm yr^{-1}. Eighty percent of the annual precipitation falls as snow. The soils are typic cryohumods over fractured andesite or moraines of andesitic material.

Findley Lake is located within the *Abies amabilis* zone (Henderson *et al.* 1992), and *Abies amabilis* is the most abundant and ecologically important tree species in the basin. *Tsuga mertensiana* and *Tsuga heterophylla* are also important, while *Abies procera* occurs in scattered locations in the basin. *Abies lasiocarpa* can be found on the high ridges that surround the basin on three sides. The floor and lower headwalls of the Findley Lake Basin are cloaked with montane forests. These stands gradate into transitional subalpine forests on the upper headwalls and ridges that rim the basin. The forest stands of the Findley Basin are mature (± 220 years old) and have a mean basal area of 74.3 m^2 ha^{-1}. The understory is well shaded, with advance regeneration of *Abies amabilis*, and ericaceous shrubs such as *Vaccinium ovalifolium*, *V. membranaceum*, *V. alaskaense*, *Menziesia ferruginea* and *Rhododendron albiflorum*. Some of the common forbs include *Xerophyllum tenax*, *Cornus canadensis* and *Clintonia uniflora*. Del

Moral (1973) is a good source for more comprehensive information on the vegetation of the Findley Lake Basin.

The managers of the Cedar River Watershed have given special status to Findley Lake and its surrounding forests in recognition of its unspoiled beauty and value for research. The locale was dedicated as the Joe E. Monahan Findley Lake Reserve and Research Area in 1992. The Thompson Creek Research Center in the lower watershed and Findley Lake were coniferous biome study sites for both the International Biological Program and the Integrated Forest Study (Johnson and Lindberg 1991). The forest ecosystem of the Findley Lake Basin has been the subject of numerous published research papers, most recently by Buchmann *et al.* (1998); Martin *et al.* (1999); Martin *et al.* (2001) and Sprugel (2002).

Three sites were chosen along an approximately east-to-west transect (Figure I.1) to study canopy structure in the Findley Lake Basin: Findley Lake (elevation 1170 m), the midslope of Findley Ridge (elevation 1230 m), and the summit of Findley Ridge (elevation 1320 m). These sites were assumed to sample, respectively, the lower (57.6 ha forest, 4.9 ha talus), middle (57.9 ha forest, 16.9 ha talus) and upper (50.3 ha forest, 20.6 ha talus) environments of the Findley Lake Basin. The same sites were also used to study throughfall (Chapter II). Findley Lake was the most important and intensively studied study site, principally because it was the location of a concurrent study on canopy transpiration conducted by Martin *et al.* (2001).

Stand structure

Five 1/20th ha circular plots were established at each of the three sites. The criteria for plot location included: 1) mature forest, 2) a plot basal area of ± 75 m^2 ha^{-1}, and 3) at least two tree lengths away from large openings. The criteria for the plot basal area target came from the average for the Findley Lake Basin reported by Del Moral (1973). The purpose of the basal area target was to mitigate the influence of variation in stand density on throughfall. The species, height, diameter at breast height, crown width, crown length, height to base of crown and canopy position (dominant, co-dominant, intermediate or suppressed) were recorded for all stems > 7.5 cm DBH within each circular 1/20th ha plot. Additionally, the distance and azimuth from the plot center of all stems > 7.5 cm DBH were recorded for the five 1/20th ha plots on Findley Ridge and at Findley Lake.

Canopy architecture of Tsuga heterophylla

Nine mature, co-dominant *Tsuga heterophylla* were chosen for detailed measurements of canopy structure. Sprugel (unpublished) quantified comparable data for crowns of *Abies amabilis* at Findley Lake. Unfortunately, the intensive nature of crown dissection measurements limited the study of canopy structure to one of the two *Tsuga* species in the Findley Lake Basin. The second species of *Tsuga*, *Tsuga mertensiana*, is more widespread in the Findley Lake Basin than *Tsuga heterophylla*. However, *Tsuga heterophylla* was chosen for comparison to *Abies amabilis* due to its importance in the Pacific Northwest (Franklin and Dyrness 1973). Although tree-level data (height, diameter, live crown ratio, crown diameter, crown height) were recorded separately for *Tsuga mertensiana*, throughout this dissertation the morphology and distribution of foliage for *Tsuga mertensiana* will be assumed to be the same as for *Tsuga heterophylla*.

To initiate the canopy measurements, the total height, live crown ratio, height to crown base, four-sided crown radius, and diameter at breast height of each tree were measured. Since the cross-sectional sapwood area can be a predictor of leaf area (Kaufmann 1981), the bark thickness was determined with a penotrometer, and then the sapwood thicknesses on the north, east, west and south sides of the stem were measured with an increment borer. The height each branch was determined by ascending the tree and measuring with a 50 meter tape. Unlike *Abies* *amabilis* where the branch is largely circular, the branches of *Tsuga heterophylla* are ovate in cross section, with the longest axis of the branch parallel with the axis of the central stem. Therefore, the minimum and maximum diameters of each branch were measured with calipers and recorded separately.

To sample branches for the determination of leaf area, at least one branch was randomly sampled from the high, mid-high, mid-low and low portions of the crown. Ultimately, the leaf area data were obtained from a total of 48 branches. The length of each sampled branch was measured on the ground. Then, each branch was transported to the University of Washington Tree Physiology Lab in Seattle.

At the tree physiology lab, eight small shoots were chosen at random from each branch for leaf area measurement. First, the silhouette area of the shoots laid flat was measured with Optimas Digital Area Analyzer. Then, all needles were removed from each shoot. The needles were arranged to eliminate any overlap, and were pressed flat with a glass pane. The projected area of the needles was then determined with the Optimas Digital Area Analyzer. The silhouette-to-projected area ratio ($SPAR_{max}$), was calculated by dividing the silhouette area of each shoot by the projected area of its needles. $SPAR_{max}$ tends to be low in sun-adapted shoots, due to acute needle angles and self-shading of needles (Leverenz and Hinckley 1990). The $SPAR_{max}$ of shade-adapted shoots trends towards 1.0. This is because the needles of shade-adapted shoots are displayed at near-horizontal angles, and are arranged to minimize self-shading (Leverenz and Hinckley 1990).

The remainder of the branch material was placed into paper bags, and dried for 24 hours at 70°C. At this point, the needles readily dehisced when the stems were gently tapped over a tray. The separated needles were dried for an additional 48 hours at 70°C, and then weighed.

The projected leaf area of each branch was calculated by multiplying the total dry needle weight by the mean specific leaf area of the eight leaf area samples. For a conifer, specific leaf area (SLA) is the ratio of the one-sided projected area of all needles of a shoot, divided by the oven-dry weight of the needles. SLA is usually expressed in units of $cm^2\ g^{-1}$. Shade-tolerant conifers such as *Abies amabilis* and *Tsuga heterophylla* usually have sun-adapted needles that are thicker in cross section than shade-adapted needles. Consequently, the SLA of sun-adapted shoots is lower than the SLA of shade-adapted shoots (Leverenz and Hinckley 1990).

The projected leaf area of each branch of the crown was determined by regression, where branch height and branch diameter were the independent variables. Height was related to the light environment, and hence to the mean SLA of the foliage, while branch diameter was a predictor of the mass of needles displayed by each branch. The projected leaf area of the crown was estimated by the sum of the estimated leaf areas of all branches. Finally, the estimated projected leaf area of each sample tree was regressed on cross-sectional sapwood area.

Results

Stand structure

The mean basal area of the plots at Findley Lake was 78.5 ± 4.3 $m^2\ ha^{-1}$. *Abies amabilis* accounted for two thirds of this basal area (52.6 $m^2\ ha^{-1}$), while a mixture of *Tsuga heterophylla* and *Tsuga mertensiana* contributed the other third of the stand basal area (25.9 $m^2\ ha^{-1}$). The continuous, well-developed canopy and the quasi-normal distribution of tree sizes (Figure I.2) suggested that the Findley stand is single-aged and regenerated following a stand-replacing disturbance. The diameter distribution of the two *Tsuga* species paralleled the diameter distribution of *Abies amabilis*, except for the ten- and twenty-cm size classes, which were predominated by small stems of *Abies* (Figure I.2). With respect to the canopy-forming trees of the stand at Findley Lake (the intermediate, codominant and dominant crown classes), stems of *Abies amabilis* and *Tsuga heterophylla* at Findley Lake were similar in size with respect to both mean diameter (50.1 ± 3.2 cm DBH for *Abies amabilis* vs. 54.6 ± 3.5 cm DBH for *Tsuga heterophylla*) and height (32.4 ± 1.9 m for *Abies amabilis* vs. 31.3 ± 0.8 m for *Tsuga heterophylla*). *Tsuga heterophylla* at Findley Lake, however, had deeper crowns than *Abies amabilis*. The mean live crown ratio of *Tsuga heterophylla* (44%) was significantly greater than the mean live crown ratio of *Abies amabilis* (34%, $p>0.05$, Student's t-test).

The two *Tsuga* species had wider crowns than *Abies amabilis* (Figure I.3). The radius of the crown was related to square root of the stem DBH. For *Abies amabilis*, crown radius = $0.58 + 0.81 * DBH^{.5}$ ($r^2 = 0.83$, F-ratio = 335.4, $p<0.01$; DBH in cm). For *Tsuga heterophylla*, crown radius = $-1.31 + 1.47 * DBH^{.5}$ ($r^2 = 0.78$, F-ratio = 36.0, $p<0.01$; DBH in cm). *Tsuga mertensiana* had crowns of intermediate width (data not shown), with radius = $-0.26 + 0.95 * DBH^{.5}$ ($r^2 = 0.80$, F-ratio = 471.0, $p<0.01$).

Canopy architecture

From the top to the bottom of the canopy at Findley Lake, shoots of *Tsuga heterophylla* showed at least two kinds of morphological plasticity. The first was variation in specific leaf area (Figure I.4A). The needles from the tops of *Tsuga heterophylla* crowns were relatively thick, with SLA values ranging from 40 to 50 cm^2 LA g^{-1} dry needle weight. Needles from the lowermost portions of *Tsuga heterophylla* crowns were thinner and lighter, with SLA varying from 100 to 120 cm^2 LA g^{-1} dry needle weight. Variation in SLA was continuous in the trees examined at Findley Lake (Figure 4A), presumably reflecting the gradual diminution of light with depth in the canopy. However, for scaling purposes the point at which the mean minimum SLA of the sun-adapted (40 cm^2 g^{-1}) shoots had increased 50% and at which shoots had assumed a mostly flattened arrangement ($SPAR_{max} > 0.6$) was interpreted as the arbitrary dividing line between sun- and shade-adapted shoots.

Variation in the structure of needle displays was the second type of morphological plasticity seen in *Tsuga heterophylla* at Findley Lake. Needles from the tops of crowns tended to be attached at acute angles to vertically oriented short shoots, and arranged in whorls. Mutual shading among needles was high in these "star" shoots, resulting in $SPAR_{max}$ values of 0.38 – 0.55 (Figure I.4B). In the lower canopy, needles were arranged in flattened displays on horizontally oriented shoots. This type of needle arrangement reduced self-shading. The SPAR values of shoots from the lower canopy varied between 0.75 and 0.85—closer to the hypothetical minimum of 1.0 for a shoot perfectly designed to intercept directional light from above (Leverenz and Hinckley 1990). As with SLA, variation in $SPAR_{max}$ was continuous with height in the *Tsuga heterophylla* crowns at Findley Lake (Figure I.4B).

Height and diameter were the best predictors of the projected needle area displayed by individual branches. Large diameter branches supported a greater mass of needles. Second, since SLA increased with depth in the canopy (Figure I.5A), branches near the bottom of the canopy produced more leaf area per unit of needle biomass than branches in the upper canopy. The maximum diameter of the oval-shaped *T. heterophylla* branches proved to be a better predictor of branch needle area than either the mean branch diameter or the cross-sectional area of the branch.

The one-side projected needle area of *Tsuga heterophylla* branches at Findley Lake was predicted by the regression: natural log(m^2 one-sided projected needle area) = 0.40 – 0.03*branch height + 0.37*longitudinal branch diameter ($r^2 = 0.90$, F-ratio = 288.1, $p<.01$), where branch height is in meters and branch diameter is in centimeters. The estimated total projected needle area of the nine *Tsuga heterophylla* for which the height and diameter of all branches were known ranged from 354 to 955 m^2, with a mean of 561.9 ± 65.8 m^2 one-sided projected needle $tree^{-1}$ (Table I.1). Most of this needle area was arrayed in the middle and lower portions of the canopy (Figure I.7). Slightly less than forty percent of the needle area was born by very old, well-shaded branches less than twenty meters in height. Fifty percent of the needle area was displayed by branches between twenty and thirty meters in height, while about ten percent of the canopy leaf area was arrayed on the sun-washed branches that were thirty meters in height or more. In comparison to *Abies amabilis*, *Tsuga heterophylla* had more leaf area in the lower part of the canopy (Figure I.7).

DBH, total height and cross-sectional sapwood area were related to total needle area. DBH had a strong relationship with total needle area: ln(m^2 one-sided projected needle area) = $3.925 + 0.309*DBH^{.5}$ ($r^2 = 0.83$, F-ratio = 39.8, $p < 0.01$), where DBH is measured in centimeters. Total height proved to be better predictor of total needle area than DBH: total projected needle area = $2.59 + .071*\text{total height}^{2.5}$ ($r^2 = 0.90$, F-ratio = 62.52, $p < 0.01$), where total height is expressed in meters. Cross-sectional sapwood area was the most time-consuming to quantify, but yielded the most precise estimate for needle area: m^2 one-sided projected needle area = 190.32 + 0.36*sapwood area ($r^2 = 0.93$, F-ratio = 104.3, $p<0.001$), where sapwood area is in cm^2 (Figure I.6).

Of the three predictors of leaf area for individual stems, tree height was used to scale the stand-level needle area at Findley Lake. To estimate the leaf areas of the stands at the midslope and summit of Findley Ridge, a less-site specific equation was prepared from the data of Sprugel (unpublished): ln(projected needle area) = $0.28 + 0.66*DBH^{0.5}$, $r^2 = 0.95$. Virtually all of the leaf area at the mid- and high-elevation sites was from *Abies amabilis*. The midslope site has a leaf area of 5.9 ± 1.0 m^2 LA m^{-2}. The ridgetop site, with twice the stem density but lower stand height

(Table I.2) had an estimated leaf area of 5.8 ± 0.4 m^2 m^{-2}. Findley Lake had an estimated stand leaf area index of 8.5 ± 0.2 m^2m^{-2}, significantly greater than the leaf areas of either the midlsope or Findley Ridge sites ($p<0.01$, Tukey's multiple comparison test). Although two thirds of the stand basal area at Findley Lake was from *Abies amabilis*, crowns of *Tsuga* contributed nearly half of the canopy needle area. Site index, as indicated by the mean height of mature dominant *Abies amabilis* (Hoyer and Herman 1983), was low at Findley Lake, and then declined further with elevation along the transect to Findley Ridge (Table I.2).

Discussion

The forest canopy near Findley Lake is a fabric of *Abies amabilis* punctuated by long crowns of *Tsuga*. Although two thirds of all stems and two thirds of the stand basal area are *Abies amabilis*, the total canopy area is nearly equally divided between crowns of *Abies* and *Tsuga*. Given that photosynthesis, transpiration, and the interception and processing of precipitation occur in the crowns of individual trees, simple stem-based stand data do not fully reflect the ecological importance of *Tsuga* at Findley Lake.

The disproportionate contribution of *Tsuga* to the Findley Lake canopy was due to its massive leaf area display. The average-sized *Tsuga* had a mean height and diameter of 31.3m and 54.6 cm, respectively. The mean canopy-forming *Abies amabilis* was nearly identical in stature. Using the regression from Martin *et al.* (2001), an *Abies amabilis* of this size would have an estimated leaf area of 124 m^2. However, using the regression for the leaf area of *Tsuga heterophylla* developed in this study, a tree of similar size would have a leaf area of 375 m^2—a threefold difference.

The geometry of *Tsuga heterophylla* and *Abies amabilis* crowns can explain the much of the difference in the projected needle area of the two species. First, *Tsuga heterophylla* had a greater live crown ratio than *Abies amabilis*. *Tsuga* crowns were longer, with more foliar layers stacked on a stem. Part of the explanation for this is that mature *Tsuga heterophylla* at Findley Lake had greater plasticity with respect to SLA than *Abies amabilis*. Thick needles (low SLA) are well adapted for sun, whereas thin needles (high SLA) are better adapted to shade (Givinish 1988; Leverenz and Hinckley 1990). For both species, sun-acclimated needles in the tops of crowns had similar minimum values of SLA of about 35 $cm^2 g^{-1}$. The maximum SLA values found in the dimly illuminated lower crowns of the two species were quite different: 80 $cm^2 g^{-1}$ for *Abies amabilis*, compared to 120 $cm^2 g^{-1}$ for *Tsuga heterophylla*. Since plasticity with respect to SLA is an important part of conifer shade tolerance (Leverenz and Hinckley 1990), and *Abies amabilis* and *Tsuga heterophylla* are both highly shade tolerant species, the rarity of high-SLA needles in overstory *Abies amabilis* at Findley Lake was unexpected.

Abies amabilis has the capacity to form shade-acclimated needles with three to four times more needle area per unit of needle mass than the much thicker sun-acclimated needles in the upper crown (Sprugel *et al.* 1996). In contrast, the overstory *Abies amabilis* at Findley Lake showed a two-fold variation in SLA. The reason for the more limited morphological plasticity of the canopy-forming *Abies* at Findley Lake is not known. The abundance of advanced regeneration (< 1.5 m height) *Abies amabilis* in the understory at Findley Lake indicates that the species can produce highly shade-adapted foliage. Indeed, Tucker *et al.* (1987) found SLA of 110 $cm^2 g^{-1}$ in advanced regeneration *A. amabilis* from montane forests in the Cedar River Watershed. One explanation may be that *A. amabilis* loses some of its morphological plasticity with age. The trees studied by Sprugel *et al.* (1996) were 35 years old, compared to an age of more than 220 years for the *Abies amabilis* included in this study. Crown position may be another factor. Sprugel (2002) has noted divergent shade acclimation abilities in dominant and co-dominant versus suppressed *Abies amabilis*. For trees of similar age, the height of the last live branch, and the SLA and $SPAR_{max}$ values of the foliage were very different between the two groups. In contrast, the capacity of *Tsuga heterophylla* at Findley Lake to vary shoot morphology seemed unrelated to age, and only slightly sensitive to crown class.

Crown width was another major difference between the crown architectures of the two species. *Tsuga heterophylla* fills the space around its stem with extraordinarily long branches. The crown of *Abies amabilis* is more compact. The crown radii of the two species illustrate this point. For a tree of mean size (DBH = 50 cm), an *Abies amabilis* would have a crown radius of 6.3 m, while the crown radius of a 50 cm DBH *Tsuga heterophylla* would be 9.1 m. Snow-loading may select for branch length in this cold and wet environment. Presumably, the short and stiff branches of *Abies amabilis* are better adapted to high snow loads than the long branches of *T. heterophylla* (Tom Hinckley, personal communication).

The greater crown length and width of *Tsuga heterophylla* resulted in more crown volume. If *Tsuga heterophylla* and *Abies amabilis* are assumed to have crowns shaped like cones, then the crown volume of an *Abies amabilis* with a DBH of 50cm and height of 32m would be 683 m^3. The crown volume of a same-sized *Tsuga heterophylla* would be 1825 m^3. The difference between the estimated crown volumes of the two species can largely account for the large differences in needle area per unit of stem basal area.

Martin *et al.* (2001) reported that the stand leaf area index at Findley Lake was 6.4 $m^2\ m^{-2}$. The difference between the LAI reported in their study and the Findley LAI estimated in this dissertation (8.5 ± 0.2 $m^2\ m^{-2}$) cannot be attributed to a mensurational error, since in both studies the average height, diameter, density and basal area of trees were similar. However, Martin *et al.* assumed that the crown allometry of all species at Findley was the same. If the same assumption had been made in this study, then the estimate for the LAI of the Findley Lake stand would have been reduced to 6.8 m^2 m^{-2}. The actual LAI at Findley Lake may lie somewhere between the estimate by Martin *et al.* (2001) and the estimate made in this study, since *Tsuga mertensiana* is intermediate in crown length and crown radius between *Abies amabilis* and *Tsuga heterophylla*.

Summary

This chapter described the forest structure at three sites representative of the lower (Findley Lake), middle (midslope of Findley Ridge) and upper (summit of Findley Ridge) zones of the Findley Lake Basin. Nearly all of the fieldwork described in this dissertation was conducted at these three sites. The estimated leaf areas of the study sites—important values that will be used throughout this dissertation—were 8.5 m^2 m^{-2}, 5.9 m^2 m^{-2} and 5.8 m^2 m^{-2}, respectively. Most of this leaf area was from *Abies amabilis*, although at Findley Lake, nearly half of the total leaf area was contributed by the two *Tsuga* species, despite their low total basal area relative to *Abies amabilis*. *Tsuga heterophylla,* due to its long branches and deep crown, was estimated to have three times more leaf area per unit of basal area than *Abies amabilis*.

Table I.1. DBH, height, estimated one-sided projected needle area, and sapwood area at DBH for nine mature co-dominant *Tsuga heterophylla* at Findley Lake, WA.

Tree	DBH (cm)	Height (m)	Projected leaf area (m^2)	Sapwood area (cm^2)
H1	50.0	33.9	475.7	826.1
H2	40.6	30.3	426.5	682.2
H3	57.4	35.1	480.4	672.9
H4	68.8	---	641.4	1380.1
H5	52.1	34.1	475.6	879.0
H6	46.2	32.6	331.1	454.0
H7	84.9	39.4	789.9	1783.4
H8	80.1	45.4	954.7	1876.6
H9	52.2	35.2	482.1	634.0

Table I.2. A comparison of stands at three sites in the Findley Lake Basin, WA. The data are the means ± SE.

Site	Site index[a]	DBH (cm)	Canopy height (m)	Stem density n ha^{-1}	Leaf area (m^2 m^{-2})
Findley Lake	100	39.9 ± 2.3	32.3 ± 1.3	488 ± 24.4	8.5 ± 0.2
Midslope	80	44.7 ± 2.1	27.7 ± 0.8	388 ± 29.4	5.9 ± 1.0
Findley Ridge	60	30.5 ± 2.2	23.5 ± 0.8	880 ± 103	5.8 ± 0.4

[a] source: Hoyer and Herman (1989).

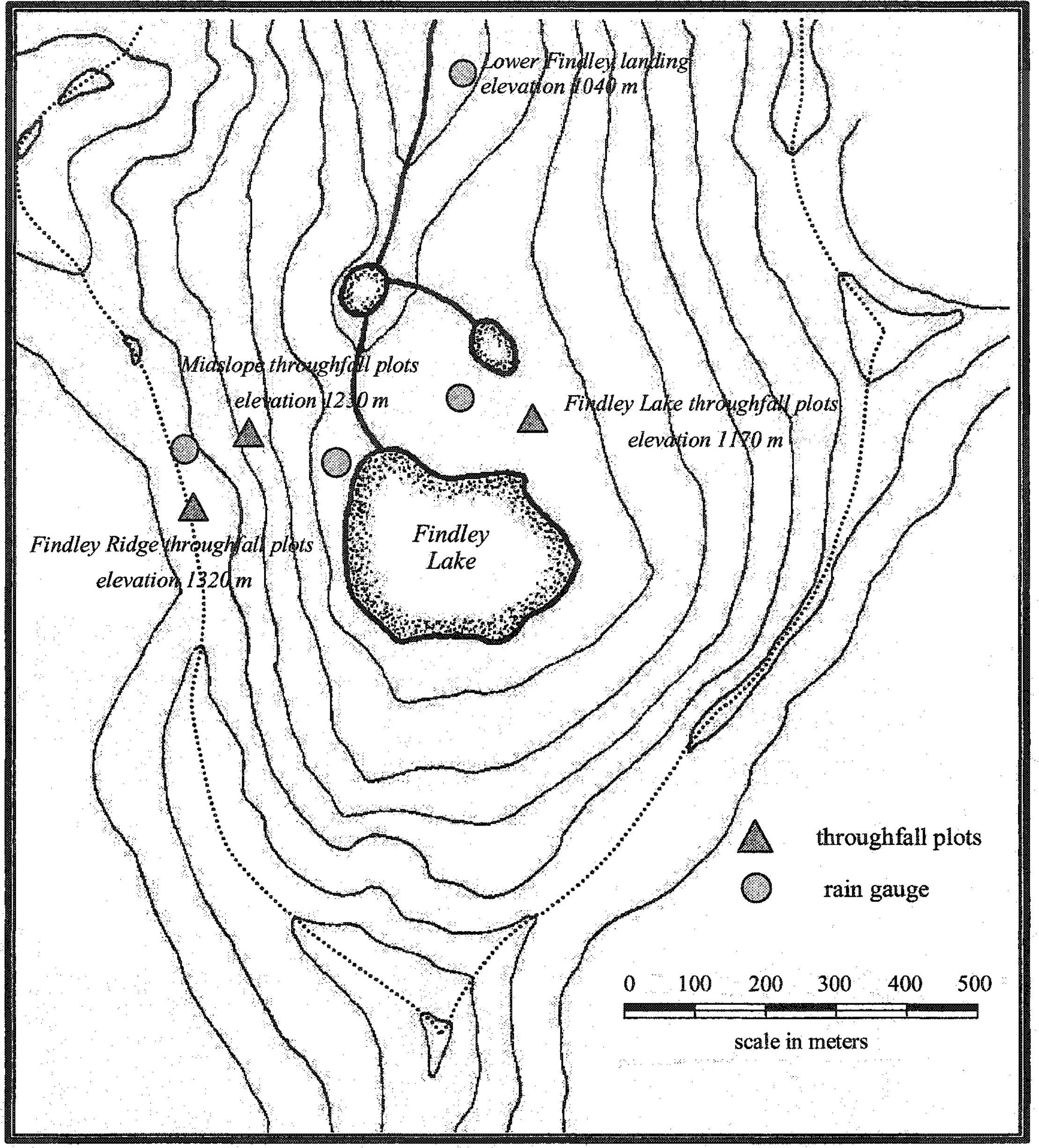


Figure I.1. Map of the Findley Lake Basin, Cedar River Watershed, WA. Elevation contours are shown in 50 meter intervals.

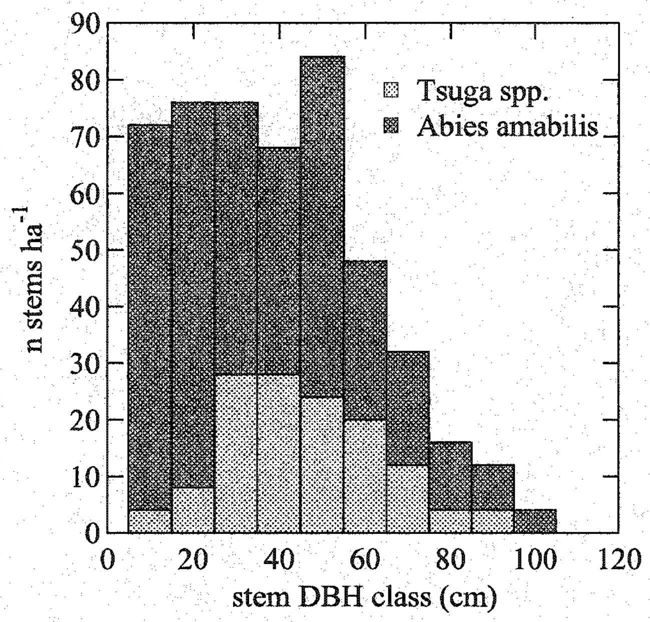


Figure I.2. The diameter (cm DBH) distribution of trees at Findley Lake, WA. The data were obtained from five 1/20th ha circular plots. The data for *Tsuga* spp. are a composite of records for *Tsuga heterophylla* and *Tsuga mertensiana*.

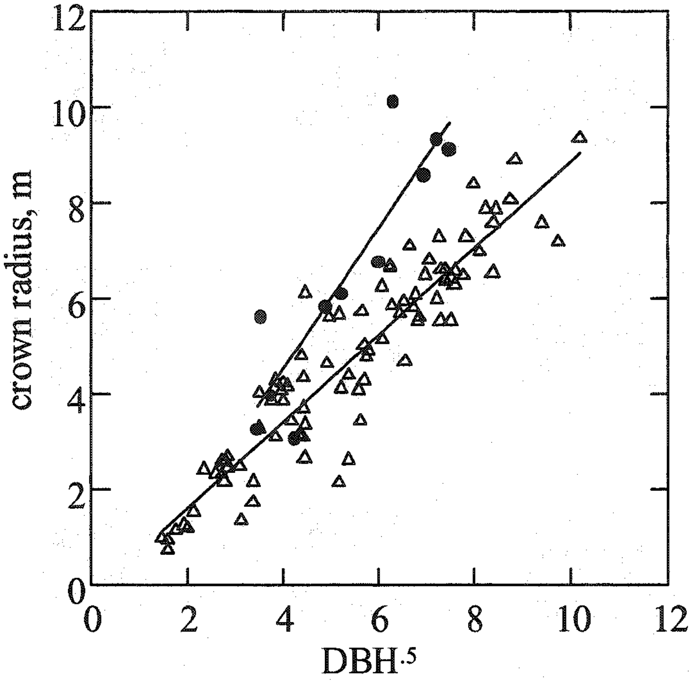


Figure I.3. Crown radius as a function of the square root of DBH for mature codominant *Tsuga heterophylla* and *Abies amabilis* at Findley Lake, Washington. Solid circles, *Tsuga heterophylla*; open triangles, *Abies amabilis*. DBH was measured in centimeters.

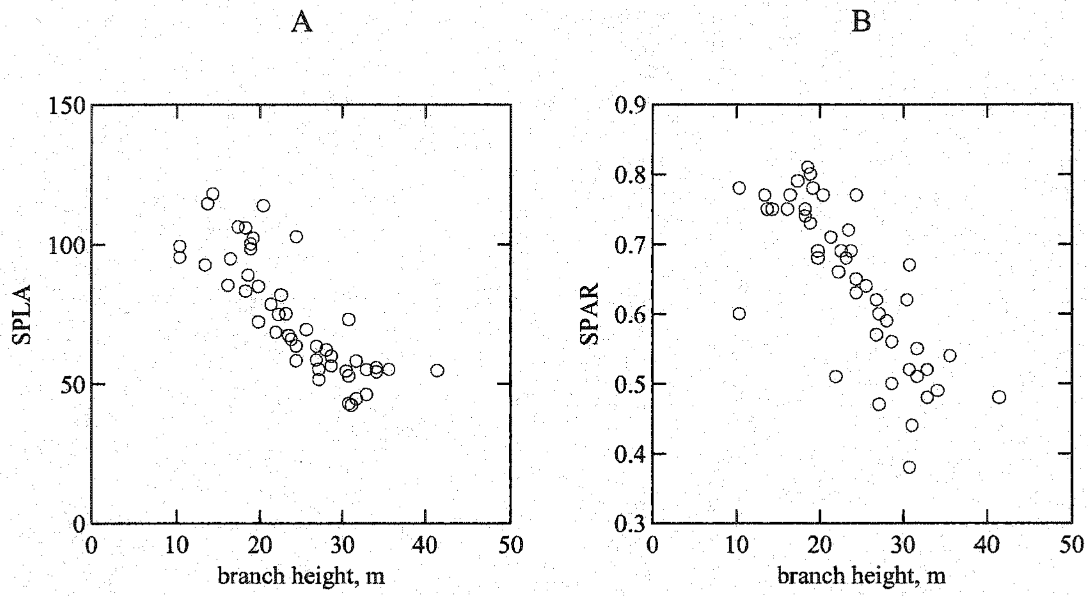


Figure I.4. A) Specific leaf area (SLA, cm^2 g^{-1}), and B) the silhouette-to-projected area ratio (SPAR$_{max}$) as a function of branch height for *Tsuga heterophylla* at Findley Lake, WA. The gradient of height in the canopy is also a gradient of light. The data are from 48 branches sampled from eight mature, co-dominant trees. Each data point is the mean of eight shoots randomly sampled per branch.

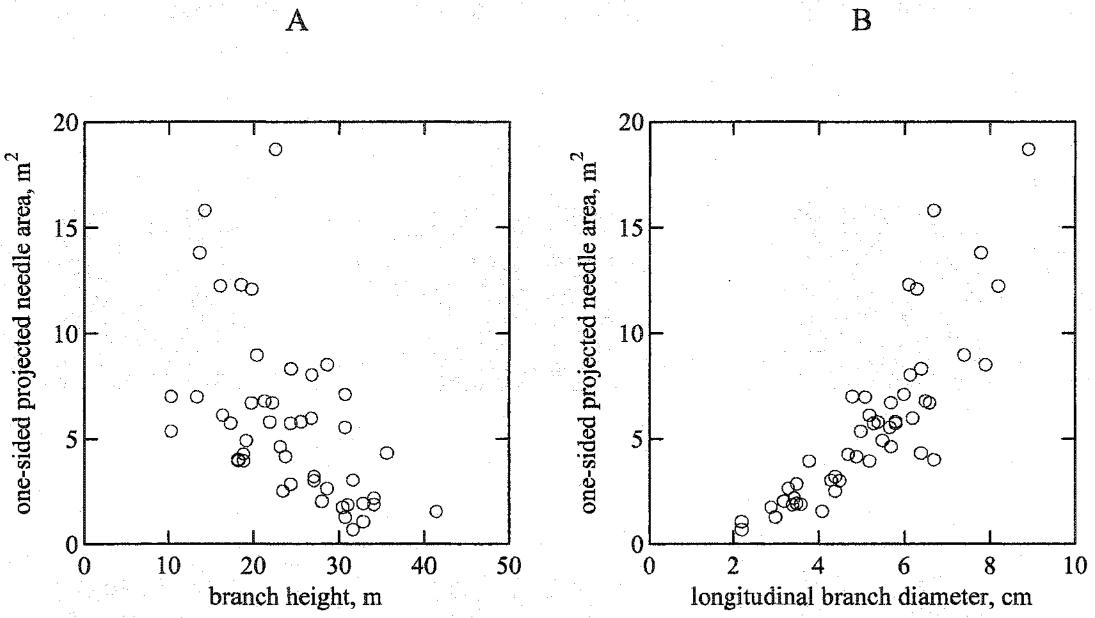


Figure I.5. The relationship between the one-sided projected needle area displayed by branches (m^2) and A, branch height (m); and B, branch diameter (cm). The data are from 48 branches sampled from eight mature co-dominant *Tsuga heterophylla* at Findley Lake, WA.

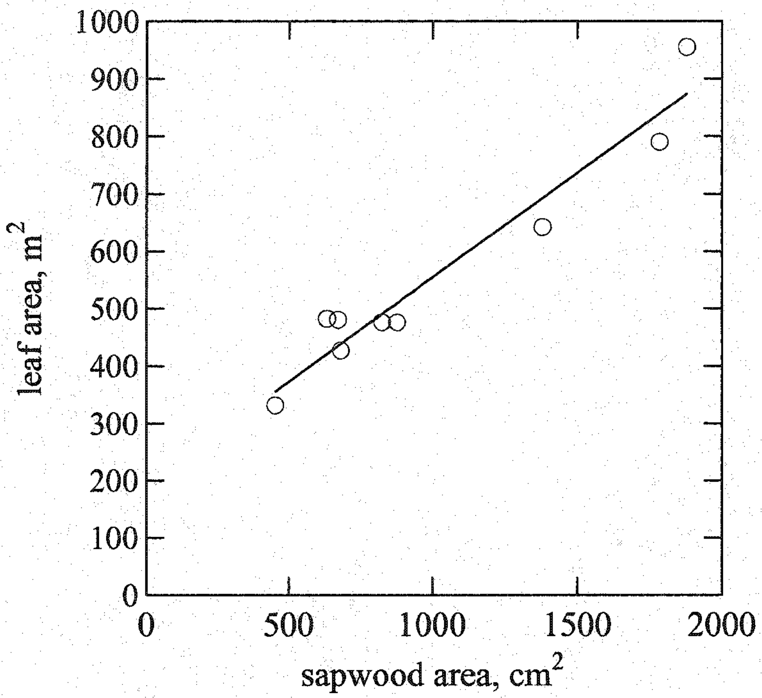


Figure I.6. The relation between sapwood area (cm^2) and one-sided projected leaf area (m^2) for nine mature codominant *Tsuga heterophylla* at Findley Lake. One-sided projected leaf area is predicted by the regression: leaf area (m^2) = 190.32 + 0.36*sapwood area at DBH (cm^2), r^2 = 0.93, F-ratio = 104.3, $p<0.001$.

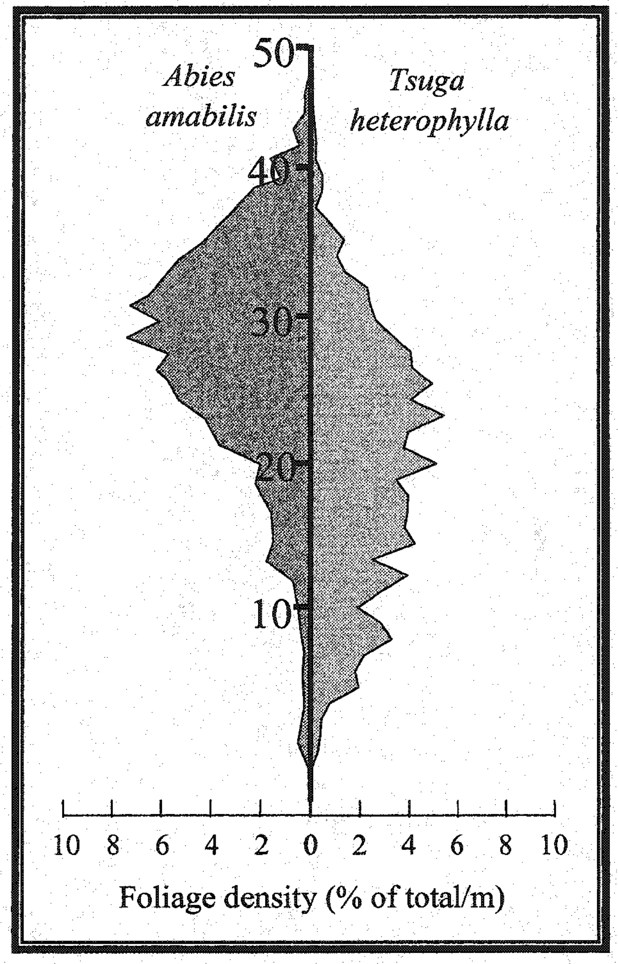


Figure I.7. The distribution of leaf area by one-meter height intervals for *Tsuga heterophylla* and *Abies amabilis* at Findley Lake, WA. The mean leaf area per tree for *Tsuga heterophylla* ($\overline{DBH}$ = 59.2 cm, *n* = 9) was 562 ± 63 m^2; the mean leaf area for *Abies amabilis* ($\overline{DBH}$ = 58.2 cm, n=11) was 239 ± 50 m^2. The data for *Abies amabilis* were from Sprugel (unpublished).

Chapter II. Precipitation and throughfall in the Findley Lake Basin.

Introduction and objectives

In forests, most rain droplets fall first upon the canopy. A portion of the diverted precipitation coats leaves and stems, or is absorbed by epiphytes. Water held in the canopy is vulnerable to evaporation (McNaughton and Jarvis 1983; Calder 1990; Calder 1998). Indeed, in mesic forests, the loss of water due to evaporation usually exceeds water loss due to transpiration (Zinke 1967; McNaughton and Jarvis 1983; Calder 1990; Calder 1998). However, evaporation losses can be at least partially offset by canopy harvest of cloud water (Harr 1982; Calder 1990). Whether precipitation is from rain or fog, throughfall commences once the water-holding capacity of the canopy is exceeded (Calder 1990; Gash *et al.* 1995). Because the path of water penetrating the canopy is re-directed by inclined surfaces, the pattern of throughfall on the forest floor is non-random and related to the structure of the canopy above (Ford and Deans 1978; Kimmins 1987). Thus, forest canopies modulate both the quantity and distribution of throughfall arriving at the forest floor.

Accordingly, the study of canopy interactions with precipitation had four objectives. The first was to determine the frequency and duration of canopy wetness in the Findley Lake Basin. The Washington Cascades have a high frequency, low intensity precipitation regime. Snoqualmie Pass, located 16 km NE of Findley Lake, has an average of 173 days yr^{-1} of measurable precipitation, with a mean of 15.3 mm precipitation per event. Stampede Pass, 18 km E of Findley Lake, receives a mean of 201 days yr^{-1} of measurable precipitation, with a mean of 11.1mm per event; and Stevens Pass has 184 days yr^{-1} of measurable precipitation, with an average of 11.3 mm per event (Western Regional Climate Center 2002). Forest canopies in the northern, west-facing Cascades are moistened nearly half of all days during the course of a typical year. "Wet" is a pervasive condition in these forests, with potentially important consequences.

The second objective was to determine if below-canopy throughfall is less than or greater than measured precipitation in the Findley Lake Basin. Isaac (1946), Harr (1982), Dawson (1998) and others have shown that moisture raked from low clouds by trees can be hydrologically and ecologically significant. On the other hand, the rate of evaporation from a wet canopy can be high, even during rains when the atmosphere is presumably well charged with water vapor (McNaughton and Jarvis 1983; Calder 1990). Additions of water harvested from clouds by forest canopies, and the evaporation of intercepted precipitation from wet canopies, are key determinants of the hydrology of forested watersheds.

The third objective was to identify patterns of throughfall distribution on the forest floor. Forest canopies affect the pattern of water (Ford and Deans 1978; Pederson 1992) and chemical (Seiler and Matzner 1995) influxes to the forest floor. The canopy also influences the pattern of cation and anion inputs to the forest floor. However, the patterns of chemical additions do not always correspond to patterns of throughfall input, and tend to be specific for different groups of chemical elements (Seiler and Matzner 1995). Canopy-induced gradients of water and nutrients on the forest floor could influence the structure of plant communities. Ford and Deans (1978), for example, found that the density of fine roots tended to be highest where the input of throughfall was high.

Finally, the point-to-point distribution of throughfall on the forest floor should be predictable if the interactions between canopy structure and precipitation can be understood. The fourth objective was to develop a spatial model that would predict throughfall as a function of canopy structure. The development of a spatially explicit model of throughfall would enable tests of putative relationships between the patterns of water supply to the soil and understory processes such as seedling establishment and mortality.

Materials and methods

Throughfall study sites

Three sites along an approximately east-west transect were established to examine effects of elevation and slope exposure on fog interception and throughfall (Figure I.1). These sites, described in Chapter I, were Findley Lake (elevation 1170 m), the midslope of Findley Ridge (1230 m), and the summit of Findley Ridge (1320 m). Findley Lake was the most important and intensively studied of the three study sites, principally because it was the site for a concurrent study on canopy transpiration described by Martin *et al.* (2001). Two other sites on the west side of Findley Ridge were repeatedly dismantled by unsympathetic large mammals (*Cervus canadensis* and *Ursus americanus*), and therefore abandoned in 1994.

Meteorological measurements

Rainfall was recorded with Taylor wedge-type rain gauges. Two gauges were mounted on a tower near Findley Lake. Another set of two gauges was mounted on a tower on Findley Ridge. The data from the Findley Ridge and Findley Lake stations varied by less than 5%. In 1994, an automated weather station was mounted on a 39-meter tower at Findley Lake. In 1995, the weather station was operated at the lower Findley Landing (elevation 1040 m). Rainfall was logged with a tipping bucket rain gauge. A pyranometer (Li200S, Li-Cor, Lincoln, NE) measured net radiation. A shielded combination capacitative humidity sensor/thermisistor probe (HMP35C, Campbell Scientific, Inc., Logan, UT) recorded air temperature and relative humidity. Wind speed and wind direction were monitored with a cup anemometer and wind vane, respectively (Model 03001-5, R.M. Young, Traverse City, MI). Variables were read every minute and averages were recorded at 30-minute (1994) or 5-minute (1995) intervals. Meteorological data were collected during the period 17 July – 10 Oct 1994 and 28 July- 31 October 1995.

The weather station was powered with a 12-volt deep cycle car battery recharged with a solar panel. In 1994, the battery source was also used to power an array of stem-mounted heat balance sap flow gauges. Due to the high power demand from the sap flow gauges, the solar-charged power supply was often exhausted during overcast days. Accordingly, the 1994 Findley Lake meteorological data were incomplete, particularly for days corresponding to precipitation events. The 1995 meteorological record from the lower Findley Landing had fewer gaps.

Canopy wetness

Canopy wetness was measured from 7 July 1995 to 30 October 1995. Leaf wetness sensors were installed in the upper (height = 43 m) and lower canopy (height = 20 m) at Findley Lake, and in the upper (height = 24 m) and lower (height = 6m) canopy at Findley Ridge. The sensors were constructed by winding two parallel gold wires around an artificial fir needle, constructed of a piece of green 14-gauge copper wire insulation mounted on a stainless steel wire. The mean contact angle of water droplets placed on the artificial fir needles (107.1 ± 1.1°, n=20) was not significantly different than the mean contact angles of water droplets applied to current year, sun-adapted needles of either *Abies amabilis* or *Tsuga heterophylla* ($p>0.20$, Bonferroni's multiple comparison). The sensors were installed on foliated shoots, normal to the orientation of the foliage. An electrical current was supplied to the sensors from two alkaline D cell batteries. Moisture on the surface of the artificial needle completed the electrical circuit between the two gold wires. The voltage passing between the two wires was measured at 12-minute intervals with a Hobovolt data logger (Onset Instrument Company).

Throughfall

Ten throughfall collectors were positioned in the previously described 1/20th ha circular plots (see methods, Chapter 1). With five plots at each site, fifty throughfall collectors were installed at each measurement site. The throughfall collectors were made from 27.4 cm diameter funnels mounted on two liter plastic bottles. The collectors were mounted on posts 0.9 meters above the soil surface. Within each throughfall plot, ten throughfall collectors were located at a random azimuth and distance (0 m $\leq d \leq$ 12.62 meters) from the plot center. The collectors were emptied after each precipitation event. Randomly located funnels have been shown to more accurately estimate throughfall than systems of collection troughs (Dolman 1987; Teklehaimanot *et al.* 1991). Canopy throughfall was measured 25 July – 18 October 1994, and 16 June – 18 October 1995. The late initiation of measurements in 1994 was due to the time required to install the throughfall collectors and to construct a canopy access tower at Findley Lake.

In some forest ecosystems, stem flow can be an important conduit for water delivery to the forest floor. The importance of stem flow in coniferous forests is closely linked to stand age (Table II.1). Young conifers often have uplifted branches that direct intercepted precipitation to the stem, and smooth bark that guides water to the forest floor (Ford and Deans 1978). However, as most species of conifers mature, branch angles decrease, bark becomes rough, and stemflow declines as a percentage of above-canopy precipitation (Table II.1). Cole *et al.* (1992) found that stemflow in the old growth forest at Findley Lake during the period 1 August 1986 – 1 August 1988 was 0.57% of total precipitation. Given its minor contribution to water flux at Findley Lake, stemflow was not quantified, and was assumed to equal 0.6% of precipitation.

Fog

In July 1995, fog collectors were installed at Findley Lake and at Findley Ridge. The fog collectors were constructed of 50 cm Teflon filaments tensioned over a collection plate. The filaments had a total surface area of 0.25 m^2. The fog collectors were shielded with a flat, 2.0-meter diameter aluminum disc to prevent rain from falling onto the collection plate. The collectors were suspended at the height of the upper canopy strata from a long boom mounted to a steel tower. Aerosols intercepted by the collectors were measured after each precipitation event. The quantity of water harvested by each collector was converted to water equivalents of precipitation by dividing the amount collected by the interception area.

Relationship of canopy structure to throughfall

Tree canopies are intricate structures, and can be described by measurements ranging from elementary to amazingly complex. Given that a desired product of this study was a model that would relate simple canopy measurements to on-the-ground patterns of throughfall, the tools used to quantify gross canopy structure were the same tools used by foresters and field ecologists: a diameter tape, a distance tape and a clinometer. Using these tools, the canopy structure above each throughfall collector in each of the five throughfall plots at Findley Lake and Findley Ridge was described by recording: the distance and bearing from each collector to adjacent trees and their crowns; the diameter and canopy position of each of these trees, the height, length and width of each crown that overlapped the position of a throughfall collector; and the number of overlapping crown layers. In all, more than twenty-five descriptors of canopy structure were quantified to explore their relationship to throughfall (Table II.2). Relationships between forest structure and throughfall were examined using the MGLH program for multivariate analysis

(SPSS 1996). Hypothesized relationships between throughfall and canopy structure were tested at three levels: between sites, between plots at each site, and between points in each plot.

Needle wettability

The wettability of needle cuticles was quantified by measuring the contact angle of a water droplet with the cuticle surface using methods similar to those described by Fogg (1948). Branches were collected from sunlit and well-shaded zones of the canopy from a co-dominant *Abies amabilis* and *Tsuga heterophylla* at Findley Lake, WA, and transported to the tree physiology lab for analysis. At the lab, the branches were separated into current year, one year-old, two year-old, three year-old, and four year-old segments. Ten needles per combination of species, shoot type (shade- or sun-acclimated) and age were obtained from the middle of a subsample of the shoot segments. To determine the contact angle, the needles were secured in a clip that held the needles horizontally. A 5 µl droplet of deionized water was placed with a syringe at the needle's midpoint. The silhouette of the needle was projected onto an opaque screen, and the contact angle was measured with a protractor.

Results

Canopy wetness

Canopy wetness was monitored for a total of 94.5 days between 7 July 1995 and 30 October 1995 (Figure II.1). Forty wetting events were recorded in the upper stratum of the Findley Lake canopy during the monitoring period. Over this same time interval, 30 wetting events were detected in the lower potion of the canopy. Canopy-wetting events during the 1995 growing season were concentrated in July and October. The greater frequency of wetting events in the upper canopy was most likely due to sparse rains or to fog, since dew was never observed on the canopy at Findley Lake, even after clear, still nights.

While the frequency of wetting events was higher in the upper canopy, the duration of wetness was greater in the lower canopy. The mean duration of a wetting event in the upper Findley Lake canopy was 16.4 hours. In the lower Findley Lake canopy, the mean duration of wetness was 24.3 hours. During the measurement period, the upper canopy at Findley Lake was wet during 28.9% of all hours. The lower canopy was wet 32.1% of all hours over the same time interval. There was no difference between the frequency of canopy wetting events at Findley Lake and at Findley Ridge (Figure II.1). However, the duration of canopy wetness was greater at Findley Ridge. The upper canopy at Findley Ridge was wet during 32.8% of all hours, while the lower canopy at Findley Ridge was wet during 34.4% of all hours.

Rain, fog and throughfall

The 1994 growing season was drier than normal, with 199.8 mm of precipitation, compared to a 42-year mean for the same period of 391 mm at Snoqualmie Pass (Western Regional Climate Center 2002). The growing season of 1995 was wetter, with 375.5 mm of above-canopy precipitation (Figure II.2). Canopy throughfall during the 1994 and 1995 growing seasons was less than above-canopy precipitation (stemflow was assumed to equal 0.6% of precipitation, and is reported here as throughfall). 1994 and 1995 throughfall at Findley Lake (137.2 ± 2.7 mm and 270.7 ± 5.7 mm) was 31.3 and 27.9 percent less, respectively, than above-canopy precipitation (Figure II.2). Throughfall at the midslope throughfall plots (170.7 ± 2.5 mm and 343.0 ± 10.1 mm) was 14.5 and 8.6 percent less than precipitation, while 1994 and 1995 throughfall at Findley Ridge (166.2 ± 8.9 mm and 338.0 ± 8.6 mm) was 16.8 and 10.0 percent less than precipitation. Throughfall at Findley Lake in 1994 and 1995 was significantly lower than throughfall at either of the two upper sites, while interception losses at Findley Lake were greater ($p<0.01$, Bonferroni's multiple comparison test). Throughfall and interception losses were not significantly different between the midslope and Findley Ridge throughfall plots.

In 1995, tower-mounted fog collectors were installed at Findley Ridge and at Findley Lake. Fog precipitation data were collected for six events, until the Findley Ridge fog collector was destroyed by a strong east wind in mid-September 1995. Between 8 August 1995 to 6 September 1995, the quantity of fog intercepted by the tower-mounted collector at Findley Ridge was three to four times greater than the fog collector at Findley Lake, varying from 0.06 to 3.46 mm water equivalents per event (Figure II.3). Over the same period, fog precipitation at Findley Lake varied between 0.04 mm to 0.92 mm water equivalents per event.

Qualitatively, when fog was present in the Findley Lake Basin, Findley Ridge and the midslope throughfall plots were immersed in cloud, while Findley Lake was typically clear. The data from the two fog collectors data support this observation. Between 8 August and 6 September, a total of 12.9 mm water equivalents of fog precipitation were collected at Findley Ridge. During the same period, 3.4 mm water equivalents of fog precipitation were collected at Findley Lake, while cumulative above-canopy precipitation at both sites was 121.3 mm

Given that the quantity of water droplets intercepted by the Findley Ridge fog collector during the period 8 August – 6 September 1995 was ten percent of above-canopy precipitation, it was expected that the amount of intercepted fog would be a significantly related to the amount of throughfall. At first analysis, this hypothesis appeared to be false. Irrespective of the quantity of water aerosols intercepted by the fog collectors, precipitation from rain was a robust, stand-alone predictor of throughfall. For all precipitation events during the 1994 and 1995 growing seasons in the Findley Lake Basin, the natural log of canopy throughfall was a linear function of the natural log of precipitation (Figure II.4). At Findley Lake site, throughfall was predicted by: ln(throughfall, mm) = -1.54 + 1.34[ln(precipitation, mm)], r^2=0.98, F-ratio = 992.6, $p<0.01$. Throughfall at the midslope site was predicted by: ln(throughfall, mm) = -0.51 + 1.13[ln(precipitation, mm)], r^2=0.98, F-ratio = 1422.0, $p<0.01$. At Findley Ridge, throughfall was predicted by: ln(throughfall, mm) = -0.74 + 1.17[ln(precipitation, mm)], r^2 = 0.98, F-ratio = 1423.1, $p<0.01$. For the six 1995 precipitation events for which data on fog precipitation were available, the quantity of fog intercepted by the fog collectors was not related to the *amount* of throughfall arriving at the forest floor ($p>0.50$). Thus, to predict throughfall at the three sites, it was not important to know how much moisture was contributed by fog.

However, a closer examination of the data revealed a more complex relationship between above-canopy precipitation, throughfall and fog in the Findley Basin. Several precipitation events featured quantities of throughfall that were greater than above-canopy precipitation (Figure II.5). On 6 July 1995, throughfall was 7.1% greater than precipitation at the midslope plots. Similarly, on 8 August 1995 and 6 September 1995, throughfall was 4.0% and 23.8% greater than above-canopy precipitation at the midslope plots, and 12.7% and 24.0% greater than above-canopy precipitation at Findley Ridge. Throughfall was never greater than precipitation at Findley Lake during the study period.

For the six events for which data for precipitation, throughfall, and water aerosols intercepted by the fog collectors are available, the *percentage difference* between throughfall and precipitation was significantly related to the quantity of water droplets intercepted by the fog collectors (Figure II.6). The percentage difference between throughfall and precipitation was predicted by the regression:

$$100\left(\frac{throughfall - precipitation}{throughfall}\right) = -33.95 + 12.37(mm\ fog\ water\ equivalents);\ r^2 = 0.62,$$

F-ratio = 30.0, $p<0.01$. Thus, while precipitation alone was an excellent predictor of the total amount of throughfall at each of the three throughfall measurement sites in the Findley Basin (Figure II.4), nested within this trend were events when throughfall was apparently enhanced with respect to above-canopy precipitation. These events appear to be related to the harvest of fog water by the forest canopy.

Forest structure and throughfall

Within the plots where throughfall was measured, some points on the forest floor were moisture-rich, while other areas were consistently dry. This was most true on Findley Ridge, where the point-to-point quantity of throughfall varied by a factor more than 3.4 (Figure II.7). During the 1994 and 1995 growing seasons, throughfall was greater than precipitation for nearly one-third of the collectors on Findley Ridge. Throughfall was also patchy at the midslope plots, varying by a factor of 2.2. Throughfall was greater than precipitation for one quarter of the collectors at the midslope collection site. Throughfall at the Findley Lake plots was less spatially variable, differing by a factor of 1.5. Throughfall was less than precipitation for all of the fifty throughfall collectors at Findley Lake (Figure II.7).

The variability of throughfall in the Findley Lake Basin was assessed at three levels: between sites, between plots at each site, and between points in each plot. The first level of analysis, between-site variation in throughfall, integrated some of the large-scale variability in the Findley Lake Basin, including elevation, landform, slope, aspect, soil depth and forest type. The categorical variable site accounted for 78% of the variability in throughfall (F-ratio = 22.0, $p<0.01$).

In most forests, water retention and evaporation have been consistently found to be the most important determinants of the quantity of precipitation penetrating the canopy to arrive at the forest floor (McNaughton and Jarvis 1983; Calder 1990; Gash *et al.* 1995). These two processes are strongly influenced by the total surface area of the forest canopy. Consistent with results by other investigators, the between-plot variability in throughfall was related to estimates of stand leaf area index in the Findley Lake Basin (Figure II.8). Throughfall was related to leaf area by the regression: throughfall = 630.6 – 23.8(estimated leaf area m^2m^{-2}); $r^2 = 0.56$, F-ratio = 18.8, $p < 0.01$. Thus, the estimated canopy leaf area of each plot accounted for more than half of the variability in throughfall at the fifteen throughfall plots in the Findley Lake Basin.

The analysis of within-plot patterns of throughfall focused on the two sites with the greatest contrasts: Findley Ridge and Findley Lake. More than twenty-five descriptors relating to canopy structure were tested for relatedness to the point-to-point variability of throughfall on the forest floor (Table II.2). The results were unique for each of the two sites.

At Findley Ridge, throughfall was predicted by the multivariate expression: $TF = 645.6 - 59.02(THS) + 0.88(\ln(TWC)*BC)$; $r^2 = 0.35$, F-ratio = 12.3, $p<0.01$. *TF* is the predicted cumulative throughfall for a given throughfall collector in mm for the 1994 and 1995 growing seasons. *THS* is the tangent of the angle from a given throughfall collector to the top of the most dominant "shielding" tree. "Shielding" trees were located upwind (generally to the west) of throughfall collectors, and were too far away for drip from their crowns to fall into the collector under consideration. *TWC* is the tangent of the angle from a given throughfall collector to the crown perimeter of the most dominant "collecting" tree nearest to the throughfall collector. "Collecting" trees were trees exposed to incoming streams of windblown water droplets, and were near enough for drip from their crowns to fall into the throughfall collector under consideration. Finally, *BC* is the bearing from a given throughfall collector to the nearest "collecting" tree. One collector (R201) was sheltered by a leaning bole and did not follow the trend of these data. For this reason, R201 was excluded from the analysis and n=49.

Throughfall at the Findley Lake forest floor was predicted by the multivariate expression: $TF = 355.12 + 6.42N - 48.61BA - 16.92A$ ($r^2 = 0.39$, $p<0.01$). N is the cumulative sum of the distances (in meters) from the collector to the four nearest stems, and is thus an inverse measure of the stem density in the immediate region of the collector. BA is the cumulative basal area (m^2) of stems more than 2 meters but less than 6 meters from the collector of interest. Since basal area is a good predictor of leaf area, BA is an index of the canopy leaf area above a given throughfall collector. Finally, A is a 0,1 categorical variable that describes the location of a throughfall collector relative to the nearest dominant or codominant stem. For collectors on the north or east sides of the nearest large tree, $A = 0$, while $A = 1$ for collectors on the south and west sides of adjacent large crowns.

Needle wettability

Needle age accounted for 74% of the variability in water droplet contact angles (Table II.3). The current year needles of both *Abies amabilis* and *Tsuga heterophylla* were barely wettable, which was reflected in the large contact angles of water droplets placed on their cuticles (Figure II.9). However, much of the water repellency of the newly formed needles was lost by the time the needles had aged one year. Thereafter, water droplet contact angles continued to decrease incrementally with age on the surfaces of *Tsuga heterophylla* needles. Two-year and older *Abies amabilis* needles did not show a trend of increasing wettability with age.

The effects of shoot type and species on needle wettability were minor in comparison to the influence of needle age. Shoot type explained 14% of the variability in contact angles. For both species and at all ages, sun-acclimated needles were less wettable than shade adapted needles. Species accounted for 9% of the variability in the contact angle data. Current year *Abies amabilis* needles were less wettable than current year *Tsuga heterophylla* needles. Thereafter, all other classes of *Abies amabilis* needles were slightly more wettable than *Tsuga heterophylla* needles of similar age and shoot type.

Discussion

Canopy wetness

The greater duration of wetness in the lower canopy is attributable to a combination of: 1) slightly lower vapor pressure deficit, 2) lower atmospheric turbulence, and 3) substantially lower net radiation in the interior canopy microenvironment. The greater duration of canopy wetness at Findley Ridge was likely due to: 1) the greater frequency and intensity of fog at Findley Ridge; and 2) slightly lower temperatures at Findley Ridge as a result of adiabatic cooling. The smaller difference in the duration of wetness between the upper and lower levels of the canopy at Findley Ridge may have been related to the shorter stature of the ridge-top stand. The average canopy height at Findley Ridge was 25 m (versus 42 m at Findley Lake; see Table I.2), thereby establishing a lesser microenvironmental gradient between the upper and lower canopies.

The forest canopy in the Findley Lake Basin was wet 29-34% of all hours during the growing season of 1995, a year with near-normal precipitation. Wolfe (1988) reported that the forest canopy in the Thompson Forest Research Center in the lower Cedar River Watershed (elevation = 220 meters) was wet during 21% of the growing season, and 39% of all hours during the winter. The difference between the two canopy wetness estimates is not large and is close to what would be expected, given the more than 950 meter difference in elevation between Thompson Forest and Findley Lake. The films of water that often coat the needles in the canopy may have important implications for the function of the forest ecosystem in the Findley Lake Basin.

Exchange is one of the potential consequences of needle wetness. Ca^{2+}, Cl^-, H^+, K^+, Mg^{2+}, NH_4^+, NO_3^-, SO_4^-, as well as organic ions can be exchanged by diffusion between needles and surface solutions in accordance with concentration gradients (Kylin 1960; Reiners and Olson 1984; Schaefer *et al.* 1988; Bowden *et al.* 1989; Eilers *et al.* 1992; Hansen and Garten 1992; Lindberg and Lovett 1992; Lovett and Lindberg 1993; Macklon and Armstrong 1994; Boyce *et al.* 1996; Macklon *et al.* 1996). The quantity of nutrients exchanged in this way is not inconsiderable: approximately 13% of the annual foliar nitrogen requirement of the *Pseudotsuga menziesii* forest at the Thompson Research Center is acquired in the canopy (Johnson and Lindberg 1992). Exchange can also be detrimental. Frequent foliar wetting is a predisposing factor to leaching-induced nutrient imbalances from acid rain. However, sulfate and nitrate concentrations are lower in northwest precipitation than elsewhere in the United States (Johnson and Lindberg 1992). Water uptake is another type of exchange that can occur when the forest canopy is wet. Despite its potential importance, rates of water uptake have not been well quantified for whole leaves and needles (Kersteins 1996; Reiderer and Schreiber 2001). This topic will be explored in subsequent chapters of this dissertation.

Foliar wetting should have depressed the transpiration rates of the forest canopies in the Findley Lake Basin. The general expression for transpiration is (Nobel 1999):

$$J_j = g_j \Delta c_j \qquad 2.1$$

where J_j is the transpirational flux through layer j, g_j is the conductance of layer j, and Δc_j is the concentration gradient of water on either side of layer j. When a leaf is coated with water, Δc_j is zero, reducing J_j to zero. For this reason, wet forest canopies are considered to be decoupled from the atmosphere, with near zero rates of transpiration (McNaughton and Jarvis 1983). Seasonal estimates of canopy transpiration will be overestimated if wetness is not taken into account. This is particularly true for environments characterized by frequent light rains, fog and other events that moisten the forest canopy.

Throughfall and interception losses

While the rate of transpiration is near zero when the canopy is wet, the concurrent rate of evaporation from a wet canopy can be suprisingly high. The evaporation of water from a wet shoot is controlled by the conductance of the shoot boundary layer (g_{aS}), which in turn is a function of leaf size and wind speed (Nobel 1999). For fine needled conifers such as *Abies* and *Tsuga*, and at moderate wind speeds, g_{aS} is at least an order of magnitude greater than stomatal conductance, g_s (Table 2.3). While it is commonly assumed that the atmosphere is saturated during precipitation events, a modest saturation deficit is often maintained by advection (McNaughton and Jarvis 1983). The high value of g_{aS} relative to g_s at least partially compensates for the small Δc during precipitation events. Typical rates of evaporation from wet canopies proceed at roughly two thirds the value of typical rates of transpiration (McNaughton and Jarvis 1983). For these reasons, canopy evaporation is an important driver of forest hydrology, accounting for 35 to 75% of annual water losses from forests (McNaughton and Jarvis 1983).

Canopy evaporation is the largest part of the explanation as to why throughfall was less than precipitation in the 1994 and 1995 growing seasons in the Findley Lake Basin (Figure II.2). The estimated interception loss at Findley Lake was comparable to losses reported from other mature coniferous forests (Table II.1). The study by Cole *et al.* (1992), also performed at Findley Lake, is an exception.

The figure by Cole *et al.* (1992) was a full-year estimate that includes precipitation from snow. Canopy-held snow is less susceptible to interception loss than rain: approximately 13-27% of the snow intercepted by forest canopies in the western United States is lost due to sublimation or evaporation, as compared to 30-49% of intercepted rain (Zinke 1967). Findley Lake is set in a dark, north-facing basin that receives little radiation during the winter months, and the region receives more than eleven meters of snow year^{-1}—80% of the Basin's annual precipitation (Western Regional Climate Center 2002). The full year estimate for canopy interception at Findley Lake by Cole *et al.* 1992 (15.5%, versus a mean of 29.6% for the 1994 and 1995 growing seasons) probably reflects the disproportionate contribution of snow to total precipitation in the Findley Lake Basin.

Given the age of the forest and other figures for interception losses in mature forests (Table II.1), the interception losses at the midslope and Findley Ridge throughfall collection plots seem unusually low. The estimated leaf area at the midslope and ridge sites was lower than at Findley Lake (Table I.2). When, as is often the case for coniferous forests, canopy aerodynamic conductance (g_{aM}) is high (Table II.4), transpiration and evaporation are directly related to canopy leaf area (McNaughton and Jarvis 1983). Throughfall was indeed related to the estimated leaf area index at the three throughfall collection sites (Figure II.8), but the relationship was not the one-to-one correspondence that would have been anticipated. Instead, while the estimated LAI of the upper sites was 65 – 70% of Findley Lake, presumed canopy evaporation was half of the amount at Findley Lake in 1994, and one third in 1995.

Apparent evaporation was surprisingly low at the midslope and ridge sites, compared with the site on the basin floor. Since the throughfall estimates are robust, one possible source of error would be if the canopy received water not included in the estimates of above-canopy precipitation. Fog could have supplied this extra source of water.

Fog was an enigmatic factor in the Findley Lake Basin. Precipitation explained most of the variability in throughfall (Figure II.4), and the quantity of water aerosols intercepted by the fog collectors at Findley Lake and at Findley Ridge did not have a significant relationship with the amount of throughfall arriving at the forest floor (p>0.50). The mean water content and droplet size of fogs in the basin may not have been sufficient to initiate intense, canopy-drenching fog events (Merriam 1973). However, the fog collector at Findley Ridge intercepted more water aerosols than did the collector at Findley Lake (Figure II.3). Furthermore, for six precipitation events the percent interception loss was significantly related to the quantity of water aerosols intercepted by the fog collectors (Figure II.6).

Instead of contributing directly to throughfall through drip, the effects of fog on throughfall in the Findley Lake Basin may have been indirect. Above-canopy precipitation is routed to the forest floor once the capacitance of the canopy is saturated (Gash *et al.* 1995). By saturating canopy capacitance, fog sedimentation may have improved the efficiency of precipitation. Additionally, regardless of its intensity, when fog forms the atmosphere is saturated. The presence of fog may have been symptomatic of very low rates of evaporation in the Findley Lake Basin, particularly at the two upper sites where fog was frequently observed.

If the ridge and midslope did not receive extra inputs of water from fog, then the observation that apparent evaporation was lower at the two upper sites than at the basin floor must have been correct. Evaporation from forests is related to the water storage capacity of the forest canopy. In turn, the water storage capacity of the canopy is linked to its leaf area (McNaughton and Jarvis 1983; Gash *et al.* 1995). At least three explanations could account for the observed differences in evaporation rates.

The first is that the rate of evaporation per unit of leaf area may have been similar at the three sites, but the leaf areas at the midslope and ridge were over-estimated. The estimated leaf area of the stands on the midslope and summit of Findley Ridge was obtained from the relationship between stem diameter and crown leaf area for *Abies amabilis* at Findley Lake. Given the large decline in site quality along the gradient from Findley Lake to Findley Ridge

(Table I.2), the allometric relationship between diameter and leaf area may have changed, resulting in an incorrect estimate for the canopy leaf areas at the two upper sites. If the leaf areas of the two upper sites were overestimated, then these errors could account for a portion of the differences in apparent canopy evaporation in the Findley Lake Basin. However, error in estimates of leaf area cannot explain the year-to-year differences in apparent evaporation in 1994 and 1995.

If the leaf area estimates for the three sites were accurate, then the second explanation to account for the differences in apparent evaporation would be that evaporation per unit of leaf area was lower at the ridge and midslope sites than at the basin floor. Since evaporation is equal to conductance times the water vapor pressure gradient, differences in either could have reduced evaporation from the midslope and ridge relative to the basin floor.

With respect to conductance, the coupling coefficient Ω describes the efficiency of gaseous transfer between a plant canopy and the atmosphere (McNaughton and Jarvis 1983). For a transpiring forest, Ω is proportional to the ratio g_a/g_{canopy}, where g_{canopy} is the sum of canopy stomatal conductance (Jarvis and McNaughton 1986). For a wet forest, g_{canopy} is the sum of canopy shoot boundary layer conductance, g_{aS}, and Ω becomes proportional to the ratio g_{aM}/g_{aS}. When $\Omega \approx 0$, the canopy is well mixed with the bulk atmosphere. At values of Ω close to 1.0, the transfer of water vapor from the canopy to the atmosphere is impeded, and layer of air entrained by the canopy can become more humid than the atmosphere aloft. Consequently, the rate of evaporation is slower than would otherwise be expected (McNaughton and Jarvis 1983).

Martin *et al.* (1999) examined the boundary layer conductance of *Abies amabilis* branches and found that they are generally well coupled with the atmosphere, with values of the coupling coefficient Ω typically less than 0.1. In a subsequent study conducted at Findley Lake, Martin *et al.* (2001) reported that the Ω of a large *A. amabilis* exceeded 0.3 for 9% of daylight hours during the 1994 growing season. The relatively high values of Ω observed by Martin *et al.* (2001) at Findley Lake were induced by the combination of low wind speed and high radiation.

Conditions in the Findley Lake Basin forest canopy during precipitation events were quite different to those that led to higher values of Ω in the study by Martin *et al.* (2001). The average radiation during 1995 precipitation events was 23 Wm^{-2} (for comparison, the radiation from full sunlight is generally > 800 Wm^{-2}), while the average wind speed during rain was 6.1 m s^{-1} (T.A. Martin, unpublished). As discussed, the transpiration rate of wet leaves is assumed to be close to zero and, therefore, there is no positive feedback to consider between g_s and radiation. If g_{aM} is $\approx$ 0.335 x w, where w is the wind speed in m s^{-1} (Martin *et al.* 2001), the mean value of g_{aM} during these events was 2.0 m s^{-1}. This is considerably higher than typical values for a coniferous forest (Table II.4), but turbulent winds usually accompany rains in the Findley Lake Basin. The boundary layer conductance for *Abies amabilis* shoots is approximated by the regression: $g_{aS} = 0.005 + 0.0744 * w * \text{LAI}$ (Martin *et al.* 1999). The assumption will be made that wind velocity attenuates by half at the midpoint of the forest canopy. Finally, Ω is estimated by (modified from Jarvis and McNaughton 1986):

$$\Omega = \frac{s/\gamma + 2}{s/\gamma + 2 + (g_{aM}/g_{aS})} \qquad 2.2$$

where s is the slope of the saturation vapor pressure versus temperature curve at air temperature (kPa K^{-1}), and γ is the psychrometric constant (kPa K^{-1}). The resulting estimates of Ω for wet canopies at Findley Lake, the midslope of Findley Ridge, and Findley Ridge are 0.80, 0.74, and 0.73, respectively.

The above estimates of Ω are approximations. Nonetheless, the values of Ω are surprisingly near 1.0, and suggest that wet canopies in the Findley Lake Basin were at least partially decoupled from the bulk atmosphere. Even when the estimate of g_{aM} is doubled to 1.34 m s^{-1}, the canopy is still relatively uncoupled (Ω near 0.6). While the assumption $g_{aM} >> g_{aS}$ does not seem appropriate, g_{aM} was certainly large enough that the rate of evaporation from the wet canopies was high. Given the similarity of the three estimates of Ω, differences in canopy ventilation cannot explain the discrepancy between the interception losses at Findley Lake and the two upper throughfall collection sites. If anything, the estimates of Ω would indicate that the rate of evaporation should be greater from the canopies at the midslope and summit of Findley Ridge than at Findley Lake.

Since differences in conductance were unlikely to have been responsible for the apparent differences in evaporation between Findley Lake and the midslope and ridge sites, evaporation may have been affected by elevation-related changes in the water vapor pressure gradient. The rate of water evaporation varies with elevation. The decrease in atmospheric pressure with elevation affects two important factors related to evaporation: the saturation water vapor content of the atmosphere, and temperature (Nobel 1999). The saturation mole fraction of water vapor in air (N_{wv}^{*}) decreases with elevation. Since air becomes drier with altitude, the driving force for evaporation ΔN_{wv} increases (Nobel 1999). At the same time, lower atmospheric pressure allows rising air to expand and cool. Cooling diminishes the water-holding capacity of air, bringing air parcels closer to saturation and decreasing ΔN_{wv}. Cooling is the more important of the two elevation-related influences on ΔN_{wv} (Nobel 1999).

Elevation becomes a critical determinant of evaporation when the atmosphere is near saturation. For example, during a typical rain-free day in the Findley Lake Basin, temperature and relative humidity at the lower Findley landing might be 20°C and 40%, respectively. Under these conditions, elevation-related effects should reduce the rate of evaporation at the midslope and Findley Ridge throughfall plots by two and five percent, respectively, compared to the expected rate of evaporation at Findley Lake. However, during the low temperature and high humidity characteristic of precipitation events in the Findley Lake Basin, the 190-meter difference in elevation between Findley Lake and Findley Ridge becomes significant. When the relative humidity is 96.5 percent and temperature 6.1 °C (means for 1995 precipitation events at the lower Findley landing; T. Martin unpublished data), the anticipated rate of evaporation at the midslope of Findley Ridge would be 68 percent less than evaporation at Findley Lake, while Findley Ridge should be immersed in saturated air (Table II.5). Elevation is a tentative explanation for the observed differences in interception losses in the Findley Lake Basin.

The third and final possibility to consider is that the canopy may have absorbed some of the precipitation. The ten to fifty percent interception loss of rain in forest canopies (Zinke 1967) is usually attributed to evaporation (McNaughton and Jarvis 1983). This assumption is probably sufficient from a hydrological perspective. However, the possibility that canopy processes other than evaporation may consume precipitation has been left largely untested. Stone (1957a; 1957b), Katz *et al.* (1987); Boucher *et al.* (1995) and others have proposed that the shoots of coniferous trees can directly absorb water. Direct water uptake by wet shoots has not been quantified at the level of a tree canopy. If water was absorbed in the canopy, and the canopy at the basin floor absorbed more, then canopy uptake could account for at least a portion of the apparent differences in evaporation from the three throughfall measurement sites. Canopy uptake will be explored in subsequent chapters of this dissertation.

Forest structure and throughfall

The quantity and pattern of throughfall was variable during the summer growing season in the Findley Lake Basin. Throughfall varied at different scales on the landscape: between sites, between plots at a given site, and between points within plots. The large-scale variability of throughfall is pertinent to investigations of the hydrology of the Findley Basin. At a smaller scale of resolution, the distribution of throughfall is relevant to biological questions such as seedling recruitment, understory organization and the pattern of additions of airborne pollutants.

At the largest scale, the categorical variable "site" accounted for 78% of the between-site variability in throughfall (Figure II.7). Among the sites where throughfall was sampled, physical (temperature, humidity, radiation, wind speed and wind direction) and biological (soil type and depth, species composition, stand structure) parameters varied in concert with the primary factors of elevation, landform and aspect. As discussed, elevation appeared to be a determinant of throughfall variability, but little can be concluded regarding the importance of landform and aspect. The study design was not adequate to assess these larger-scale variables.

A single variable, leaf area, explained more than half of the between-plot variability in throughfall. The relationship between throughfall and canopy leaf area in the Findley Lake Basin was linear (Figure II.8), as hypothesized by McNaughton and Jarvis (1983). Again however, the study was not explicitly designed to test the influence of this variable. To compare throughfall between plots within a given site, the centers of the throughfall plots were located in regions with similar basal area. Since the basal area at each plot center was similar, between-plot variation in leaf area within a site was accidental. A better test of the effect of leaf area on throughfall would be to hold site constant, while varying stand basal area (see study by Teklehaimanot *et al.* 1991). In this study, the effects of site and leaf area are not clearly separable, because leaf area was related to site ($r^2 = 0.49$, F-ratio = 5.6, $p < 0.05$).

The point-to-point variability of throughfall within a site was assessed only at Findley Ridge and at Findley Lake. The relationships between throughfall and forest structure were unique for each of these two sites. At both sites, the relationships between structural features of the overhead canopy and throughfall to the forest floor were suprisingly weak. Findley Ridge provides clues as to why this was so.

Gusty winds accompanied most of the precipitation events in the Findley Lake Basin. This was particularly true on Findley Ridge, where strong, mist-charged winds whipped water onto the canopy. Due to variation in wind speed and wind direction, throughfall arriving at a discrete point on the forest floor likely had many points of origin in the canopy, from directly overhead, to many meters from the vertical.

The importance of windblown precipitation helps to interpret the variables that were related with the spatial distribution of throughfall on Findley Ridge. *THS*, the tangent of the vertical angle from a given throughfall collector to the top of the nearest "shielding" crown, partially quantifies the importance of rain shadow effects on Findley Ridge. Shielding crowns intercepted horizontally moving precipitation. The larger the value of *THS*, the larger the profile of the shielding crown and the lower the supply of precipitation. The importance of *THS* suggests that canopy gaps—in the rain shadows of surrounding trees—should be xeric during the growing season on Findley Ridge.

The horizontal transport of precipitation also helps to explain the significance of *TWC*, the tangent of the horizontal angle from a throughfall collector to the outside edges of the nearest "collecting" crown. Like shielding crowns, collecting crowns harvested wind-pushed aerosols, but were close enough for the intercepted precipitation to drip to the throughfall collector. The significance of *TWC* indicates that points under the crowns of trees that are exposed to streams of horizontally moving precipitation receive more throughfall during the growing season on Findley Ridge. This finding is corroborated by the significance of the interaction variable *BC*TWC*. *BC* was the bearing from a throughfall collector to the nearest collecting crown. Points on the western side of collecting crowns received more throughfall than points on the eastern side of collecting crowns. Since winds on Findley Ridge usually came out of the west, the interaction of *BC*TWC* described the supply of water to a collecting surface.

The interception of wind blown precipitation at Findley Ridge created a heterogeneous pattern of dry and wet zones on the forest floor. The input of throughfall exceeded precipitation at one-third of the sampled points at Findley Ridge. Overall, throughfall varied by a factor of three on Findley Ridge (Figure II.7)—a potentially significant ecological factor given the thin, well-drained soils of the ridge top environment. The capture and redistribution of wind blown precipitation may be one of the positive feedback processes that create small openings in this transitional subalpine forest.

Interestingly, patterns of throughfall deposition described by Weathers *et al.* (1995) resemble patterns found on Findley Ridge. Weathers *et al.* (1995) studied a subalpine forest in the Catskill Mountains of New York. As observed on Findley Ridge, throughfall was augmented by a factor of three at the wettest versus the driest sites in the Catskill forest. The pattern of throughfall was highly variable for separate precipitation events, and small differences in crown size or crown exposure resulted in large differences in throughfall. Weathers *et al.* (1995) found that the sedimentation rates of lead, sodium and other ions at the forest edge tracked the threefold enhancement observed for throughfall. Some sites had ion concentrations as much as fifteen times greater than amounts found under less-exposed canopies. Thus, the patchy distribution of throughfall on Findley Ridge may also correspond to aestival hotspots of nutrient and heavy metal influxes.

The structural variables associated with throughfall patterns on Findley Ridge were related to the interception of horizontally moving rain. In comparison, the variables linked to throughfall at Findley Lake more strongly described the sequestration of precipitation by the canopy. Findley Lake is less exposed than Findley Ridge; it has taller trees, fewer gaps and many more leaf surfaces per unit area. For these reasons, the canopy at Findley Lake had a higher throughfall capacitance, and those canopy attributes related to the retention of precipitation had the strongest influence on throughfall.

The three structural variables significantly related to throughfall at Findley Lake were *N*, *BA* and *A*. *N* was inversely related to the stem density in the region of a throughfall collector. Throughfall was positively related to the value of *N*, suggesting that the area of precipitation-trapping canopy surfaces varies with stem density. *BA* was the sum of the basal areas of all stems more than two meters but less than six meters from a given throughfall collector. Like *N*, *BA* was negatively related to the quantity of throughfall at a given point. Leaf area is functionally related to basal area, so again, *BA* indicates the function of canopy surfaces as substrates that consume precipitation at Findley Lake. The third structural variable related to throughfall at Findley Lake, *A*, described the position of a given throughfall collector relative to the crown of the nearest co-dominant or dominant tree. Unlike Findley Ridge, where the prevailing wind came from the west, at Findley Lake winds that accompanied precipitation typically ascended the drainage and arrived from the north. The yield of throughfall tended to be greater under the north (windward) side of trees at Findley Lake, and less under the south side.

The spatial distribution of throughfall under the Findley Lake canopy was more homogenous than on Findley Ridge (Figure II.7). Findley Lake is sited in a more sheltered location than the exposed ridge; it likely had lower mean wind velocities during precipitation events (wind speed was not monitored at Findley Ridge). Thus the angle of incipient precipitation at Findley Lake was probably more vertical than at Findley Ridge, and the distribution of precipitation onto the Findley Lake canopy was more uniform. The larger amount of leaf area at Findley Lake implied that the canopy had greater throughfall capacitance, and a larger surface area for evaporation. At Findley Ridge, the predominant function of the canopy was the capture and spatial redistribution of throughfall; relatively little evaporation occurred. At Findley Lake, the most important process was the evaporation of intercepted precipitation from the canopy.

The empirical approach used in this study to examine the spatial patterns of throughfall is limited. Less than a third of the spatial variability in throughfall was accounted for at either Findley Ridge or Findley Lake, and the statistical models that described the pattern of throughfall are highly specific in place and time. A more precise empirical model would require the stratification of the forest floor into different throughfall environments. Each environment would then be sampled separately, with the number of data points dependent on the variability of throughfall for each stratum. This was the approach used by Ford and Deans (1978) to study throughfall in a young *Picea sitchensis* plantation, and was one of the approaches tested and abandoned in this study. The stratification of an old growth forest floor into throughfall environments, where the canopy is structured of irregularly spaced crowns of different species, size and shape is a more complex task than assessing throughfall in a plantation setting. Furthermore, this approach assumes that throughfall environments can be related to structural features of the canopy. This becomes problematic when the source areas in the canopy for throughfall at a particular point on the forest floor are dynamic, and may be several to many meters from the vertical.

The analysis of the spatial pattern of throughfall would require a spatially explicit mechanistic model. Such a model would depict the canopy as three-dimensional surface, and would include meteorological variables such as temperature, relative humidity, wind speed and direction, droplet size, and precipitation intensity. Given that the size, direction, and speed of droplets change as they collide with canopy surfaces, coalesce, travel along stems, and then drip to the forest floor, the development of such a model would be challenging.

Needle wettability

Contact angles are a measure of the attraction between water and the surface of a cuticle. The greater the contact angle, the weaker the attraction of the leaf surface for water (Fogg 1948). Non-wettable leaves have contact angles greater than 130°, while wettable leaves have contact angles less than 100° (Holly 1976). The wettability of current-year needles of *Abies amabilis* and *Tsuga heterophylla*, with contact angles ranging between 105° and 115°, was intermediate. All needles one or more years in age were wettable (Figure II.9). The contact angles reported here for *Abies amabilis* and *Tsuga heterophylla* are comparable to those reported by Boyce *et al.* (1991) for *A. balsamea* and *Picea rubens* in the White Mountains of northern New England.

The water repellent properties of leaves are due to the nature of chemical groups exposed on the cuticle surface, and to surface roughness established by fine crystals of epicuticular wax or by leaf hairs (Fogg 1948). The large change in wettability after the needles of *Tsuga heterophylla* and *Abies amabilis* had aged one year (Figure II.9) suggests that the crystalline structure of the epicuticle was almost certainly degraded. Changes in the wettability of conifer needles are usually linked with a breakdown in the microstructure of epicuticular wax on needle surfaces (Boyce *et al.* 1991; Percy *et al.* 1992). The cause of this change is not known. Given that contact angles changed abruptly sometime between the first and second growing seasons and thereafter declined slowly or stabilized (Figure II.9), physical erosion during the winter may have been responsible. Boyce *et al.* (1991) noted a similar abrupt change in foliar wettability between the first and second growing seasons for *Abies balsamea*, and postulated that the decrease was a discrete change that occurred during the winter.

The change in the wettability of the *Abies amabilis* and *Tsuga heterophylla* needles may be interpreted to suggest that as the needles aged, their cuticle surfaces became polar. However, there is little evidence that the chemical nature of the cuticle surfaces changed with age. Crystals of epicuticular wax prop water droplets above the cuticle surface. Water does not physically contact the cuticle surface until epicuticular wax crystals degrade. Contact angles for water droplets on the needles of either species changed little after the second growing season (Figure II.9).

Given that water repellency is lost by the second growing season, its significance is not clear. The change in water repellency with age certainly did not appear to be related to needle viability. Many shade-adapted shoots of *Abies amabilis* had twelve or more age classes of needles, while the number of age classes of *Tsuga heterophylla* needles ranged from four (sun-adapted shoots) to at least seven (shade-adapted shoots).

One function may be that the water repellency of current-year needles enhances throughfall. The adherence of a water droplet to a plant leaf is a function of the contact angle: water beads and then rolls off leaves with large contact angles (Brewer *et al.* 1991). At the level of a whole tree crown, the outermost shell of mostly new, water-repellent foliage may establish an "umbrella" effect that augments throughfall under the canopy perimeter. This same effect should also decrease evaporation from the canopy, since the total surface area of wet needles will be less than if all needles were coated with a uniform film of water.

Another possibility is that the water repellency of current-year *Abies amabilis* and *Tsuga heterophylla* needles may be related to gas exchange. Water can occlude stomata and block the uptake of carbon dioxide. Ishibashi and Terashima (1995) found that photoinhibition occurred in *Phaeseolus vulgaris* plants exposed to six or more hours of simulated rain. Brewer and Smith (1994) linked the trichome density of wet *Glycine max* leaves with photosynthetic performance. They found that trichomes supported water droplets above the surfaces of wet leaves, thereby avoiding the occlusion of stomata and permitting gas exchange to continue. Smith and McClean (1989) examined the effect of foliar wetting on an array of 57 subalpine plant species. Photosynthesis was enhanced 10-34% when species with non-wettable leaves were moistened with artificial dew, while the same treatment lowered the photosynthetic rate of species with wettable leaves by 64-89%. A comparable study is not known for conifers.

The short duration of the growing season in the Findley Lake Basin, coupled with the prevalence of canopy wetness, may make it advantageous for trees to have water repellent needles. Tranquillini (1974) hypothesized that in subalpine forests, the cuticles of new needles may not have adequate time to fully develop. If water repellency does improve photosynthesis in *Abies amabilis* and *Tsuga heterophylla*, then the extra carbon gain may help to complete the construction of the cuticle before the onset of winter. Even if the completion of cuticle synthesis is not a constraint in the Findley Lake Basin, water repellency may give new needles a one-season boost in carbon production that would otherwise be forfeited. Water repellency, and consequently, the presumed enhanced rate of gas exchange may be lost after the first growing season because *Abies amabilis* and *Tsuga heterophylla* lack mechanisms to re-coat mature needles with crystalline epicuticular wax.

Finally, water repellency may be spurious in relation to a more important function of the epicuticular wax deposits on the needles of *Abies amabilis* and *Tsuga heterophylla*. Epicuticular wax has been shown to reflect ultraviolet light (Barnes *et al.* 1994), reduce leaf temperatures (Richards *et al.* 1986), and improve disease resistance (Martin and Juniper 1970). Nonetheless, when scaled to the vast surface area of the canopy of a mature forest, an unusual property of a single age class of needles can affect forest function.

Summary

The forest canopies of the Findley Lake Basin were wet approximately one third of all hours during the 1995 growing season. The canopies of the basin are not sources of precipitation, but neither are they strong sinks, except at the basin floor. The measured interception losses at the midslope and summit of Findley Ridge (9-17%) were lower than the interception losses at Findley Lake (28-31%). The distribution of throughfall on the forest floor was patchy on Findley Ridge, while the pattern was more homogenous at Findley Lake. On Findley Ridge, structural features of the canopy related to the pattern of throughfall were associated with the interception and redistribution of horizontally-moving water droplets. At Findley Lake, structural predictors of throughfall patterns were related to the storage and evaporation of intercepted precipitation. A mechanistic model linking canopy structure with patterns of throughfall delivery to the forest floor could not be synthesized with the data that were collected in this study.

Table II.1. Stemflow, throughfall, and interception losses in coniferous forests of different ages.

Study	Forest type	Age (yrs)	Precipitation (mm)	Interception loss (%)	Through-fall (%)	Stem-flow (%)
Loustau *et al.* 1994	*Pinus pinaster*			12-21	77-83	1-6
Ford and Deans 1978	*Picea sitchensis*	14	1639	30	43	27
Delfs 1967	*Picea abies*	15		11	87.8	1.2
Anderson and Pyatt 1986	*Picea abies*	25		29	58	13
Teklehaimanot *et al.* 1991	*Picea sitchensis*	27		33	50	17
Titak and Bouter 1994	*Pseudotsuga menziesii*	29	834	38		
Hudson 1988	*Picea abies*	29		25	62	13
Johnson 1990	*Picea sitchensis*	50	5791	28	69	3
Houle *et al.* 2000	*Abies balsamea/ Picea rubens*	50		18	81	1
Anderson and Pyatt 1986	*Picea abies*	63		49	50	1
Delfs 1967	*Picea abies*	80		36	63.5	0.5
Cole *et al.* 1992	*Abies amabilis/ Tsuga*	220	2730	15.5 [b]	83.9	0.6
Findley Lake (this study)	*Abies amabilis/ Tsuga*	230		28-31[a]	68-71	0.6[c]
Upper Findley Lake Basin (two sites, this study)	*Abies amabilis*	230		9-17 [a]	82-90	0.6[c]

[a] growing season estimate only
[b] full year estimate
[c] assumed rate from Cole *et al.* 1992

Table II.2. Descriptors of canopy structure tested for relationships to spatial patterns of throughfall on Findley Ridge (5 plots) and at Findley Lake (5 plots) in the Findley Lake Basin, WA. The throughfall data were from the 1994 and 1995 growing seasons.

Variable	Variable type
bearing to the nearest "scoop" tree	continuous
bearing to the nearest "shield" tree	continuous
vertical angle to the top of the nearest "scoop" tree	continuous
vertical angle to the top of the nearest "shield" tree	continuous
horizontal angle tangent to the maximum crown perimeter of the nearest "scoop" tree	continuous
horizontal angle tangent to the maximum crown perimeter of the nearest "shield" tree	continuous
number of overlapping crown layers above the throughfall collector	continuous
horizontal distance from the throughfall collector to the nearest stem	continuous
height of the tree nearest to the throughfall collector	continuous
species of the tree nearest to the throughfall collector	categorical
basal area of the tree nearest to the throughfall collector	continuous
height of the crown base of the tree nearest to the throughfall collector	continuous
live crown length of the tree nearest to the throughfall collector	continuous
mean crown width of the tree nearest to the throughfall collector	continuous
estimated crown volume of the tree nearest to the throughfall collector	continuous
aspect of the throughfall collector relative to the nearest tree	categorical
distance from the nearest crown edge ("dripline") to the throughfall collector	continuous
crown class of the tree nearest to the throughfall collector	categorical
horizontal distance from the throughfall collector to the second nearest stem	continuous
horizontal distance from the throughfall collector to the third nearest stem	continuous
sum of the distances from the throughfall collector to the first and second nearest stems	continuous
sum of the distances from the throughfall collector to the first, second and third nearest stems	continuous
cumulative basal area of all stems within 2 meters of the throughfall collector	continuous
cumulative basal area of all stems more than 2 but no more than 4 meters from the throughfall collector	continuous
cumulative basal area of all stems more than 4 but no more than 6 meters from the throughfall collector	continuous
cumulative basal area of all stems within 4 meters of the throughfall collector	continuous
cumulative basal area of all stems within 6 meters of the throughfall collector	continuous
basal area of all stems more than 2 but less than 6 meters from the throughfall collector	continuous
canopy environment over the throughfall collector	categorical

Table II.3. Analysis of variance on the effects of age, shoot type (sun- or shade acclimated) and species on the contact angle of a 5μl droplet of deionized water placed on the surface of horizontally held conifer needles. The data are from current year, one year-old, two year-old, three year-old and four year-old needles of *Abies amabilis* and *Tsuga heterophylla* collected from two codominant trees on 5 July 1996 at Findley Lake, WA. N=10 for each combination of species, age and shoot type.

Source	df	MS	F-ratio	P
age	4	11008.5	557.5	0.000
shoot type	1	2020.0	102.3	0.000
species	1	1372.7	69.5	0.000
age*species	4	260.0	13.2	0.000
shoot type*species	1	139.7	7.1	0.008
age*shoot type	4	88.7	4.5	0.002
age*shoot type*species	4	35.3	1.8	0.133
Error	177	19.7		

Table II.4. Representative estimates of stomatal, boundary layer and atmospheric conductances for moist montane forest ecosystems.

conductance	value (m s^{-1})	remarks	source
g_s	1.43×10^{-3}	typical for *Abies amabilis*	Martin *et al.* 2001
g_{aS}	2.0×10^{-2}	*A. amabilis,* low windspeeds	Martin *et al.* 1999
	8.0×10^{-2}	*A. amabilis,* typical windspeeds	Martin *et al.* 1999
	1.5×10^{-1}	*A. amabilis,* high windspeeds	Martin *et al.* 1999
g_{aM}	1.56×10^{-1}	Vancouver Island	Beaudry and Sagar 1995
	2.86×10^{-1}	United Kingdom	Calder 1990
	$0.9\text{-}2.4 \times 10^{-1}$	United Kingdom	Teklehaimanot and Jarvis 1991
	$1.0\text{-}2.0 \times 10^{-1}$	general range	McNaughton and Jarvis 1983

Table II.5. The simulated effects of elevation on the estimated fluxes of evaporation from wet forest canopies during precipitation events at three sites in the Findley Lake Basin, WA.

variable	lower Findley landing	Findley Lake	midslope of Findley Ridge	Findley Ridge
elevation (m)	1100	1170	1230	1320
temperature (°C) [a]	6.1	5.8	5.5	5.0
rH (%) [b]	96.5	98.0	99.4	100
p_{atm} (MPa) [c]	0.0888	0.0880	0.0874	0.0864
N^{*}_{wv} [d]	0.01063	0.01046	0.01032	0.01011
N^{atm}_{wv}	0.01026	0.01026	0.01026	0.01011
ΔN_{wv}	3.72×10^{-4}	2.06×10^{-4}	6.56×10^{-5}	0
E_I (m s^{-1}) [e]	8.06×10^{-4}	4.46×10^{-4}	1.42×10^{-4}	0
ΔE_I (%)			-68	-100

[a] mean climatic parameters during 1995 precipitation events at the lower Findley landing include $t = 6.1$°C; rH = 96.5%; and $w = 6.1$ m s^{-1} (T. Martin, unpublished data).

[b] mean relative humidity during 1995 precipitation events at the lower Findley landing

[c] pressure of the atmosphere = $0.1013\left[1-\left(.02255*\frac{elevation}{1000}\right)\right]^{5.256}$

[d] saturation mole fraction of water vapor = $\frac{610.78e^{\frac{17.34t}{t+238.3}}}{p_{atm}*10^{6}}$

[e] evaporative flux = $.355w\Delta N_{wv}$

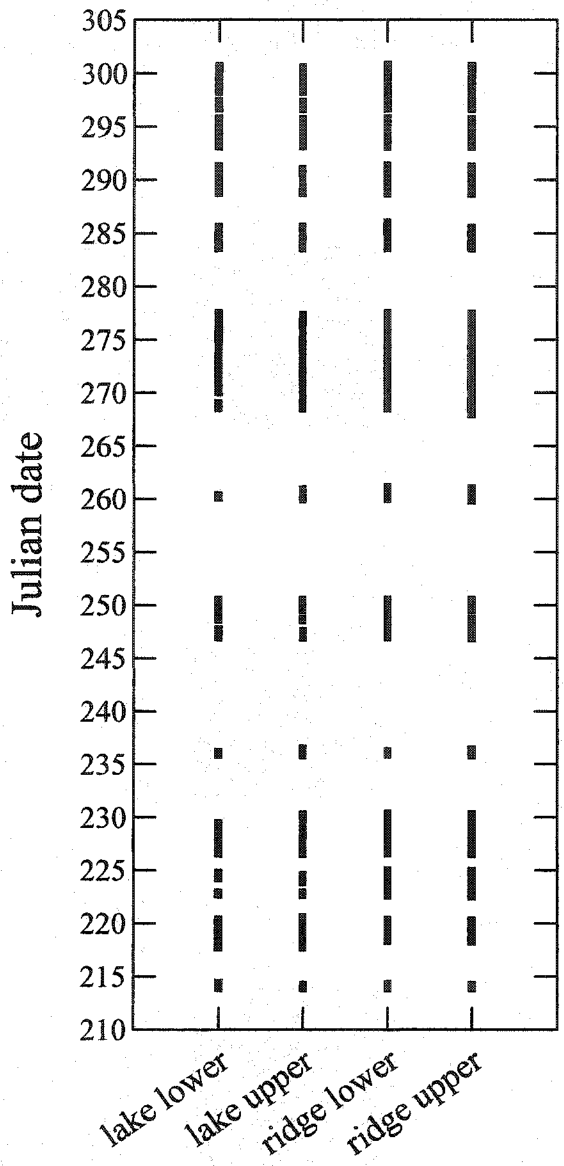


Figure II.1. Canopy wetness during the period 28 July – 31 October 1995 at Findley Lake (1170 m) and Findley Ridge (1320 m) in the Findley Lake Basin, WA. Canopy wetness was monitored by measuring the electric current passing between two gold wires wound around an artificial *Abies* needle. At Findley Lake the wetness sensors were mounted at heights of 43 m (lake upper) and 20 m (lake lower). At Findley Ridge, the sensors were installed at 26 m (ridge upper) and 6 m (ridge lower). The heights of the upper and sensors corresponded to the top and bottom of the forest canopies at the two sites.

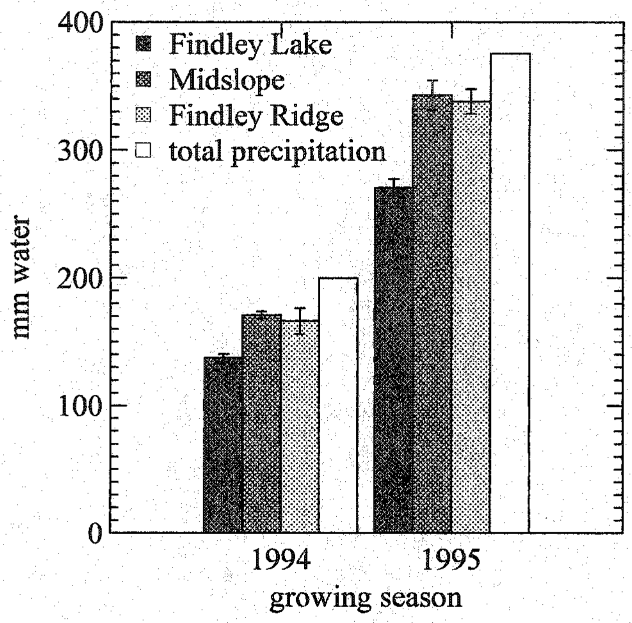


Figure II.2. Above-canopy precipitation and water inputs to the forest floor (throughfall + stemflow) during the 1994 and 1995 growing seasons at three measurement sites in the Findley Lake Basin, WA. The three measurement sites (Findley Lake, 1170 m; midslope Findley Ridge, 1230 m; Findley Ridge, 1320 m) were arranged in a west to east elevational gradient. Stemflow was assumed to be 0.6% of precipitation. The data are the means (n=5 for each site) ± SE. Three minor precipitation events in July 1994 for which data were not available for the midslope throughfall plots were excluded from this dataset.

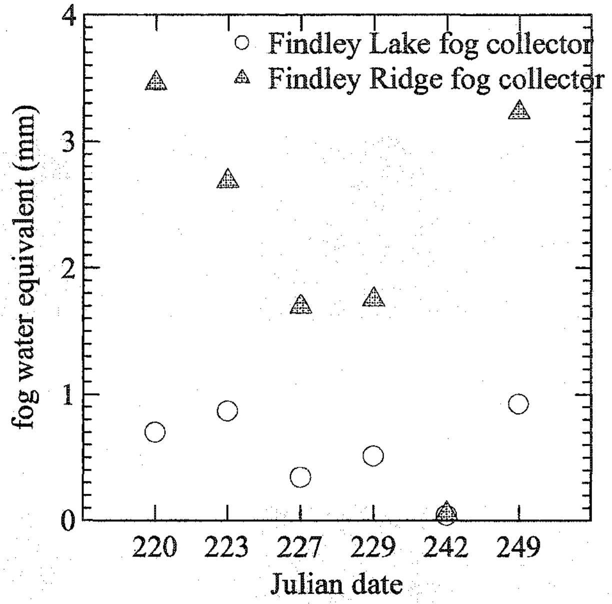


Figure II.3. Fog precipitation at Findley Lake and Findley Ridge during the period 8 August 1995 to 6 September 1995. The gross yield per event of each fog collector was converted to fog water equivalents by dividing by the total surface area of each collector (0.25 m^2).

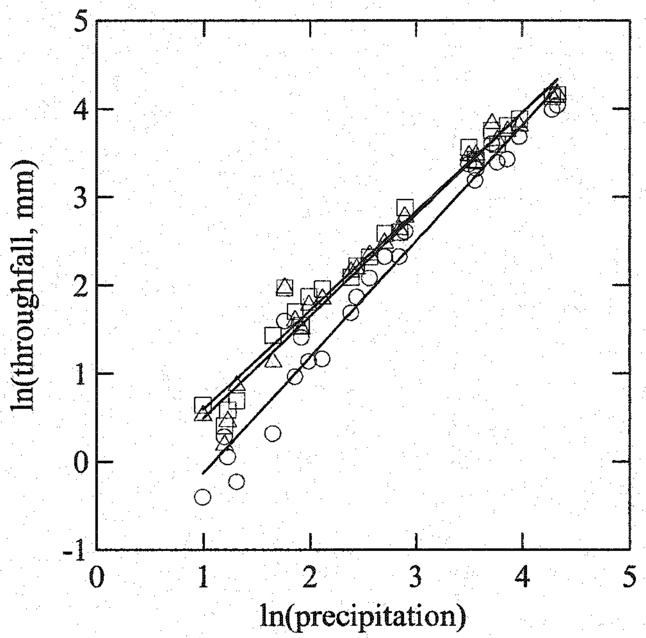


Figure II.4. The relationship between throughfall and precipitation at Findley Lake, the midslope of Findley Ridge, and Findley Ridge during the period 8 August – 6 September 1995. Circles, Findley Lake; squares, midslope of Findley Ridge; triangles, Findley Ridge. Throughfall at Findley lake is predicted by: ln(throughfall, mm) = -1.54 + 1.34[ln(precipitation, mm)], r^2=0.98, $p<0.01$. Throughfall at the midslope site was predicted by: ln(throughfall, mm) = - 0.60[ln(precipitation, mm)], r^2=0.98, $p<0.01$. For Findley Ridge, throughfall is predicted by: ln(throughfall, mm) = -0.74 + 1.17[ln(precipitation, mm)], r^2 = 0.98, $p<0.01$.

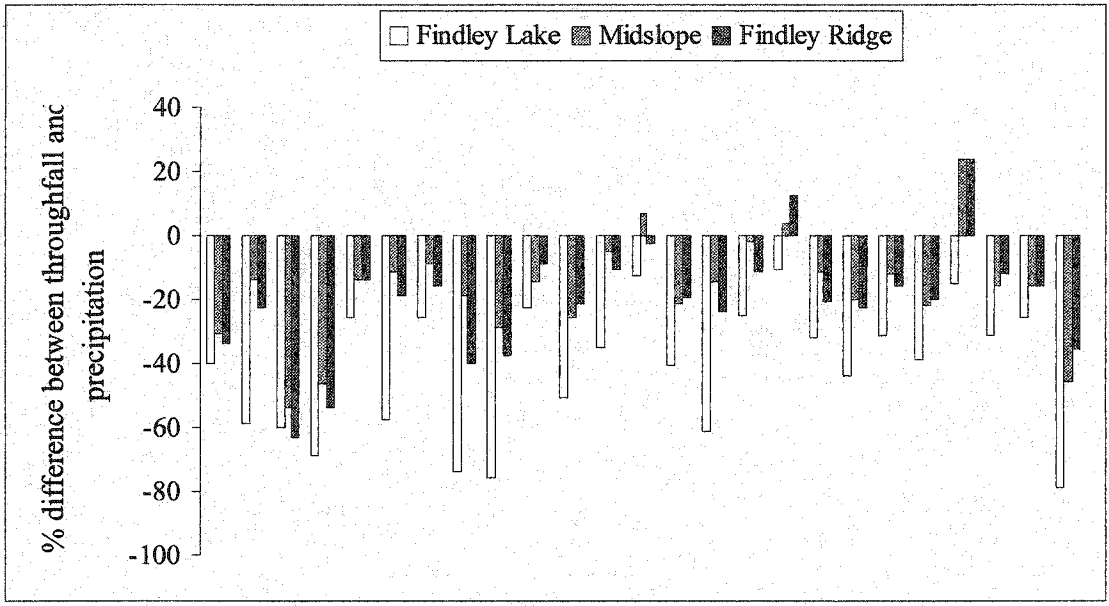


Figure II.5. The percentage difference between throughfall and precipitation at three throughfall measurement sites during the 1994 and 1995 growing seasons in the Findley Lake Basin, WA. The data are from 25 separate precipitation events.

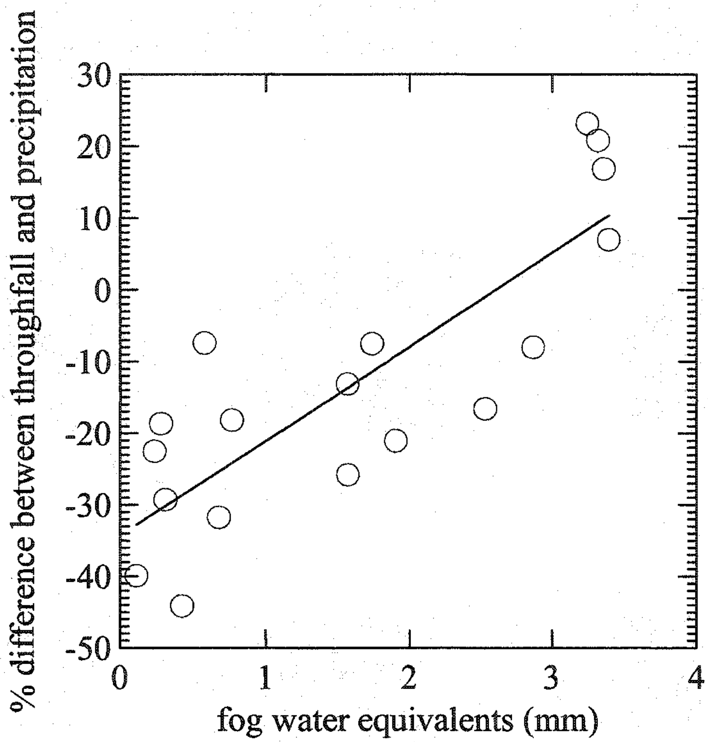


Figure II.6. The relationship between the percentage difference between throughfall and precipitation, and the quantity of water aerosols intercepted by two fog collectors installed at Findley Ridge and at Findley Lake in the Findley Lake Basin, WA. The data are from six precipitation events during the 1995 growing season (8 August, 11 August, 15 August, 17 August, 30 August and 6 September). The percentage difference between throughfall and precipitation was predicted by the regression:

$$100\left(\frac{\mathit{throughfall} - \mathit{precipitation}}{\mathit{throughfall}}\right) = -33.95 + 12.37(\mathit{mm\ fog\ water\ equivalents})$$; $r^2 = 0.64$, F-ratio = 29.0, $p<0.01$.

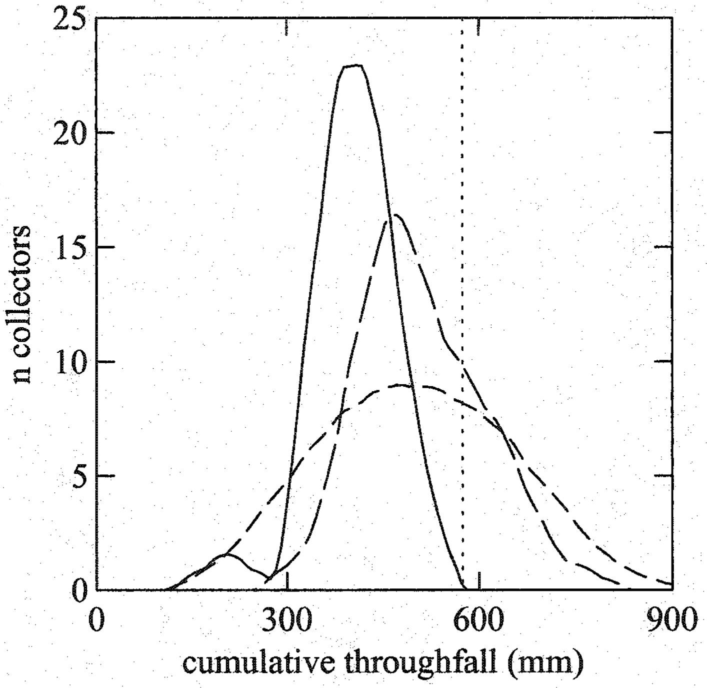


Figure II.7. Distribution of throughfall at Findley Ridge and Findley Lake for 25 precipitation events during the 1994 and 1995 growing seasons (8 August 1994 - 18 October 1995; see the appendix for the full data set). Solid line, cumulative throughfall at Findley Lake throughfall plots; long dashes, cumulative throughfall at the midslope throughfall plots; short dashes, cumulative throughfall at the Findley Ridge throughfall plots. The data are from fifty throughfall collectors randomly located at each site. The cumulative amount of above-canopy precipitation from the same precipitation events (fine dashed line) was 575.3 mm.

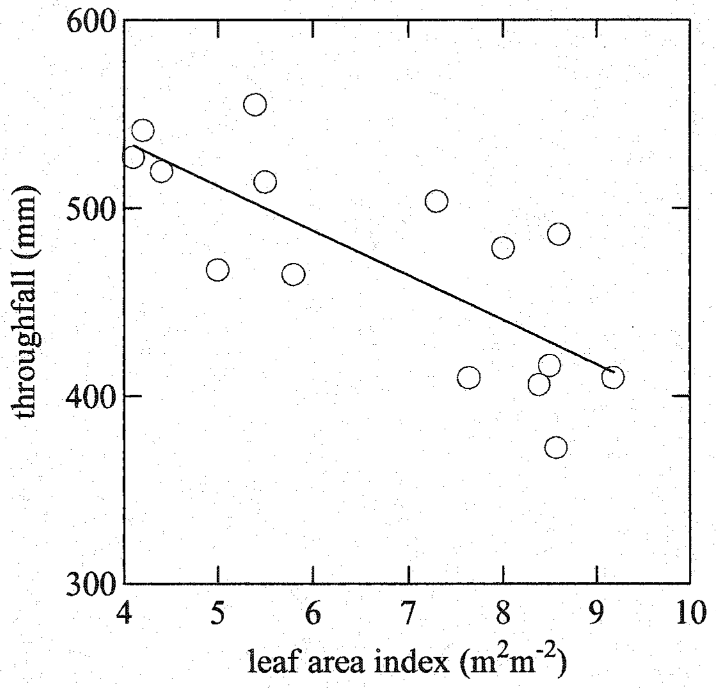


Figure II.8. The relationship between cumulative 1994 and 1995 growing season throughfall (mm) and estimated canopy leaf area ($m^2 m^{-2}$) in the Findley Lake Basin, WA. Each data point is the mean amount of throughfall recorded during the period 8 August 1994 – 18 October 1994 and 6 June 1995 – 18 October 1995 of ten throughfall collectors randomly located within a 1/20th ha circular plot. Five plots were established at each of the three throughfall collection sites (Findley Lake, the midslope of Findley Ridge, and Findley Ridge). Throughfall at each plot is predicted by the regression: throughfall = 630.6 – 23.8(stand leaf area index $m^2 m^{-2}$); $r^2 = 0.56$, $p<0.01$.

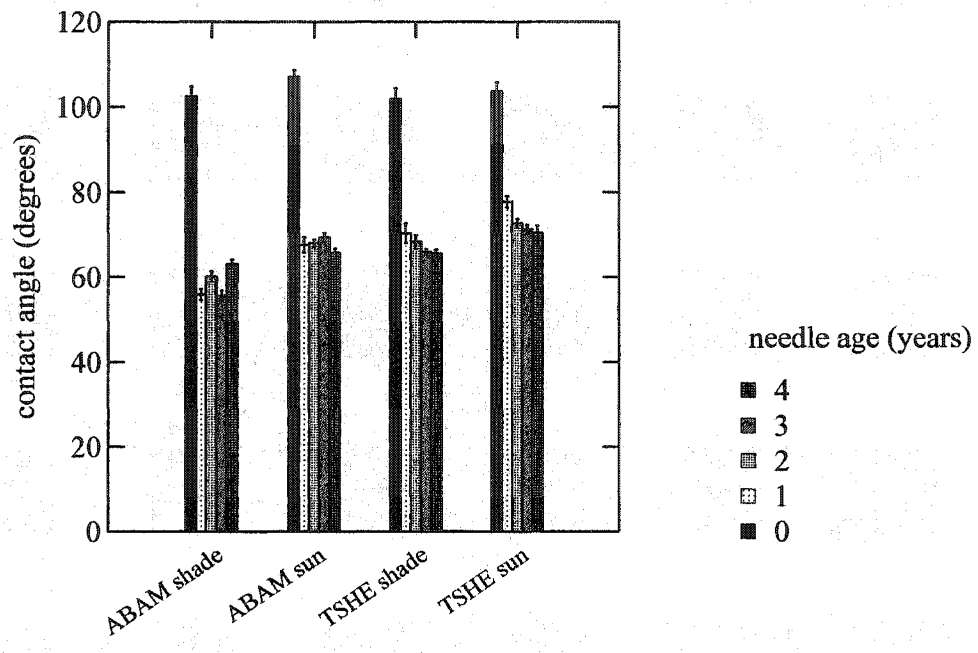


Figure II.9. Contact angles of a 5 μl droplet of deionized water placed on current year, one year-old, two year-old, three year-old and four year-old sun- and shade-acclimated needles of *Abies amabilis* and *Tsuga heterophylla* collected 5 July 1996 at Findley Lake, WA. N=10 for each combination of species, age and shoot type.

Chapter III. A review of the synthesis, structure and properties of plant cuticles.

Introduction

Two of the most important findings from the measurements of canopy wetness and throughfall were that the canopy is often wet during the growing season, and a considerable proportion of the precipitation that is intercepted by the canopy never reaches the forest floor. The interception loss, defined as the difference between precipitation and throughfall, is usually assumed to be caused by evaporation (Rutter *et al.* 1972; Gash and Morton 1978; McNaughton and Jarvis 1983; Teklehaimanot and Jarvis 1991). This assumption has rarely been examined, perhaps because the quantitative models that predict throughfall are robust (Gash *et al.* 1995). Alternatively, interception is treated as a "black box"—a convenient approach in the hard-to-access and structurally complex forest canopy.

Wet angiosperm and conifer shoots can absorb water (Breazeale *et al.* 1950, 1951; Slatyer 1956; Stone 1957b; Leyton and Juniper 1963; Vaadia and Waisel 1963; Johnston 1964; Daum 1967; Suárez and Gloser 1982; Katz *et al.* 1983; Karman 1986; Bowden *et al.* 1989; Grammatikopolus and Manetas 1994; Boucher *et al.* 1995; Yates and Hutley 1995; Munné-Bosch *et al.* 1999). Therefore, part of the interception loss may represent absorption. Some of the consequences of moistening the surfaces of shoots or leaves with water include change in water xylem water potential (Katz *et al.*1989; Grammatikopolus and Manetas 1994; Boucher *et al.* 1995; Munné-Bosch *et al.* 1999; Yates and Hutley 1995), increased survival (Stone 1957a, 1957b), and at least one report of increased growth (Boucher *et al.* 1995). Isotopic techniques (Vaadia and Waisel 1963) and fluorescent markers (Katz et al. 1983; Grammatikopolus and Manetas 1994; Munné-Bosch et al. 1999) have demonstrated the penetration of water into plant tissues. There is one published estimate of the quantity of water absorbed by wet conifer shoots (Katz *et al.* 1983). The significance of water absorption on the scale of whole canopies has not been studied, nor have there been attempts to examine the contribution of shoot water uptake to the interception loss of above-canopy precipitation.

One of the principle objectives of this dissertation was to quantify water fluxes in wet shoots of *Abies amabilis* and *Tsuga heterophylla*. Chapters IV and V will describe experiments that explored the absorbance of water by detached shoots of *Abies* and *Tsuga*. First, it is important to have an understanding of the properties of cuticles with respect their roles as barriers to water movement. This chapter will present a review of the synthesis, structure and properties of plant cuticles.

Structure, synthesis and properties of plant cuticles

Generalized structure of plant cuticles

The cuticle seals the leaf, and is armor against predators, pathogens and abrasion. The design of the cuticle meets important requirements. Since leaves shrink, bend and expand, the cuticle needs to be flexible. It is transparent to useful wavelengths of light, stable under temperature extremes, and extremely resistant to both chemical breakdown and decay.

The classical model of cuticle structure is that of a bi-layered membrane (Mérida *et al.* 1981; Hallam 1982; Holloway 1982a; Fahn 1990) As will be seen later, there are significant exceptions to this model, but for now it useful to review the important features of the structural model that prevails in the literature. According to this model, the materials that accomplish the critical functions of the cuticle—water conservation and protection—are segregated into two discrete layers. The outer layer is rich in soluble, 12- to 62-carbon compounds, and is referred to as the lipid layer. The lipid layer is responsible for the water-repellent properties of the cuticle. The inner layer, or polymer matrix, is insoluble, and has a tough superstructure of cutin. Cutin is a durable polymer made of interlinked strands of fatty acids. Strands of cellulose, pectin and hemicellulose are interwoven throughout the cutin superstructure, and waxes may be encrusted on the outer portion of the polymer (Holloway 1982a). The polymer matrix is a durable shell that protects the vulnerable contents of the mesophyll.

The terminology in the literature used to describe the different cuticular layers is confusing. "Lipid layer" and "polymer matrix" are respectively synonymous with the terms "cuticle proper" and "cuticular layer" that were introduced by Sitte and Rennier (1963) and used by Holloway (1982a) in an authoritative review. The latter terms are needlessly ambiguous. Both the cuticle proper and the cuticular layer are layers of the cuticle. Therefore, throughout the discussion that follows, "lipid layer" will refer to the outer wax-rich barrier to water, and "polymer matrix" will refer to the tough inner layer of cutin and cellulose.

Synthesis of the lipid layer

The lipid layer is made of large molecules, many of which are too massive to pass through the intermolecular spaces in the underlying polymer matrix. The mechanism by which waxes are synthesized in the epidermis and then delivered to the cuticle surface has long been debated (Jeffree *et al.* 1975; Sargent 1976; Hallam 1982; Anton *et al.* 1994). Several investigators have presented evidence that waxes are delivered to the surface via a system of tubes or ducts (Hall and Donaldson 1962; Miller 1985; Miller 1986a; Miller 1986b). Such a network would influence the transport properties of the cuticle, since ducts navigable by large wax molecules would facilitate the movement of water, gases and other substances.

Wax molecules are so large that a system able to transport them should be visible in micrographs. Alvin and Boulter (1974) published micrographs of cuticles of *Cryptomeria japonica*, *Metasequoia glyptostroboides*, *Sequoia sempervirens* and *Sequoiadendron giganteum* that featured apparently porate surfaces. Miller (1986a) reported large transcuticular pores 0.5-

0.75 μm in diameter for 53 out of 69 plant species examined. Pores of this size would be observable with a light microscope, yet have not been noted by investigators who routinely scan enzymatically isolated cuticles for cracks, holes and other defects. Fluxes of water through a network of large pores would be nearly instantaneous, but again, this result has not been reported from studies of isolated cuticles (Kerler *et al.* 1984). It is likely that the cuticular pores described by Miller and others are artifacts of preparing cuticles with strong acids, solvents and other cuticle-degrading substances.

The sequence in which the lipid layer and polymer matrix are assembled seems to make a network of transcuticular pipes unnecessary. Cuticle construction is centripetal: the outermost layers are synthesized first. The building blocks of the lipid layer are manufactured from fatty acids in the epidermal cells. The lipid layer is developed while the epidermal cells are still expanding (Mérida *et al.* 1981; Anton *et al.* 1994; Gilly *et al.* 1997). The lipid mixture is deposited upon the pectinaceous epidermal cell wall (Schieferstein and Loomis 1959). Epicuticular wax appears shortly after the lipid layer is established (Schieferstein and Loomis 1959). The epicuticular wax forms intricate crystals on leaf surfaces and inside stomatal chambers (Gunthardt 1985; Riding and Percy 1985; Turunen *et al.* 1995). The formation of the underlying polymer matrix does not commence until the leaves emerge from the bud and the epidermal cells have fully expanded. As the synthesis of the polymer matrix begins, the accretion of the lipid layer slows, then halts. Since the lipid layer is assembled first, the large molecules of wax are not required to negotiate a barrier of polymerized cutin. Instead, they diffuse through large gaps between the polysaccharide fibers of the cell wall before the polymerized barrier of cutin is constructed. A transcuticular transport system to shunt waxes to sites of deposition is not a requirement during leaf construction.

Cuticular waxes have the interesting property of being self-organizing systems. Epicuticular wax can be dissolved in a solvent and then recrystallized on porous ceramic disks to form intricate crystals in the shape of plates, tubes, and stars, identical to those found on the surfaces of leaves (Jeffree *et al.* 1975). Similarly, extracted and recrystallized intracuticular waxes have the same transport properties as waxes from intact cuticles (Schreiber and Riederer 1996a; Merk *et al.* 1998). The property of self-organization implies that waxes can spontaneously re-establish protective barriers after physical damage to the cuticle. Even dead leaves have been shown to retain this property: *Picea abies* needles dead for three months can restore tubular crystals of epicuticular wax (Bermadinger-Stabentheiner 1995).

Characteristics of the lipid layer

The lipid layer has a complex chemical makeup. It is composed of a mixture of 12- to 62-carbon compounds that include alkanes, esters, alkyl aldehydes, primary alcohols, secondary alcohols and monobasic fatty acids. The most important of these are the 22- to 56-carbon alkane and ester waxes. The alkane and ester waxes are represented by series of homologue molecules that differ in carbon number. Consequently, the lipid layer of a cuticle will typically include fifty or more different types of wax molecules. The composition of the wax fraction varies between species, within species on different sites, with age, or with exposure to stress.

Although cuticular waxes are diverse, all wax-forming compounds found in plant cuticles have three important characteristics. First, the carbon skeletons of cuticular waxes are highly saturated. Saturated carbon chains resist oxidation, and form the backbone of durable compounds (Kollatukudy 1976; Morrison and Boyd 1976). Furthermore, saturated hydrocarbon chains have symmetrically distributed electron clouds, and are consequently either completely non-polar or only weakly so. The second property of cuticle-forming waxes is that they tend to have high carbon numbers. Due to the size-dependent effect of Van der Waals forces, the melting and boiling points of alkanes increase with chain length, and up to a certain threshold, the degree of packing in crystals (Morrison and Boyd 1976; Small 1986). The third property of cuticular waxes is a rod-like backbone of aliphatic carbon atoms. Straight hydrocarbon chains have the highest possible surface area to volume ratio, and therefore the strongest possible attraction between lipid molecules. The orthorhombic crystal lattices formed by straight-chain alkanes are the most stable crystals formed by lipid compounds (Small 1986). Branching and functional side groups reduce the surface area to volume ratio and symmetry of organic molecules (Morrison and Boyd 1976) and, therefore, are rare in cuticular waxes.

Properties of the lipid layer with respect to the movement of water

Many investigators have searched for links between cuticle chemistry and the efficacy of the cuticle as a barrier to water loss. These studies have focused on forest trees in areas heavily affected by air pollution, where ozone and acid rain have been implicated as agents of cuticular degradation. Attempts to find a link between the chemistry and water permeability of cuticular waxes have been disappointing (Riederer and Schneider 1990; Merk *et al.* 1998). It appears that with respect to establishing a barrier against the movement of water, many types of waxes are similarly effective. Riederer and Schneider (1990) identified three key attributes that relate to the barrier properties of cuticular waxes: the variability of the carbon number of the wax mixture, the percent functional group content of the waxes, and the molecular mass of the wax molecules. The arrangement of waxes in the lipid layer needs to be considered to understand why these three factors are important.

As mentioned, the most dominant structural feature of a wax molecule is a long aliphatic carbon chain. Due to their long carbon backbones, wax molecules readily form crystalline solids.

Within these crystals, wax molecules are tightly packed with side-to-side linkages established by Van der Waals forces (Small 1986). Alkane and ester waxes form flat crystalline flakes the width of one molecule, with the long axis of each wax molecule oriented perpendicular to the plane of the cuticle (Small 1986; Riederer and Schneider 1990). The crystalline wax flakes are stacked in the lipid layer in two to twenty bands (Figure III.1), or lamellae, that can be observed with polarized light (Sitte and Rennier 1963; Merk *et al.* 1998).

In contrast to the orderly arrangement of hydrocarbon chains within the crystalline domains, the zone of contact between flakes of wax crystals is more chaotic, with variable interactions between chain-ending functional groups (Riederer and Schneider 1990). A variable percentage of these end groups are polar (Hauke and Schreiber 1998). The solid amorphous zone (Figure III.1) is the disorganized region influenced by the ends of the long hydrocarbon wax and their functional groups (Schreiber *et al.* 1996). Twenty-five to seventy percent of the carbon structure of wax molecules may occupy this zone, while the remaining portion lies within crystalline domains (Reynhardt and Riederer 1994; Schreiber *et al.* 1996; Merk *et al.* 1998). The mobile amorphous zone is the non-solid matrix in which the crystalline wax flakes are embedded (Figure III.1). The mobile amorphous zone features cutin, cyclic hydrocarbons and short-chain lipids with low melting points (Schreiber *et al.* 1996).

The crystalline wax flakes are superb barriers to water movement. Within the wax crystals, the space between the well-packed hydrocarbon chains (0.158 nm) is smaller than the mean diameter of a water molecule (0.390 nm). Furthermore, the structure of the tightly held carbon chains minimizes thermal movement (Small 1986; Kersteins 1994). Together, these two attributes bar water diffusion through the wax crystals. Since water molecules cannot pass through the wax flakes, they must detour around the crystalline moats through the two amorphous zones (Riederer and Schneider 1990; Schreiber *et al.* 1996). The net effect of the wax crystals is to increase the path length for the water diffusion through the cuticle by a factor of 150 times or more (Baur *et al.* 1999). Artificial membranes with properties similar to cuticles have been prepared by imbedding flakes of mica in an amorphous matrix (Cussler 1984).

With this discussion in mind, the importance of the chain length distribution, the functional group content, and the mean mass of wax molecules in the lipid layer is clear (Riederer and Schneider 1990). The lipid layer would be perfectly crystalline if it were constructed of a single type of wax molecule. In a homogenous wax, the size of crystalline flakes is large, and the volume of the wax layer occupied by the amorphous hydrocarbon matrix is small. Large flakes divert aqueous pathways across the lipid layer at many points, resulting in a highly sinuous, tortuous route for water movement. The net effect is to increase the path length for diffusion, and to decrease permeability. In contrast, large crystals cannot be formed if many wax types with variable chain lengths are present. As the chain length distribution of a wax becomes more variable, the volume of amorphous matrix between the flakes is greater, and the aqueous pathways are less tortuous. The wax layer is relatively permeable when the chain length variability is high (Riederer and Schneider 1990).

The functional group content relates to the degree of substitution of functional groups for hydrogen atoms on the central aliphatic axis of the wax molecules. Since functional groups diminish the ability of wax molecules to form crystalline solids, the higher the percentage of functional groups, the more permeable the wax. Mean molecular mass is an index to carbon chain length. Chain length is inversely related to permeability: the longer the carbon chain, the greater the force of attraction between molecules, and the larger the crystals of wax that form in the lipid layer. Therefore, the long-chain waxes found in plant cuticles are good barriers to water (Schönherr and Riederer 1989; Hauke and Schreiber 1998). The functional group content and mean molecular mass of waxes are interrelated, since as the carbon number decreases, the properties of terminal functional groups become more important (Riederer and Schneider 1990).

Finally, in addition to preventing water loss, cuticle waxes must be durable if leaves are to persist one or more growing seasons. While a layer made of a pure wax would be crystalline and impermeable to water, it would also be brittle, and susceptible to fracture during thermal expansion and contraction. This is why cuticle waxes are mixtures of different classes of straight-chain lipids and their homologues. Although the size of the crystalline flakes is reduced and water permeability increased in wax mixtures, the resulting compound has a tough, plastic texture that resists mechanical injury, and will not crack during heating or cooling. Thus, the limited permeability demonstrated by mixtures of waxes is a necessary trade-off between durability and water conservation (Riederer and Schneider 1990).

Synthesis of the polymer matrix

The polymer matrix develops by interposition within the outer walls of the epidermal cells. Bubbles of 16-carbon fatty acid precursors are manufactured by Golgi bodies in epidermal cells, and secreted through plasmalemma into the pectinaceous outer cell wall of the epidermis (Chabot and Chabot 1977; Kolattukudy 1977; Holloway 1982b). The bubbles enlarge with the addition of fatty acids at their surfaces. The globules merge upon mutual contact, forming a continual matrix (Mérida *et al.* 1981). Prior to polymerization, unesterified side groups render the mixture of fatty acids moderately polar (Mérida *et al.* 1981) and the fatty acid chains are ordered by the clustering of their polar functional groups (Tyree *et al.* 1990).

The cutin-forming polymerization reactions involve cross-linkages between secondary hydroxyl groups (Deas and Holloway 1977; Riederer and Schönherr 1988), and are likely catalyzed by extra-cellular enzymes embedded in the cutin matrix proximal to the zones where the precursor fatty acids are secreted (Kollatukudy 1977; Holloway 1982b; Mauseth 1988). As cutin polymerization proceeds and cross-linkages are formed, the polymer matrix becomes increasingly lipophilic. However, the esterification of the carboxyl and hydroxyl groups is incomplete, and therefore, weak polarity is retained (Mérida *et al.* 1981; Riederer and Schönherr 1988; Tyree *et al.* 1990).

Polysaccharide fibers—predominantly hemicellulose, cellulose, and pectin—are plentiful in the polymer matrix of most plant cuticles. Of these, cellulose is the most important (Villena *et al.* 2000). The long cellulosic fibers that intermingle with cutin in the polymer matrix originate from the outer wall of the epidermis (Martin and Juniper 1970; Holloway 1982a; Viougeas *et al.* 1995). The fibers become incorporated into the globules of cutin precursors that arise within the fibrous cell wall (Mérida *et al.* 1981). It is not clear if the intermingled polysaccharide fibers have an important function, or if their presence in the cuticle is an unavoidable consequence of the construction of the polymer matrix. Presumably, the fibers add strength to the cuticle (Baur *et al.* 1999). Cellulose and pectin may provide hydrophilic corridors within the predominantly non-polar domain of the polymer matrix (Kersteins 1994).

In evergreen leaves, lignin often encrusts the outer epidermal cell wall (Lendzian *et al.* 1986; Riederer 1989; Fahn 1990). Lignin is the last element to be added to the cuticle, appearing after the construction of the polymer matrix is complete (Lendzian *et al.* 1986; Riederer 1989). Lignin reduces the permeability of the cell wall, adds rigidity, and provides protection against herbivores and pathogens (Taiz and Zeiger 1991).

Characteristics of the polymer matrix with respect to water transport

The lipid layer is nearly an absolute barrier to water movement. In contrast, the polymer matrix is porous, featuring discrete regions that are penetrable by water. Schönherr (1976a) termed these regions micropores. There are two types of micropores in the polymer matrix. Polar micropores are localized in zones where the functional groups of fatty acids aggregated prior to polymerization (Tyree *et al.* 1990). The second class of micropores is structural features that pass through gaps in the carbon skeleton of the cutin polymer. These gaps are created by the dynamic dissociation of hydroxyl and unesterified carboxyl groups, as well as the dissociation of polyuronic acids associated with cellulose and pectin embedded in the matrix of cutin (Schönherr and Bukovac 1970; Schönherr and Bukovac 1973; Chamel *et al.* 1991). Water flowing through this second type of pore would circulate along a hydrophobic, convoluted route past hydrocarbon chains bound or trapped at discrete sites into the polymer matrix. The more highly cross-linked the polymer, the smaller the gaps in the polymer skeleton, and the more highly obstructed the route for water transport (Luque *et al.* 1995).

The structural differences between the lipid layer and the cutin matrix determine their relative permeabilities to water. In the lipid layer, water transit can only occur through the small regions of amorphous materials sandwiched between broad flakes of crystalline wax. The length of the torturous and interrupted path is many times greater than the width of the lipid layer (Baur *et al.* 1999). In the polymer matrix, routes for water passage pass more directly through gaps between cross-linked carbon chains. As a result, cutin is many times more permeable to water than are the waxes of the lipid layer. Lipid removal with solvents increased the permeability of isolated *Citrus aurantium* cuticles 350-500 times (Schönherr 1976b) and of isolated *Abies alba* cuticles by a factor of twenty (Lendzian *et al.* 1986). The lipid layer is clearly the predominant barrier to the movement of water through leaf cuticles.

Cuticle structure in Pinaceae

The generalized model of cuticle structure has been discussed to this point. This model depicts the cuticle as a two-layered system, with a waxy lipid layer affixed to an underlying polymer matrix. Structures of plant cuticles are more diverse than the general model implies. The lipid layer can be distinctly lamellate, amorphous, or the cuticle may not have a discrete lipid layer. On the other hand, some cuticles do not have a discrete polymer matrix. Holloway (1982a) used the morphology of the lipid layer and the polymer matrix to devise his widely used classification scheme, which recognizes six types of cuticles (Table III.1). Instead of a single, bi-layered cuticle structural type, Holloway identified at least six. An implication of the diversity in cuticle structures is that transport properties may vary between Holloway cuticle types, and findings from some of the more intensively studied plant cuticle systems—*Ficus elastica*, *Hedera helix*, *Clivia minata*, and *Citrus aurantium*—may not apply broadly to all plants.

The Pinaceae have Holloway type IV cuticles, while Holloway type I cuticles are characteristic of other major gymnosperm groups (Riederer 1989; Taylor *et al.* 1989; Guignard *et al.* 1998). Holloway type IV cuticles with a deeply thickened polymer matrix are recurrent features in plant species with sclerophyllous leaves or needles (Orgell 1955; Mérida *et al.* 1980; Holloway 1994; but see Jordaan and Kruger 1998). There is at least one exception to this rule: *Pseudotsuga menziesii* has a type I cuticle (Holloway 1982a). Beyond defense against water loss, the thick polymer matrix may protect expensive-to-construct leaves from herbivores and physical damage (Walters and Reich 1999), or increase desiccation tolerance by reducing leaf and cell wall elasticity (Kramer and Boyer 1995; Wiedemann and Neinhuis 1998).

Cuticles that differ in structure may also differ in transport properties. Two features of Holloway type IV cuticles may set their transport properties apart from other cuticle types. First, Holloway type IV cuticles lack a discrete lipid layer—the portion of plant cuticles that most clearly limits the passage of water (Schreiber and Riederer 1996a; Baur *et al.* 1999). Instead, water-conserving waxes are embedded in the polymer matrix. The inner surface of Pinaceae cuticles is typically encrusted with lignin (Lendzian *et al.* 1986; Reina *et al.* 2001). The cutins in the polymer matrix of the Pinaceae are qualitatively different from cutins in other plants (Holloway 1982b), and may contribute importantly toward enhancing the resistance of the cuticular membrane to water movement (Baur *et al.* 1999).

Second, Holloway type IV cuticles tend to be unusually rich in hydrophilic materials. Chamel *et al.* (1992) estimated that isolated *Abies alba* cuticles are composed of 3.5 to 5.0 percent soluble lipids (waxes), 38.0 to 55.6 percent hydrolyzable polysaccharides, with the remainder classified as cutin. Reina *et al.* (2001) produced a comparable estimate for the composition of *Araucaria bidwillii* cuticles (4.2% wax and 42.3% hydrolyzable compounds), but differentiated between cutin (26.8%) and lignin (26.7%). The abundance of hydrophilic substances in coniferous cuticles results in a high affinity for water. Chamel *et al.* (1992) found that exposure to moist air increased the percentage water content of *Abies alba* cuticles from 2.9 percent to 17.2 percent. Humidified *Araucaria bidwillii* cuticles contain up to 16.2 percent water (Reina *et al.* 2001). In comparison, angiosperm cuticles appear to have a lower affinity for water. Exposure to moisture increased the water content of *Pyrus communis* cuticles to 4.3%, and that of *Ficus elastica* cuticles to 2.3% (Reina *et al.* 2001).

Environmental influences on the structure of plant cuticles

Cuticle synthesis requires carbon, and factors that influence rates of carbon fixation can affect the structure of the cuticle. Light is the most important of these. Juniper (1960) grew *Pisum sativum* plants for eight days in the dark, and then exposed the leaves to bright light. In the dark, little wax was present on the leaves, while crystals of epicuticular wax formed rapidly after exposure to light. Hallam (1970) demonstrated that leaves of *Eucalyptus* species produce less wax when shaded. Baker (1974) found that epicuticular wax production by *Brassica oleracea* doubled when radiant intensity was doubled from 40 to 80 J m^{-2} s^{-1}. Finally, Hunt and Baker (1982) observed that *Pisum sativum* produced 40-100% more wax when irradiance was doubled.

Nutrient availability can also affect cuticle formation. Turunen *et al.* (1995) found that the extra nitrogen and sulfur provided by experimental treatments with pH 4 simulated acid precipitation increased photosynthesis by *Pinus sylvestris* and *Picea abies* seedlings. In response to the nutrient influx, acid-treated seedlings produced waxy, water repellent needles. Interestingly, seedlings grown under a pH 3 precipitation regime had less epicuticular wax, and more wettable needles. Turunen *et al.* (1995) suggested that the strong acidity of the pH 3 treatment outweighed the benefit of extra nitrogen and sulfur, and had a negative effect on carbon fixation by the seedlings. Chiu *et al.* (1992) fertilized *Pseudotsuga menziesii* with 228 kg ha^{-1} of nitrogen and potassium. The needles of the fertilized trees were more glaucous, an effect they attributed to the enhanced production of crystalline epicuticular waxes.

Once carbon has been fixed, stress-sensitive switch mechanisms seem to have a role in allocating extra carbon to the cuticle. In Baker's 1974 study, wax deposits on *Brassica oleracea* leaves more than doubled when the plants were grown in a xeric (40% rH) versus humid (98% rH) atmosphere. Hunt and Baker (1982) reported that *Pisum sativum* manufactured more epicuticular wax when the atmospheric vapor pressure deficit was intensified, or when the plants were provided with less water. Bondada *et al.* (1996) applied water stress treatments to *Gossypium hirsutum*, and found that the leaves of plants growing in the drier soil medium produced 69% more epicuticular wax than well-watered plants. Dixon *et al.* (1997) conducted a similar experiment with two clones of *Picea abies*, and reported that drought increased the production of cuticular waxes by 14-50%. Gilly *et al.* (1997) doped *Hedera helix* plants with ^{14}C-labeled acetate to show that extra carbon was shunted to developing cuticles of plants growing in a xeric (50% rH) atmosphere. No labeled carbon was diverted to the cuticles of fully developed leaves. Svenningson and Liljenberg (1986) subjected *Avena sativa* seedlings to short, four-hour periods of moisture stress. After two episodes of stress, cuticular transpiration was reduced by 30-47%. Svenningson and Liljenberg provided evidence that the stress treatments induced the synthesis of long-chain intracuticular waxes, which in turn sealed the cuticle surface and slowed cuticular transpiration.

Damaging agents such as acidic precipitation, ozone, and sulfur dioxide can directly interfere with cuticle construction. Kerfourn and Garrec (1992) treated *Picea abies* seedlings and *Hedera helix* vines with a combination of ozone and acidic mist. For the treated plants of both species, the carbon chain length of alkane waxes in the cuticle decreased. Since the permeability of cuticular waxes is a function of their chain length (Mérida *et al.* 1981), the shift from long-chain to short-chain waxes potentially increased cuticular transpiration. In another acid mist study, Percy and Baker (1991) reported that the cuticle of a *Picea sitchensis* clone treated with pH 2.6 acid mist was more wettable (contact angle 47°) than the same clone treated with pH 5.6 acid mist (contact angle 62°). The acid mist treatment interfered with the synthesis of epicuticular waxes, which largely determine the wettability of leaves. Percy *et al.* (1994) observed that acid precipitation can affect wax biosynthesis enzymes below a threshold of pH 5.0-5.5. McQuattie and Rebbeck (1994) experimented with seedlings of *Liriodendron tulipifera* fumigated with ozone at concentrations twice greater than the ambient dose. They found that elevated exposure to ozone reduced cuticle thickness from 200 to 150 nm. Interestingly, seedlings treated with twice ambient levels of both ozone and carbon dioxide had the same cuticle thickness as the control group, but the cuticular ultrastructures of the treated and untreated seedlings were different.

Ultraviolet light can be another factor that influences cuticle formation. Barnes *et al.* (1994) found that the leaves of *Nicotiana tabacum* plants illuminated with light filtered of ultraviolet wavelengths were more wettable (contact angle 95°) than plants receiving ambient ultraviolet light (contact angle 105°). They further reported that the leaves of *Nicotiana* illuminated with ultraviolet light 25% more intense than ambient were more wettable (contact angle 75°) than leaves receiving ambient light. These results suggest that leaves can plastically respond to ultraviolet light. Beyond a threshold, however, metabolic damage from intense ultraviolet light may cripple the integrity of the cuticle.

In summary, there appears to be three classes of environmental influences on cuticle formation. The first of these are constraints on the capacity of plants to fix carbon, such as light and nutrient availability. The second are environmentally induced stresses that affect the allocation of carbon. The third are abiotic factors that directly interfere with plant metabolism, and consequently interfere with cuticle biosynthesis. The process of cuticle formation is plastic, and will vary in accordance with constraints on photosynthesis and controls on carbon allocation during the period of active cuticle development. The cuticles of sun and shade leaves can be expected to have different properties, as might cuticles from plants grown in xeric or mesic environments. This last point may also apply to leaves formed along spatial and temporal gradients of decreasing water potential as trees increase in height or age.

Change in the structure of cuticles over time

Cuticles pass through four distinct phases: formation in the bud, expansion, maturation, and senescence. As reviewed earlier, cuticle formation is virtually complete by the time leaf expansion has ended. This is especially true for the leaves of deciduous plants. Evergreen plants require a tough, thickened cuticle to protect their leaves, and their cuticles consequently require more time to fully develop. In evergreens, the cessation of leaf expansion and the beginning of leaf maturation is marked by the appearance of lignin in the outer wall of epidermal cells, slower rates of cutin accumulation, and dramatically slower rates of wax accretion. The weight of coniferous cuticles peaks at the end of the first (Prügel *et al.* 1994), and in some cases the second (Günthardt 1984; Riederer 1989) growing season. Thereafter, the mass of coniferous cuticles declines. This is the senescence phase, and is marked by the slow degradation of the cuticle, accompanied by few additions of new cuticular material from the epidermal cells.

A senescing leaf becomes increasingly vulnerable to water loss as its cuticle loses integrity. Heinsoo and Koppel (1999) explored the relationship between the age, shoot position, and the minimum epidermal conductance of *Picea abies* needles. In their study, minimum epidermal conductance did not vary with shoot position, while it became greater with needle age, increasing 0.625×10^{-5} m $s^{-1}yr^{-1}$. Similarly, Hadley and Smith (1994) found that the maximum diffusion resistance of *Picea engelmannii* and *Abies lasiocarpa* needles decreased forty percent after the needles had aged one year. Schreiber (1994) assessed the cuticular permeability of aging *Picea abies* needles. As the needles aged, their permeability to water increased from 7.3×10^{-9}m s^{-1} in current-year needles, to 10.1×10^{-9}m s^{-1} in one year-old needles, and $12.9 \times 10^{-9}ms^{-1}$ for two year-old needles. In the same study, Schreiber obtained nearly identical results for needles of *Abies alba* and *Pinus sylvestris*, and attributed the increase in cuticular permeability to either the erosion of needle surfaces, or to an inability to synthesize new wax compounds as the needles increased in age.

The erosion of cuticles is unavoidable once leaves have been formed. Collisions with rain drops, ice particles and flailing tree branches wear away the surface of the cuticle, and are probably the major cause of cuticle degradation (Van Gardigen *et al.* 1991). Certain species of leaf-dwelling fungi manufacture cutinase and esterase that enable them to penetrate cuticles (Peece and Dickinson 1971; Kolattukudy 1977). Chemical alteration of the cuticle is a less likely mechanism of erosion. Some researchers have speculated that pollutants are capable of oxidizing or hydrolyzing plant cuticles (Turunen and Huttenen 1990). The refractory nature of the cuticle, however, implies that if a plant has been exposed to a pollutant potent enough to damage the cuticle, other tissues will have sustained more severe damage.

Schreiber's (1994) second hypothesis—that the manufacture of new materials may be required for cuticle maintenance—is not broadly supported by the literature. Jetter and Riederer (1995) found that the admixture of alkanediol enhances the tubular habit of nonacosan-10-ol, the most common crystal-forming epicuticular wax in coniferous cuticles. In the absence of alkanediol, nonacosan-10-ol is most stable as a flat crystal or amorphous wax. They speculated that the synthesis of alkanediol is necessary to maintain tubular crystals on the surfaces of conifer needles. However, Percy and Baker (1987; 1990) and Percy *et al.* (1992) noted that subtle shifts in the ratios of cuticular waxes at the time of synthesis can lead to changes in wax structure. If disrupted by stress, wax manufacture may result in meta-stable compounds that become disorganized and lose their characteristic structure a few weeks or months after deposition. Furthermore, the deposition of new waxes to maintain the cuticle would likely require a duct-like delivery system, which, as discussed earlier, has not been demonstrated in leaves. Finally, carbon-labeling studies have not demonstrated carbon flow to the cuticles of fully formed leaves (Gilly *et al.* 1997).

The slow decline of the cuticle may be part of the explanation why many of the Pinaceae have cuticles with an extensively thickened polymer matrix: the lipid layer—primarily made of wax—is an excellent barrier to water loss, but fragile in comparison to the tough polymer matrix. A durable cuticle may not be an important requirement for deciduous plants, but becomes essential for shade tolerant species such as *Abies amabilis*, which may retain a cohort of needles upwards of twenty plus years (T. Hinckley, personal communication). Coniferous cuticles likely have excess capacity with respect to their resistance to water loss. Several to many years may be required before the rate of cuticular transpiration approaches a critical threshold.

Water movement through plant cuticles

Two hypotheses have been developed to describe the movement of water through plant cuticles: the dynamic micropore hypothesis (Schönherr 1976a), and the simple diffusion or free-volume hypothesis. The two hypotheses may be incompatible, since each proposes very different driving forces and mechanisms of water movement (Kersteins 1996; Schreiber and Riederer 1996a). However, other evidence suggests that the two hypotheses may describe one pathway of a parallel transport network through plant cuticles (Tyree *et al.* 1992; Schönherr 2000; Schreiber *et al.* 2001).

The dynamic micropore hypothesis.

The dynamic micropore hypothesis (Schönherr 1976a) posits the formation of two types of pores in the polymer matrix. The first type of micropore is formed in cutin. Polar functional groups attached to long carbon chains of cutin- and wax-forming fatty acids are postulated to be clustered in the cutin polymer. The aggregation of these hydroxyl and unesterified carboxyl groups during cuticle synthesis would result in the lowest energy and, therefore, the most stable molecular configuration for cutin and waxes in the cuticle (Tyree *et al.* 1990). Hydration induces the polar groups to dissociate and form micropores. The pores are quite small, with radii ranging between 0.41 to 0.50 nm (Schönherr 1976a, Schönherr 1976b; Schönherr and Schmidt 1979). Water moving through these pores would circulate along a convoluted route that would be interrupted by moats of crystalline waxes, and by the carbon chains of the polymerized carbon skeleton. The more highly cross-linked the polymer, the smaller the gaps in the polymer skeleton, and the more obstructed the route for water transport (Luque *et al.* 1995).

The second type of micropore is proposed to be associated with fibrils imbedded in the cutin polymer. These fibrils account for about half of the mass of Holloway type IV cuticles (Chamel *et al.* 1992; Reina *et al.* 2001). The fibers have a diameter of 5-10 nm (Kersteins 1994), and are mostly made of cellulose (Holloway 1982a; Villena *et al.* 2000). Cellulose microfibrils are highly crystalline (Brett and Waldron 1990), which would imply that they would be largely impregnable to water. However, MacKay *et al.* (1988) proposed that the 10-nm wide cellulose microfibrils in *Phaeseoulus* hypocotyls are sheathed with a 2-nm thick, non-crystalline layer of hemicellulose II molecules. The hemicellulose sheath would account for more than two-thirds of the fibril cross-sectional area, and would be negotiable by water. Water molecules moving along microfibrils in the cuticle could pass through small amorphous domains associated with crystalline cellulose, or move through their more permeable outer sheaths (Kersteins 1994). Two features of polar micropores linked with polysaccharide fibrils would be different from pores in cutin. First, the pore diameter would be greater. Since the flow rate through a tube varies with the fourth power of the radius (Nobel 1999), a few strands of polysaccharides would have a disproportionate influence on the cuticle's transport properties. Assuming a diameter of 2 nm, micropores created by polysaccharide strands would have a flow rate more than five hundred times greater than micropores in cutin. Second, the diffusion path could be less tortuous. While micropores in cutin follow a path highly interrupted by cutin-embedded wax and cross-linked polymer chains, the route along a single fibril that spans the breadth of the cuticle may be more direct.

The flux of water through a cuticle is equal to the product of the driving force, times the conductance of the cuticle. If water-filled micropores are involved in water flux through cuticles, then there would be a continuum of liquid water from the surface of a wet needle to the epidermis. Water would move through micropores by viscous flow (Schönherr 1976a). The driving force for this movement would be the difference in water potential on either side of the cuticle. This is given by the expression:

$$\textit{driving force} = \Psi_{leaf} - \frac{RT}{\overline{V}_w}\ln\left(\frac{a^o}{a^i}\right) \quad (3.1)$$

where a^o is the activity of water on side o, a^i is the activity of water on side i, and $\overline{V_w}$ is the partial molal volume of water (Nobel 1999). The water potential gradient furnishes the driving force for water movement through the network of micropores in the cuticle. When the exterior surface of the cuticle is wet, the quantity a^o/a^i will be unity. Thus, the direction of the driving force when needles are wet will be from the cuticle surface into the tissues of the plant. When the cuticle is dry, a^o/a^i will have some value less than one, and the net direction of water movement will be towards the atmosphere.

The simple diffusion hypothesis.

According to the simple diffusion hypothesis, water can penetrate a hydrophobic lipid domain when the carbon chains of a lipid are in thermal motion. The movement of the carbon chains creates temporary gaps in the membrane's molecular matrix that can be occupied by a water molecule. As spaces are opened, randomly moving water molecules jump from donor to acceptor holes (Barnes and LaMer 1962; Lieb and Stein 1969). Water traverses the membrane in accordance with the concentration gradient. Fick's First Law (Nobel 1999) expresses the flux of water across the lipid membrane:

$$J_w = -D\frac{\Delta c}{\Delta x} \qquad (3.2)$$

Where J_w is the flux of water across the cuticle, D is the diffusion coefficient of water in the cuticle (expressed in units of m^2s^{-1}), Δc is the concentration gradient of water across the cuticle, and Δx is the thickness of the cuticle. The diffusion coefficient quantifies the mobility of water in a membrane. It is described by the Stokes-Einstein equation:

$$D = \frac{k^B T}{6\pi r \bullet \eta_{cm}} \qquad (3.3)$$

where k^B = the Boltzmann constant (1.3806 x 10^{-23} JK^{-1}), T = the absolute temperature, η_{cm} = the viscosity of the cuticular membrane, and r = the molecular radius of the diffusing substance (Price 1982). The diffusion coefficient is directly proportional to temperature, because as temperature increases, the motion of the carbon chains of cutin and wax increases and spaces that can be occupied by diffusing molecules are created more frequently. The diffusion coefficient is inversely related to the size of the diffusing molecules, since as the molecular volume of the diffusant increases, only the largest voids in the cuticular matrix can be occupied; and to the viscosity of the cuticular membrane, since the carbon chains of viscous membranes move more slowly.

In equation 3.2, *D* has a negative sign to denote that the movement of water is from zones of higher to zones of lower concentration (Nobel 1999, Schönherr 1976a). Because the actual concentration gradient of water in a cuticle is difficult to determine, it is approximated as the negative of the average gradient of water through the thickness of the cuticle:

$$-\frac{dc}{dx} = \frac{c_o - c_i}{\Delta x} \qquad (3.4)$$

In this expression, c_i and c_o are the concentrations of water just outside of the cuticle at the cell wall side and at the exterior surface, respectively (Nobel 1999). Since the solubility of water in cuticles is quite low, the gradient of water concentration on either side of the cuticle, c_o - c_i, is an overestimate of the gradient of water concentration inside the cuticle itself. A correction factor known as the partition coefficient, *K*, must be used to yield the actual concentration gradient of water in the cuticle. *K* is dimensionless, and is defined as the ratio of the equilibrium concentration of a solute in a membrane to the concentration outside the membrane in the aqueous phase. For cuticles, *K* is often expressed as the log of the ratio of the solubility of the diffusing substance in octanol and water. Substances with large partition coefficients tend to have rapid rates of transcuticular diffusion, because they enter the lipophilic domain of the membrane more easily (Lieb and Stein 1969). Fick's First Law rewritten for trans-cuticular diffusion now appears as:

$$J_w = D\frac{K(c_o - c_i)}{\Delta x} \qquad (3.5)$$

Equation 3.5 would describe diffusion through a membrane made of a homogenous lipid. This is an over-simplification for plant cuticles. The crystalline wax flakes—which are virtually waterproof—decrease the volume of the lipid layer available for diffusion. Twenty to seventy-five percent of the lipid layer is in crystalline form (Reynhardt and Riederer 1994; Merk *et al.*1998), and cannot be entered by diffusing molecules. The part-crystalline, part-amorphous structure of the lipid layer has two implications for cuticle transport. First, transcuticular diffusion of both lipophilic and polar molecules occurs in the amorphous zone (solid and mobile). Therefore, the partition coefficient, K, reflects the solubility of the diffusant into the amorphous matrix, and not into the domain of crystalline waxes. Estimates of K obtained under the assumption of structural uniformity need to be increased by an amount corresponding to crystalline volume of the lipid layer. Second, detours around the flake-like crystals of wax dramatically increase the length of the diffusion path. To penetrate a cuticle, diffusing water molecules must follow a convoluted path thirty to more than seven hundred times longer than the actual thickness of the lipid layer (Baur *et al.* 1999).

Equation 3.5 requires a coefficient to reflect the tortuosity of the lipid layer. Otherwise, the expression will either tend to underestimate the solubility of the diffusant into the amorphous matrix, overestimate the thickness of the lipid layer, or both (Baur *et al.* 1996). The coefficient of tortuosity, ϑ, quantifies the extra distance per unit of membrane thickness that a diffusant must travel due to deflection by crystalline waxes (Schönherr 1976b). The expression for the flux of water across the cuticle is now:

$$J = D\frac{K(c_o - c_i)}{\Delta x \bullet \vartheta} \qquad (3.6)$$

Becker *et al.* (1986) found that K is high for Holloway type IV cuticles: type IV cuticles readily sorb water. Accordingly, they attributed the low conductances of cuticles to very low values of

D, which were up to six orders of magnitude lower than the D of artificial hydrocarbon membranes with comparable conductances to water. Baur *et al.* (1996; 1999) later showed that the low D of plant cuticles was due to the extreme tortuosity of the diffusion path.

In practice, D, K, ϑ, and Δx are difficult to measure directly. They are integrated by the more convenient permeability coefficient, P:

$$P = \frac{DK}{\Delta x \bullet \vartheta} \qquad (3.7)$$

where P is measured in units of m s^{-1}. The simplified form of Fick's First Law for the flux of water across a cuticle becomes:

$$J = P(c_o - c_i) \qquad (3.8)$$

Equation 3.8 states that the flux of water across a cuticle is equal to the permeability coefficient, P, times the gradient of water vapor concentration across the cuticle, which is the driving force. Expressed in this manner, the permeability coefficient is equivalent to the cuticular conductance, g_c (Nobel 1999). In fact, P is synonymous with g_c when transcuticular water movement occurs by diffusion (Kersteins 1996). In this study, the symbol g_c will be used.

There is strong evidence in the literature that diffusion is the primary mechanism by which water moves through plant cuticles. In an important paper, Schreiber and Riederer (1996a) explored the relationship between the diffusion coefficient of cuticular waxes, D, and the permeability of isolated cuticles to water, P. Briefly, cuticular waxes were extracted with chloroform from the cuticles of 24 plant species. The mobility of ^{14}C-labelled octadecanoic acid—a highly aliphatic molecule—in the extracted wax was determined by desorption. Schreiber and Riederer then measured the permeability of isolated cuticles of the same species to water, using a gravimetric technique developed by Schönherr and Lendzian (1981).

Schreiber and Riederer found that the *D* of octadecanoic acid in extracted cuticular wax was highly correlated ($r^2=0.93$) with the g_c of the isolated cuticles to water. Other properties of the cuticle, such as thickness and wax coverage, were not significantly correlated with g_c. Their study had several important implications. First, if the *D* of waxes in a plant cuticle is known, then g_c can be predicted. Thus, it is sufficient to study the transport properties of extracted waxes to estimate g_c, versus more complex systems using isolated cuticles, whole leaves, or detached shoots. Second, their study implied that the conductance of a cuticle is directly proportional to the solubility of a diffusing molecule in the cuticle, and indirectly proportional to the mobility of the diffusing molecule in the cuticle. This condition corresponds to what would be expected for the passive diffusion of molecules through a homogeneous biomembrane (Lieb and Stein 1969). Finally, the strong correlation between measurements of g_c for small polar water molecules and large hydrophobic octadecanoic acid molecules hinted that there is only one path for diffusion through the limiting wax barrier that all permeating molecules must follow, regardless of their size or polarity. If correct, this is strong evidence against the dynamic micropore hypothesis developed by Schönherr (1976a).

The high correlation between g_c and *D* reported by Schreiber and Riederer (1996a) was due in part to a failure to transform their data. With a log-log transformation, the r^2 between g_c and *D* is reduced from 0.93 to 0.66, while the probability of the correlation is improved from $p<0.20$ to $p<0.01$. The correlation between g_c and *D* was still significant, but with less predictive power.

Nierderl *et al.* 1998 tested the results of Schreiber and Riederer's experiment, this time examining the co-permeability of water through isolated cuticles with the permeability of salicylic acid, benzoic acid, and 2,4-dichlorophenoxyacetic acid. Strong correlations were found between the transport properties of the cuticles with respect to water and to the weakly polar organic acids. The correlation between the co-permeability of water and 2,4-dichlorophenoxyacetic acid in isolated cuticles of *Prunus laurocerasus*, *Ginkgo biloba* and *Juglans regia* was 0.95, while the r^2 between the g_c of water and the g_c of both salicylic acid and benzoic acid was 0.99. Furthermore, the correlation between the log-log transformed co-permeability of water and benzoic acid in the isolated cuticles of twelve plant species representing Holloway type I, II, III and IV cuticle structures was 0.95.

The data of Niederl *et al.* supported the hypothesis of Schrieber and Riederer: all molecules—large or small, polar or lipophilic—follow a similar diffusion path through cuticles, with one significant and unexpected difference. The values of g_c for water reported by Niederl *et al.* in isolated cuticles were (excluding *Hedera helix*) an average of ten times greater than the values of g_c for water determined by Schrieber and Riederer in the cuticles of the same plants (Table III.2). Schreiber and Riederer assessed cuticular permeability using a system water:cuticle:air, where the physiological inner side of the cuticle was exposed to a chamber filled with deionized water, and the outer side of the cuticle was exposed to an air-containing chamber desiccated with silica gel. In contrast, the system used by Niederl *et al.* was water:cuticle:water, where the outer side of the cuticle faced a donor chamber filled with a solution of ^3H_2O, and the inner side faced a water-filled receiver chamber. Thus, the *P* measured by Schrieber and Riederer was the conductance of the cuticle to water efflux (cuticular transpiration), while the *P* reported by Niederl *et al.* was the conductance of the cuticle to water influx (transcuticular water uptake or absorbance). While both were measures of g_c, the two conductances may have been different. To avoid confusion in this dissertation, the conductance of the cuticle to the outward flux of water will be represented by the term "$g_{c_{out}}$". The conductance of the cuticle to the inward flux of water will be represented by "$g_{c_{in}}$". As will be seen later, the distinction between the two versions of g_c may be important.

Anomalies in cuticle properties induced by water

Water may interact with the properties of the cuticle that determine conductance. This possibility is supported by evidence from a variety of sources. Responses of cuticular conductance to changes in atmospheric water content have been demonstrated for systems ranging from enzymatically isolated cuticles (Schönherr and Schmidt 1979, Becker *et al.* 1986), to leaf discs (Hoad *et al.* 1997), to whole leaves (Seymour 1980; Boyer *et al.* 1997), to detached shoots (Sowell *et al.* 1982). Schönherr and Schmidt (1979) showed that the $g_{c_{out}}$ of isolated *Citrus aurantium* cuticles was directly related to the chemical activity of water vapor in the atmosphere (a_{wv}). As a_{wv} increased from 0.2 to 0.95, $g_{c_{out}}$ increased by a factor of two. Becker *et al.* (1986) reported a similar relationship between $g_{c_{out}}$ and a_{wv} for isolated *C. aurantium* cuticles, and for cuticles isolated from *Ficus elastica*. Thus as a_{wv} increased and the driving force for vaporous diffusion declined, the conductance of *hydrateable* cuticles became larger. The conductance of non-hydrateable cuticles (e.g. polyethylene and silicon rubber), however, was not affected by variation in a_{wv} (Schönherr and Schmidt 1979).

The relationship between $g_{c_{out}}$ and a_{wv} seen for isolated cuticles is similarly observed in studies on *in vivo* cuticles. Hoad *et al.* (1997) worked with *Fagus sylvatica* leaf discs. Over a range of vapor pressure deficits between 0.4 kPa to 2.0 kPa, $g_{c_{out}}$ (abaxial) varied by a factor of two, from 5 mmol m^{-2} s^{-1} in the most humid atmosphere, to 2.5 mmol m^{-2} s^{-1} in the driest. Seymour (1980) documented a comparable response for intact *Mahonia aquifolium* leaves, and hypothesized that the increased transpirational fluxes that accompany the intensification of the vapor pressure deficit reduced the supply of water to the inner cuticle. Dehydration reduced the conductivity of the polymer matrix and attenuated $g_{c_{out}}$.

Decreases in $g_{c_{out}}$ in whole leaves were shown by Boyer *et al.* (1997) to be related to changes in Ψ_{xylem}. In their study, the $g_{c_{out}}$ of dehydrating *Vitis vinifera* leaves dropped from 5 mmol m^{-2} s^{-1} at Ψ_{xylem} = -0.5 MPa, to 1 mmol m^{-2} s^{-1} at Ψ_{xylem} = -3.5 MPa. The dehydration effect on $g_{c_{out}}$ was greatest after the *Vitis* leaves lost turgor. De Ruiter and Meinen (1998) found a similar link between the Ψ_{xylem} of *Solanum nigrum* and the foliar uptake of glyphosphate. Compared to uptake at Ψ_{xylem} = -0.3 MPa, the uptake of glyphosphate was reduced 2.2 times at Ψ_{xylem} = -0.7 MPa, and 4.5 times at Ψ_{xylem} =-1.0 MPa. The application of a surfactant had no effect on the wettability of the cuticle, but appeared to improve the absorption of glyphosphate by improving cuticular hydration.

As discussed, $g_{c_{out}}$ has been shown sensitive to a_{wv} and Ψ_{xylem}. Leaf and shoot water content also affect $g_{c_{out}}$. Sowell *et al.* (1996) noted that the $g_{c_{out}}$ of detached *Picea engelmannii* shoots was well linked with the relative water content of the shoots. For values of shoot water content ranging from 65 to 105%, $g_{c_{out}}$ increased from 9 to 13 µm s^{-1}. Hadley and Smith (1990a) found that the $g_{c_{out}}$ of *Pinus contorta* needles dropped from 11.6 to 7.0 µm s^{-1} as the water content of the needles fell from 80 to 60%. Hadley and Smith reported even lower values of $g_{c_{out}}$ for *Pinus contorta* as needle water content fell to 20%. These values were beyond the physiological limits of living needles, since the threshold water content for needle mortality is about 60% (Hadley and Smith 1983; Hadley and Smith 1990b).

At least for this limited group of species, these studies suggest that $g_{c_{out}}$ is dynamically linked with the water status of the cuticle. The highest values of $g_{c_{out}}$ were for well-hydrated cuticles exposed to moisture-charged air. Changes that affected the hydration of the cuticle—either water loss by the leaf or an increase in the vapor pressure deficit—lowered $g_{c_{out}}$. Two mechanisms could account for these observations. The first would be that the transport properties of the cuticle change as the water content of the cuticle changes. Water absorption has been shown to decrease the strength of plant cuticles, and makes them more flexible. Petracek and Bukovac (1995) found that hydration increased the elastic extension of *Lycopersicon esculentum* cuticles five-fold, and decreased the force required to fracture the cuticle by 40%. Similarly, hydration decreases Young's moduli for isolated *Yucca* cuticles by 35-50% (Wiedemann and Neinhuis 1998). Hydrogen bonding between water and free hydroxy groups of cutin may enable formerly interlocked elements of the polymer matrix to slide past each other. In this way, water could loosen the structure of the cuticle. It is not clear that changes in cuticle strength imply that conductance would also change. Waxes have been repeatedly shown to be the primary barriers to water movement in cuticles (Kersteins 1996; Riederer and Schreiber 2001). Hydration-induced alteration of the polymer matrix could reduce cuticular strength without a large affect on cuticular conductance.

Water may also indirectly affect $g_{c_{out}}$ by changing leaf turgor. The stretching of cuticular membranes over well-hydrated, turgid leaves could create gaps in the structure of the lipid layer and increase $g_{c_{out}}$. Conversely, dehydration-caused leaf shrinkage and the loss of turgor would relax the tension of the lipid layer, close gaps between wax crystals, and reduce $g_{c_{out}}$ (Boyer *et al.* 1997). Of these two proposed mechanisms, the first is better supported than the second: the $g_{c_{out}}$ of isolated cuticles—unaffected by leaf turgor—changes with vapor pressure deficit (Schönherr and Schmidt 1979; Becker *et al.* 1986) on the same scale that the $g_{c_{out}}$ of cuticles attached to leaves fluctuates with vapor pressure deficit (Seymour 1980; Sowell *et al.* 1996; Boyer *et al.* 1997, de Ruiter and Meinen 1998).

A starting point for predicting water uptake by wet shoots

In an experiment testing the ecological influences of dew on five species of California conifers, Stone (1957b) observed that the species with the least resistance to drought seemed to have the greatest potential for the absorbance of dew. His observation outlined an important prediction of the simple diffusion model of cuticle transport: if water penetrates the cuticle by diffusion, then the conductance of the cuticle to water should be similar regardless of the direction of flux.

The hypothetical relationship between the diffusional flux, g_c and the water vapor concentration gradient would be a convenient way to predict the diffusion of water into a leaf from a wet cuticle. The conductance of a membrane penetrable only by diffusion should be insensitive to the direction of flux. The diffusion and solubility coefficients, tortuosity, and thickness of the lipid layer can change only if the structure of the lipid layer is altered. Therefore, if the transcuticular water vapor flux, leaf water potential, and moisture content of the atmosphere are known, then g_c can be estimated by the expression:

$$g_c = \frac{J_w}{c_o - c_i} \qquad (3.9)$$

which is simply equation 3.8, re-expressed in terms of g_c. Once g_c is known, the rate of transcuticular influx from the surface of a wet leaf could be predicted by substituting the appropriate values of the water vapor gradient into equation 3.8.

Quantifying water uptake by wet shoots

The next three chapters of this dissertation will explore the uptake of water by wet shoots of *Abies amabilis* and *Tsuga heterophylla*. Three approaches were considered to study water uptake by wet conifer shoots. The first involved using the Scholander pressure bomb to measure xylem water potential (Ψ_{xylem}), and then deduce water content from $\Delta\Psi_{xylem}$. The second approach was the use of shoot drying curves to determine water content. Isotopic analysis was the third tool, and had been recently used by Dawson (1998) to study the acquisition of fog water by *Sequoia sempervirens*. Of these three approaches, isotopic analysis had the most potential, but also the greatest cost, and was not adopted for that reason. The other two approaches were used to provide perspectives on the absorbance of water by wet shoots.

Table III.1. Cuticle structures based on the classification system developed by Holloway 1982a. Additional data from Gouret *et al.* 1993; Percy *et al.* 1992; and Kruger *et al.* 1996.

Holloway cuticle type	structure of the lipid layer	structure of the cuticular layer	examples
I	strongly lamellate	reticulate	*Pseudotsuga menziesii, Buxus sempervirens, Cunninghamia lanceolata, Clivia minata, Liriodendron tulipifera, Nicotiana tabacum, Vanilla planifolia*
II	faintly lamellate	reticulate	*Eucalyptus papuana, Hedera helix, Ficus elastica, Pyrus communis, Malus pumila* (adaxial side)
III	amorphous	reticulate	*Eucalyptus perriniana, Ligustrum ovalifolium, Malus pumila* (abaxial side), *Pyrus communis, Vaccinium reticulatum*
IV	absent	reticulate	*Abies alba, Abies balsamea , Picea sitchensis, Pinus sylvestris, Pinus nigra, Capsicum annuum, Citrus limon, Galium aparine, Lycopersicon esculentum, Prunus laurocerasus, Ilex aquifolium*
V	strongly lamellate	absent	*Eucalyptus cinerea, Taraxacum officinale*
VI	amorphous	absent	many species in the family Poaceae

Table III.2. Estimates of water-based g_c for ten plant species. The data were produced using two different experimental systems. Schreiber and Reiderer (1996a; 1996b) assessed g_c using the system water:cuticle:air. Niederl *et al.* (1998) used the system water:cuticle:water. See Holloway (1982a) for a description of cuticle structure types.

Species	Holloway cuticle type	water-based g_c, m s^{-1} Schreiber and Riederer 1996a, 1996b.	Niederl *et al.* 1998	ratio
Camellia sinensis	?	13.0×10^{-11}	0.2×10^{-9}	1.5
Citrus limon	IV	47.0×10^{-11}	9.3×10^{-9}	19.8
Euonymous japonica	?	35.8×10^{-11}	0.5×10^{-9}	1.4
Ginkgo biloba	?	52.2×10^{-11}	0.9×10^{-9}	1.72
Hedera helix	II	5.7×10^{-11}	4.0×10^{-9}	70.2
Juglans regia	?	45.8×10^{-11}	5.0×10^{-9}	10.9
Liriodendron tulipifera	I	42.0×10^{-11}	1.9×10^{-9}	4.5
Lycopersicon esculentum	IV	62.2×10^{-11}	6.6×10^{-9}	10.6
Monstera deliciosa	?	4.3×10^{-11}	0.6×10^{-9}	14.0
Prunus laurocerasus	IV	13.3×10^{-11}	0.7×10^{-9}	5.3

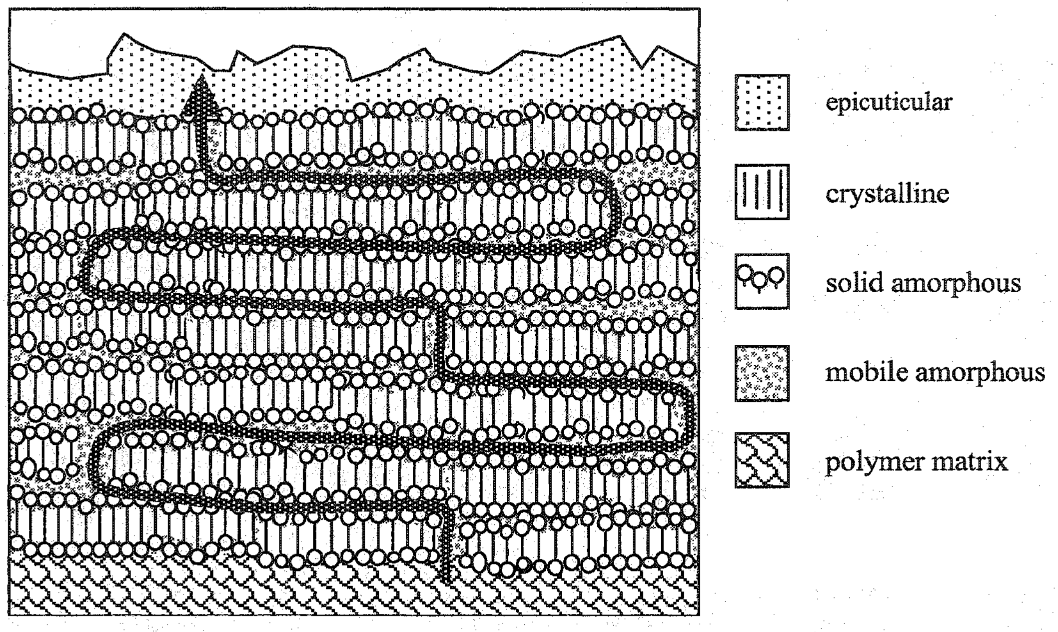


Figure III.1. Conceptual structure of the lipid layer of a plant cuticle. The lipid layer is constructed of layers of crystalline long-chain waxes, the *crystalline zone*. Alcohols, esters and acids are attached to the ends of the wax chains. Together with polar functional groups such as alcohols and carboxylic acids, the chains ends are excluded from the crystalline zone and establish the *solid amorphous zone*. The *mobile amorphous zone* is a mixture of cyclic molecules and short-chain, relatively viscous alkanes and esters with low melting points. The mobile amorphous zone is a separate, non-solid phase that fills the spaces between crystalline regions. The lipid layer is a mobility barrier to water. The moats of crystalline wax divert the path of water molecules at many points, establishing a sinuous path (line) many times greater than the actual width of the lipid layer. The figure is adopted from Schreiber *et al.* (1996) and Baur *et al.* (1999).

Chapter IV. Quantifying water uptake by wet shoots of *Abies amabilis* and *Tsuga heterophylla* with the Scholander pressure bomb.

Introduction and objectives

The xylem water potential (Ψ_{xylem}) of plant tissues is usually negative. When the surfaces of a plant shoot are moistened with water, the gradient of water potential favors the movement of water into the tissues of the shoot. Any gain of water by the shoot should result in a measurable increase in Ψ_{xylem}.

Changes in Ψ_{xylem} have been used as tool to study the uptake of water by wet shoots of conifers. Katz *et al.* (1989) found that the Ψ_{xylem} of *Picea abies* shoots increased +1.4 MPa 200 minutes after being moistened with deionized water. Boucher *et al.* (1995) showed that three treatments of artificial dew per week increased Ψ_{xylem} of *Pinus strobus* seedlings +0.35 MPa, and led to gains in shoot and root biomass. In angiosperms, changes in Ψ_{xylem} due to topical applications of deionized water have been demonstrated by Grammatikopolus and Manetas (1994), Yates and Hutley (1995), and Munné-Bosch et al. (1999).

During 1995 and 1996, a series of exploratory experiments were conducted on shoots of *Abies amabilis* and *Tsuga heterophylla* to study the effect of surface wetting on Ψ_{xylem}. Changes in Ψ_{xylem} were measured with a Scholander pressure bomb. The experiments were designed to demonstrate that: 1) water absorption by a wet shoot would increase the shoot xylem water potential; and 2) the change in xylem water potential could be quantitatively related to water uptake by inference from pressure-volume curves. The objective of these experiments was to quantify the amount of water absorbed by moistened *Abies* and *Tsuga* shoots.

Materials and methods

Two approaches were used to explore shoot water uptake. First, a forked branch was used where one fork was misted, while the other fork was covered and monitored for changes in Ψ_{xylem}. The second approach was to measure the Ψ_{xylem} of detached shoots before and after topical wetting with deionized water. Changes in Ψ_{xylem} were measured with a Scholander pressure bomb; Ritchie and Hinckley (1975) describe its use in experimental ecophysiology.

Change in the water potential of a forked branch

The objective of this experiment was to test if wetting one fork of a forked *Tsuga heterophylla* branch would increase the xylem water potential of the covered, untreated fork. The approach parallels that used by Yates and Hutley (1995) to demonstrate water uptake by *Sloanea woolsii*, a broad-leafed tree from the rain forests of Queensland. In addition, theoretical considerations of this approach have been described by Richter (1973). A forked, shade-adapted branch (end diameter = 11 mm) was harvested from a 30 cm DBH *Tsuga heterophylla* near the parking area for the Findley Lake Research Area. The branch was equilibrated for 24 hours to –1.34 MPa in a sealed plastic bag. Then, the branch was placed in a dark controlled environment chamber at 10°C. One fork of the branch was misted at half-hour intervals with deionized water. The opposite fork was wrapped in plastic and shielded from spray. After nine hours, three shoots from each branch fork were sampled for determination of xylem water potential.

The change in water potential of detached shoots of Tsuga heterophylla and Abies amabilis.

The objective of this experiment was to quantify the effect of surface wetting on the xylem water potential of detached conifer shoots. Changes in xylem water potential were then related to changes in shoot water content through the analysis of pressure-volume curves. *Abies amabilis* and *Tsuga heterophylla* branches were collected from mature codominant trees at Findley Lake, WA. Material was collected either from the upper canopy (sun-acclimated shoots), or from the lower canopy (shade-acclimated shoots). Branches were collected between the disappearance of the snowpack (30 June), and the onset of the first winter storms (15 October), with the exception of the period between the flush and reformation of the terminal buds. Although *Abies amabilis* and *Tsuga heterophylla* have determinant and indeterminate patterns of shoot growth, respectively, the overwhelming influence of the short growing season at Findley Lake tended to synchronize the phenological cycles of the two species.

Once collected, the branches were sealed in large plastic bags, and immediately transported to the Tree Physiology Laboratory at the University of Washington. At the lab, the branch ends were recut under water and the branches were rehydrated under plastic for 24 hours. After 24 hours of rehydration, the xylem water potential recovered to values ranging between -0.3 and -0.1 MPa. Following the rehydration period, 15 to 25 cm long shoots were clipped from the branches. Macroepiphytes such as lichens were gleaned from the shoots. To avoid damage to the stem periderm and needle cuticles, no attempt was made to remove molds and other coatings. The shoots were hung from a wire rack in complete darkness at 20°C for approximately 45 minutes. The objectives of imposing a period of desiccation on the shoots were: 1) to induce stomatal closure; and 2) to reduce the xylem water potential of the shoots to -0.70 to -1.5 MPa—

values that roughly correspond to the range in predawn xylem water potentials found in the top of the forest canopy at Findley Lake.

The xylem water potential of each shoot was measured at the end of the desiccation period with a Scholander pressure bomb. The cut end of the shoot was sealed by dipping the distal one cm in hot paraffin wax. The shoot was then placed in a tilted tray, thoroughly wetted for one minute with a fine spray of deionized water, and then enclosed in a ziplock plastic bag. The moistened shoots, sealed separately in ziplock bags, were placed flat on a lab bench in a darkened room at 20°C for time periods varying from 20 to 180 minutes. A non-wetted control group was originally included in these experiments. The control group was omitted after data showed that the xylem water potential either remained constant, or declined slightly if the air in the bag was pressed out before the bag was sealed (Figure IV.1). Shoots were re-wetted at half-hour intervals if the duration of the treatment period was > 60 minutes. At the end of the treatment period, free water was quickly blotted from the shoot with a paper towel, and the xylem water potential of each shoot was again measured in a Scholander pressure bomb.

Following the experimental treatments with deionized water and subsequent pressure chamber measurements, a foliated branchlet was removed from mid point of each shoot to determine its specific leaf area with an Optimas digital area analyzer. Each shoot was separated into needles and stems, dried for 48 hours at 70°C, and then both components were weighed. The total projected leaf area of each shoot was estimated by multiplying the total dry needle weight times the shoot's specific leaf area.

The change in the water content of the detached shoots was deduced from pressure-volume curves. The pressure-volume curves were prepared following the method described by

Hinckley *et al.* (1980). Water content was expressed as: 100[(wet weight – dry weight)/dry weight]. In this way, water contents greater than 100% were possible.

Results

Forked branch experiment

After nine hours, the xylem water potential of the fork treated with deionized water increased +1.00 MPa to -0.34 MPa. During the same treatment period, the water potential of the dry, covered branch fork increased +0.78 MPa to -0.56 MPa (Figure IV.2).

The change in water potential of detached shoots of Tsuga heterophylla and Abies amabilis.

Wetting with deionized water increased the xylem water potential of sun-acclimated and shade-acclimated shoots of *Abies amabilis* and *Tsuga heterophylla* (n=72; Figure IV.3A). The data were log-transformed to improve normality (Figure IV.3B). An ANOVA analysis (Table IV.1) indicated that shoot morphology (sun- or shade-acclimated) and the duration of wetness were related to the increase in Ψ_{xylem} (r^2=0.67). The length of time that the shoots were moistened with deionized water explained 38% of the change in Ψ_{xylem}: the longer the shoots were wet, the greater the change in Ψ_{xylem}. Shoot morphology explained an additional 29% of the variability in Ψ_{xylem}: the Ψ_{xylem} of shade-acclimated shoots increased more rapidly after wetting than did the Ψ_{xylem} of sun-acclimated shoots. Species (*Abies amabilis* or *Tsuga heterophylla*), phenology (pre-budburst or post-budburst), Julian date, and interaction variables were tested, but did not improve the ANOVA model.

The graphs of the pressure-volume relationships for the detached *Abies amabilis* and *Tsuga heterophylla* shoots were nearly linear for values of Ψ_{xylem} between -2.0 to -0.2 MPa

(Figure IV.4). Simple linear equations of the form: $\%\,shoot\;water\;content = m\Psi + b$ described the data over this range, where *m* is the change in percent shoot water content over the change in shoot water potential, *b* is the y-intercept, and Ψ is expressed in MPa (for *Abies amabilis*, mean r^2=0.98, n=12; for *Tsuga heterophylla,* mean r^2= 0.96, n=20). An ANOVA analysis showed that both the slopes and the y-intercepts of the linear equations fitted to the pressure-volume relationships were significantly different for pre- and post-budburst shoots. The between-species differences in these parameters were not significant (Table IV.2). When compared to pre-budburst shoots, the slopes of the pressure-volume curves were steeper for shoots with mature terminal buds. Furthermore, the y-intercepts of shoots with fully formed terminal buds were greater than the y-intercepts of the shoots before budburst (Table IV.3).

The pressure-volume relationships were used to derive estimated changes in percent shoot water content from changes in shoot xylem water potential. Wetting the detached shoots with deionized water increased the estimated water content of sun- and shade-acclimated shoots of both *Abies amabilis* and *Tsuga heterophylla* (Figure IV.5A). The mean rate of change in the water content of shade-acclimated shoots was more than three times the rate of change in sun-acclimated shoots. Since the changes in the estimated water content of the detached shoots were derived from Ψ_{xylem}, the same variables—the duration of shoot wetness and shoot morphology—that controlled changes in Ψ_{xylem} also dictated changes in percent water content. Between-species differences in changes in percent water content for shoots of similar morphological type were not significant. Similarly, calendar date, phenology, and initial Ψ_{xylem} were not related to changes in shoot water content.

Discussion

Substantial effort was invested in the experiments the Scholander pressure bomb. For reasons that will be discussed, the $\Delta\Psi/\Delta t$ techniques proved to be quantitatively less reliable than the $\Delta m/\Delta t$ techniques that will be presented later. Nonetheless the pressure bomb experiments provided insights on the uptake of water by wet conifer shoots.

The forked branch experiment—a repeat of an experiment by Yates and Hutley (1995)—supports the hypothesis that shoots absorb water from wet foliage. In this experiment, both dry and wet shoots were sampled for Ψ. The relaxation of xylem water tension due to water uptake by the misted fork of the divided branch is perhaps the best explanation for the +0.8 MPa increase in xylem water potential by the other fork.

There are at least two possibilities for the 0.2 MPa difference between the mean water potentials of the treated and untreated halves of the forked branch. The first is that Ψ_{xylem} was slow to equilibrate due to hydraulic constrictions. Zimmerman's segmentation hypothesis (Zimmerman 1983) states that structural junctions in plant stems (e.g., the union of a branch with a stem, or the attachment of a leaf to a twig) are hydraulic bottlenecks. The segmentation hypothesis has been supported for *Abies* (Ewers and Zimmerman 1984a) and *Tsuga* (Ewers and Zimmerman 1984b). Eight hours may not have been enough time for absorbed water to move through hydraulic bottlenecks and equilibrate the water potential of the forked branch.

Instrument error is a second explanation for the 0.2 MPa difference in the Ψ_{xylem} of the two branch forks. The xylem water potential of the treated (wet) shoot may have increased in the pressure chamber. The intense counter-balancing pressure of the nitrogen gas injected into the chamber could have forced water into the xylem. If this was the case, the 0.2 MPa difference between the two forks is an estimate of the measurement error. A third possibility would be that the fork shielded from mist might have continued to lose small quantities of water.

Like the forked branch experiment, the $\Delta\Psi/\Delta t$ experiments with detached *Abies amabilis* and *Tsuga heterophylla* shoots support the hypothesis that moist conifer shoots absorb water from their wet surfaces. The xylem water potential of detached shoots increased when the shoots were moistened with water (Figure IV.3A). The magnitude of the change was related to two factors (Table IV.1). First, the longer the shoots were wet, the greater the increase in Ψ_{xylem}. This is an important finding, because it suggests a proportionate response of shoots to wetting that is unlikely to have been caused by a measurement error. The relationship between $\Delta\Psi_{xylem}$ and the length of time that the shoots were wet was non-linear (Figure IV.3A), suggesting that the conductance of the shoots to water may have increased with time. Next, shoot morphology was related with change in xylem water potential. The Ψ_{xylem} of shade-acclimated shoots increased more than the Ψ_{xylem} of sun-acclimated shoots. Interestingly, the effect of species was not significant.

The ratio of surface area to mass is greater for shade-acclimated shoots than for sun-acclimated shoots of *Abies amabilis* (Sprugel *et al.* 1996) and *Tsuga heterophylla* (Figure I.4A). The greater the ratio of surface area to mass, the more rapidly the shoots should hydrate. Additionally, sun-acclimated shoots tend to grow more actively and therefore have more current-year needles than shoots from shaded microenvironments. Current-year needles are less wettable than needles aged one or more years (Figure II.9). The total area of wetted surface available for diffusion may contribute to the difference between shade- and sun-acclimated shoots with respect to $\Delta\Psi/\Delta t$ after treatment with deionized water.

Another factor not related to shoot morphology or the duration of shoot wetness may have influenced the change in xylem water potential of the mist-treated shoots. Neither of the two regression equations that model Ψ_{xylem} as a function of time (Figure IV.3B) predict that $\Delta\Psi_{xylem} = 0$ at $t = 0$. At $t = 0$, the regression for the sun-acclimated shoots predicts a +0.08 MPa increase in Ψ_{xylem}. The y-intercept of the regression for the shade-acclimated shoots is equal to a $\Delta\Psi_{xylem}$ of +0.22 MPa. Either the first few minutes of the shoot response to the experimental misting treatment were rapid and non-linear, or the results were influenced by an error.

Experimental error is the most plausible explanation. It is unlikely that the initial slope of the relationship $\Delta ln(\Psi/t)$ would change so abruptly. Furthermore, the mean difference in Ψ between the treated and untreated forks in the forked branch experiment (+0.22 MPa) is suspiciously similar to the difference between 0 and the y-intercept of the detached shade-acclimated shoots (Figure IV.3B).

Again, events in the pressure chamber are implicated. The approximate magnitude of the error was +0.2 MPa for shade-acclimated shoots, and +0.1 MPa for sun-acclimated shoots. The ratio of the two y-intercepts of the regression equations fitted to the data for the change in Ψ_{xylem} over time of the detached shoots (Figure IV.3B) was 2.7. This ratio was similar to the approximate top-to-bottom variation in SLA of *Tsuga heterophylla* canopies at Findley Lake (Figure I.4A). The large surface area-to-mass ratio of shade-acclimated shoots may render them more susceptible to the injection of water under pressure.

Pressure-volume curves were used to deduce apparent shoot water content from xylem water potential. Phenology was the lone factor with significant influence on the regression fitted to the pressure-volume curves (Table IV.2). Shoots with unexpanded buds had pressure-volume curves with steeper slopes than shoots with current-year needles (Figure IV.4). The slope of the curve may be related to differences in cell wall elasticity. In plants that have rigid cell walls, small losses in water content trigger large decreases in Ψ_{xylem} (Kramer and Boyer 1995). Lignin, which adds rigidity to the leaves of evergreen plants, does not appear in conifer needles until after expansion is completed (Lendzian 1986; Riederer 1989). Moreover, the cuticles of conifer needle have been shown to mature slowly in montane and subalpine forests (Tranquillini 1974). The importance of phenology suggests that separate pressure-volume curves may need to be established for each phase of needle development, particularly for shoot with a high percentage of current-year needles. Indeed, Teskey *et al.* (1984a) found that the pressure-volume curves of current and one-year old foliage of *Abies amabilis* were very different at the initiation of the growing season in June. The pressure-volume curves became more similar as the growing season progressed and the current year shoots developed, until October, when the two curves were essentially identical.

Water relations may not be strictly interpretable from pressure-volume curves. Specifically, the free transpiration technique (Hinckley *et al.* 1980) that was used in this study requires that shoots be artificially resaturated prior to the determination of pressure-volume curves. Artificial shoot resaturation may lead to an abnormally high percentage of water stored in cell walls (Tyree and Richter 1982). In contrast to the volume of cellular water, the volume of apoplastic water is variable (Tyree and Richter 1982). Due to this problem, Parker and Pallardy (1987) found that the water relations of leaves and shoots were not easily comparable. They showed that the quantity $\Delta(\Psi/\text{water content})$ was greater for naturally resaturated *Quercus alba* leaves when compared to *Q. alba* leaves that had been resaturated artificially. Furthermore, Parker and Colombo (1996) concluded that repeat pressurizations may alter the biophysical properties of a plant shoot and reduce the accuracy of pressure-volume curves. Each method of determining pressure-volume curves appears to have a set of unique errors that is associated with it (Ritchie and Roden 1985; Parker and Colombo 1996).

The above considerations are critical, because the quantity of water that conifer shoots absorb through wet cuticles is probably small and sensitive to measurement error. As discussed, the estimates of shoot water content were derived from xylem water potential using pressure-volume curves. The measurement of Ψ_{xylem} for wet shoots probably has a positive bias (due to the injection of water into shoot tissues under pressure), and error is associated with any technique of establishing pressure-volume curves. In the case of techniques where the shoots were artificially resaturated, there may be a bias towards over-estimating water content.

The above considerations temper any extrapolation of estimates of water uptake that were based on experiments with the Scholander pressure bomb to the level of whole canopies. Using the regression equations fitted to the estimated changes in shoot water content (Figure IV.5B), the water content of sun-acclimated shoots of either *Tsuga* or *Abies* misted for 120 minutes with deionized water would increase ± 3.0 %. The water content of shade-acclimated shoots treated for the same period would increase ± 8.7 %. Assuming a specific leaf area of 40 $cm^2\ g^{-1}$ and 100 $cm^2\ g^{-1}$ for the sun- and shade-acclimated shoots, these increases in water content translate to foliar uptake rates of 3.7 and 4.3 $ml\ m^{-2}\ hr^{-1}$, respectively. Using the mean of these two rates, the LAI estimated for Findley Lake (8.5 $m^2\ m^{-2}$), and the mean duration of canopy wetness (20.4 hours; the mean value from the upper and lower canopies at Findley Lake), estimated foliar uptake by the Findley Lake canopy scales up to 6930 $l\ ha^{-1}$ per event.

While perhaps 2-3% of one day of stand transpiration by the Findley Lake canopy (Martin *et al.* 2001), 6930 liters is a large amount of water. Foliar uptake could be at least biologically significant, but the reliability of the above estimate is not clear. The problems associated with using the Scholander pressure bomb to assess changes in water content by wet shoots have been discussed. More evidence is needed from a different method to assess the importance of foliar uptake.

Summary

The experiments with the Scholander pressure bomb provided evidence that *Tsuga* and *Abies* shoots can absorb water. The changes in the Ψ_{xylem} of moistened shoots were related to two factors: the length of time that the shoots were wet, and shoot morphology. However, it appeared that while inside the pressure bomb, water was injected into the shoot tissues.

Table IV.1. ANOVA analysis of the change in Ψ_{xylem} of moistened *Abies amabilis* (n=41) and *Tsuga heterophylla* (n=31) shoots. The shoots were collected from the upper and lower crowns of mature codominant trees at Findley Lake, WA. Changes in Ψ_{xylem} were measured with a Scholander pressure bomb. The data were log transformed.

Variable	ss	DF	F-ratio
time that the shoot was moistened	11.36	1	81.5[a]
shoot morphology (sun- or shade-acclimated)	8.70	1	62.4 [a]
Error	9.62	69	

[a] significant at $p < 0.001$

Table IV.2. ANOVA analysis of the effect of species and phenology on the slope and y-intercepts of linear equations fitted to pressure-volume relationships for detached shoots of *Abies amabilis* and *Tsuga heterophylla*. The data were limited to values of Ψ_{xylem} between –2.0 MPa to –0.2 MPa. The shoots were collected in early July (pre-budburst) and mid-August to mid-October (post-budburst).

Variable	DF	slope F-ratio	y-intercept F-ratio
Species	1	1.0	0.03
Phenology	1	49.9[a]	67.9 [a]
species * phenology	1	0.3	1.2

[a] significant at the $p < 0.001$ level of probability.

Table IV.3. Mean values of the variables for linear equations fitted to pressure-volume relationships for detached shoots of *Abies amabilis* and *Tsuga heterophylla* collected at Findley Lake, WA. The shoots were collected in early July (pre-budburst) and mid-August to mid-October (post-budburst). The equations were fitted to the pseudo-linear portion of the pressure-volume curves, corresponding to shoot water potentials between –2.0 and –0.2 MPa. The data are the means ± SE. Means for the y-intercept or slope followed by the same letter (a or b) are not significantly different ($p<0.05$; Bonferroni's multiple comparison).

Shoot type	*n*	y-intercept (% water content)	Slope $\left(\frac{dwc}{d\Psi}\right)$
Abies amabilis, pre-budburst	6	116.36 ± 2.03^{a}	7.66 ± 0.24^{a}
Abies amabilis, post-budburst	5	147.02 ± 10.17^{b}	17.65 ± 2.09^{b}
Tsuga heterophylla, pre-budburst	4	110.90 ± 2.08^{a}	7.12 ± 0.39^{a}
Tsuga heterophylla, post-budburst	16	151.08 ± 1.70^{b}	15.61 ± 0.87^{b}

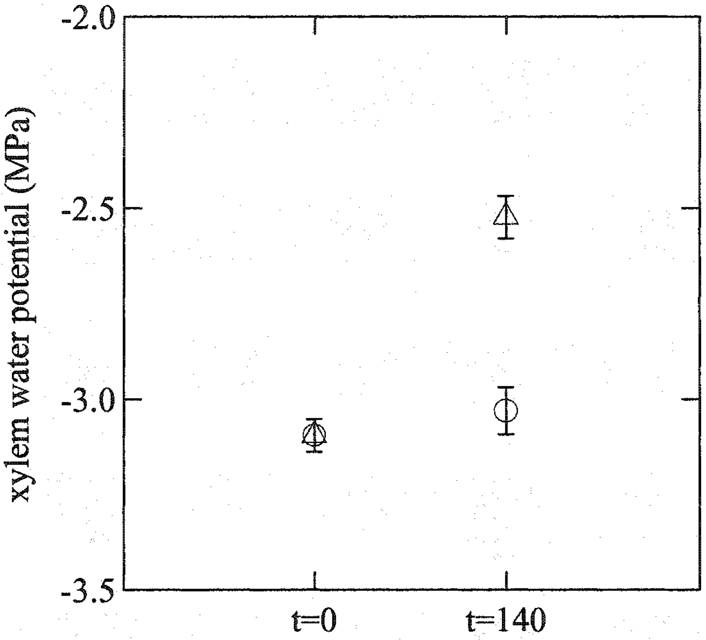
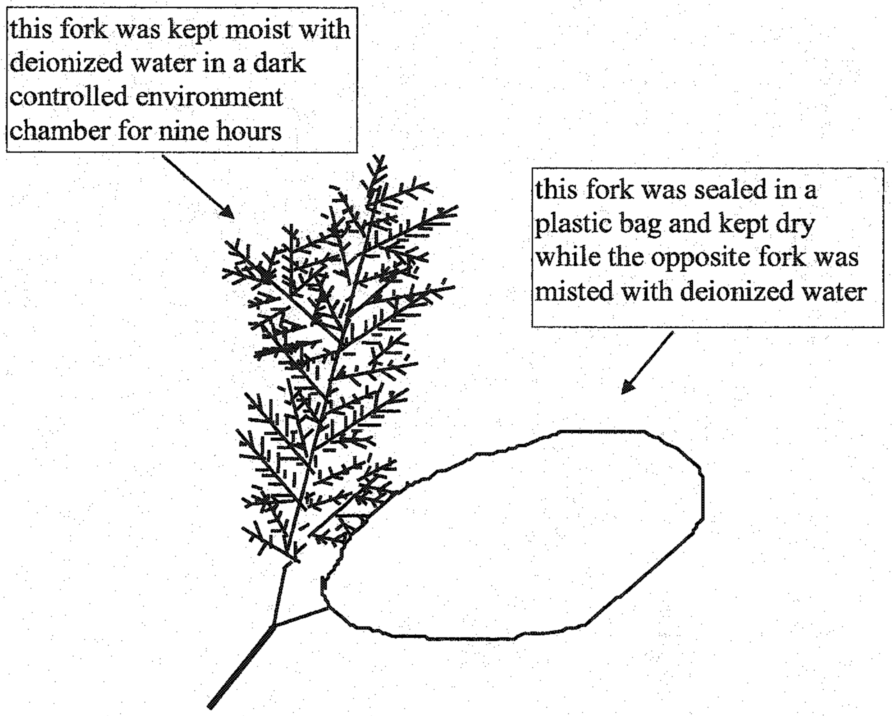


Figure IV.1. The change in Ψ_{xylem} of *Abies amabilis* sun-acclimated shoots after topical treatment with deionized water for approximately 140 minutes. Circles, control group; triangles, + mist for 140 minutes. N = 10 for both groups. The Ψ_{xylem} of the non-moistened control group was not significantly different after 140 minutes ($p>0.50$, Student's t-test), while the Ψ_{xylem} of the mist-treated shoots was significantly higher after treatment ($p<0.001$, Student's t-test). In the experiments with the Scholander pressure bomb, a control group of non-moistened shoots was often excluded because the Ψ_{xylem} of the control shoots was static.



	misted fork	shielded fork
xylem water potential prior to misting	-1.34 MPa	-1.34 MPa
post-treatment xylem water potential	-0.34 MPa	-0.56 MPa

Figure IV.2. Water uptake experiment with a forked, shade-acclimated *Tsuga heterophylla* branch. The branch was collected at Findley Lake, WA, and sealed in a plastic bag for 48 hours to allow the xylem water potential to equilibrate in all parts of the branch (Ψ = -1.34 MPa). The cut end of the branch was dipped in melted paraffin wax, one fork of the branch was sealed in a plastic bag, and the entire branch was placed in a controlled environment chamber. In the chamber, the branch was sprayed repeatedly with deionized water. After nine hours, the xylem water potential of the unshielded fork had increased to -0.34 MPa. Although the shielded fork had been kept dry, after nine hours its water potential had risen to -0.56 MPa.

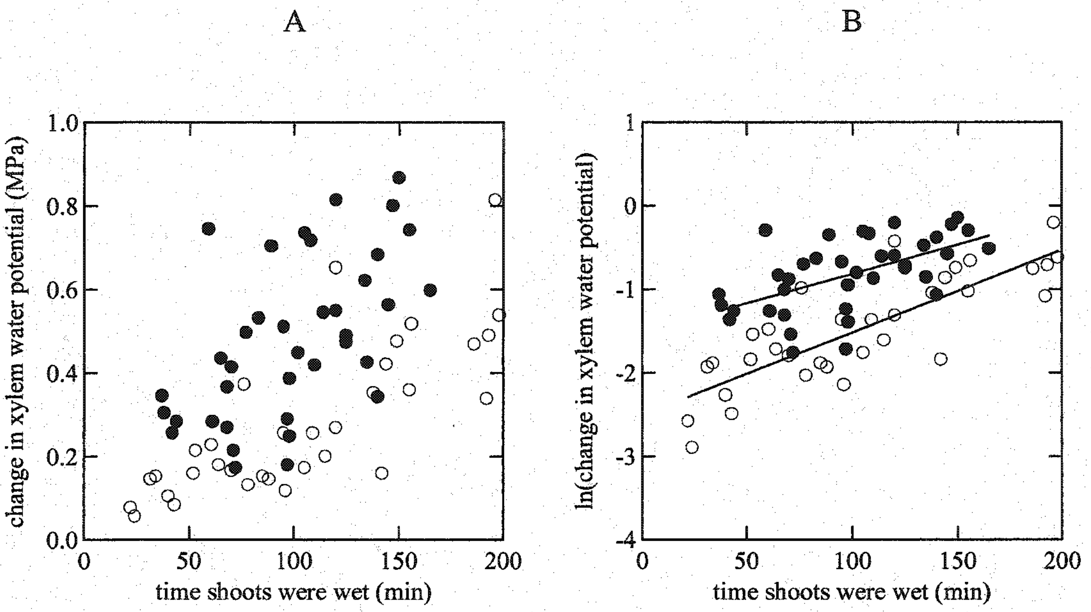


Figure IV.3. The change in Ψ_{xylem} of moistened *Abies amabilis* (n=41) and *Tsuga heterophylla* (n=31) shoots. A), untransformed data, B) log-transformed data; open symbols, sun-acclimated shoots; filled symbols, shade-acclimated shoots. The equation of the line fitted to the sun-acclimated shoots is: $\ln[\Delta\Psi_{xylem}] = 0.010\Delta t - 2.509$ ($r^2 = 0.82$, F-ratio = 63.6, $p < 0.001$). The linear equation fitted to the shade-acclimated shoots is: $\ln[\Delta\Psi_{xylem}] = 0.007\Delta t - 1.520$ ($r^2 = 0.57$, F-ratio 17.6, $p < 0.001$). The shoots were collected from the upper and lower crowns of mature codominant trees at Findley Lake, WA. Changes in Ψ_{xylem} were measured with a Scholander pressure bomb.

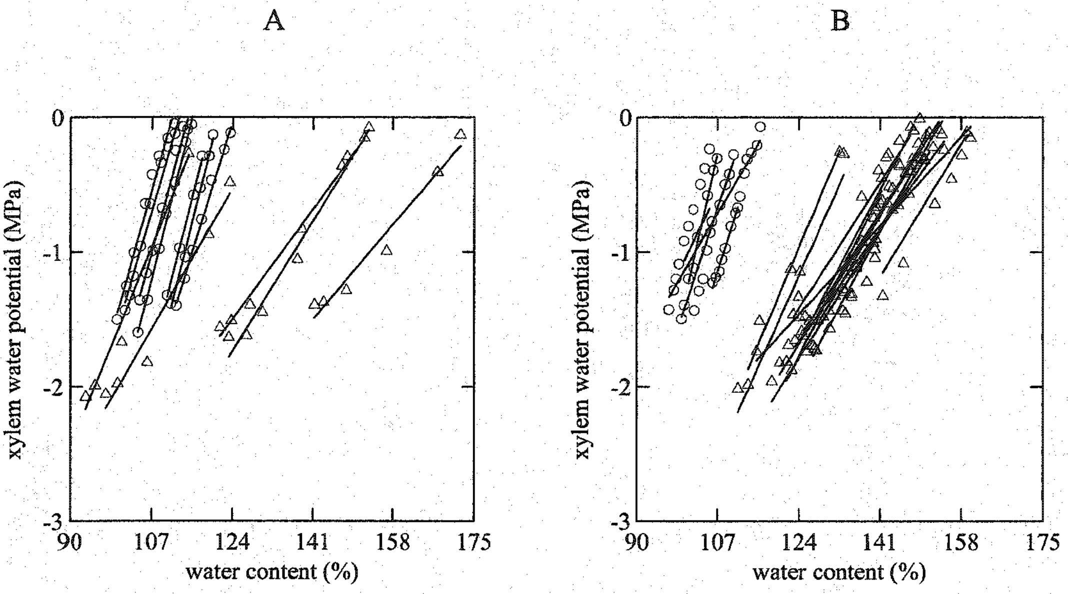


Figure IV.4. Pressure-volume relationships for detached shoots of *Abies amabilis* (n=12) and *Tsuga heterophylla* (n=20). A), *Abies amabilis*; B) *Tsuga heterophylla*. Circles, pre-budburst shoots; triangles, shoots with fully-expanded current-year needles. The shoots were collected from mature codominant trees at Findley Lake, WA.

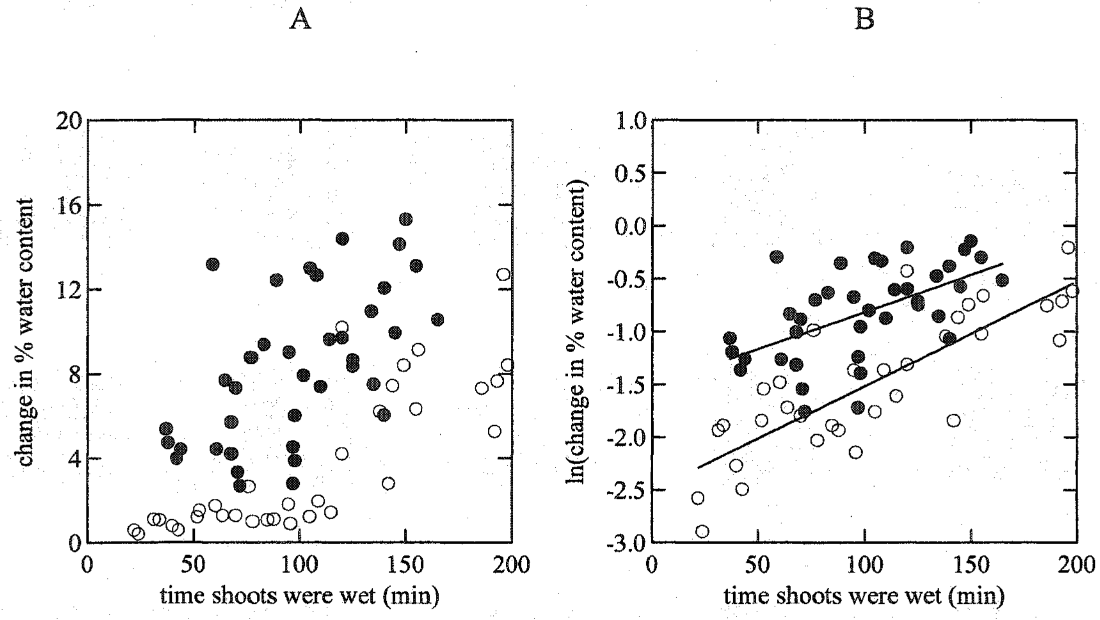


Figure IV.5. The change in percent water content of moistened *Abies amabilis* (n=41) and *Tsuga heterophylla* (n=31) shoots. Open symbols, sun-acclimated shoots (n=33); filled symbols, shade-acclimated shoots (n=39). The equation of the line describing the change in water content by sun-acclimated shoots is: ln[Δ% water content] = $0.016\Delta t - 0.828$ ($r^2 = 0.88$, F-ratio = 107.1, $p < 0.001$). The linear equation fitted to the change in percent water content for shade-acclimated shoots is: ln[Δ% water content] = $0.008\Delta t + 1.201$ ($r^2 = 0.59$, F-ratio = 19.3, $p < 0.001$). The shoots were collected from the upper and lower crowns of mature codominant trees at Findley Lake, WA. Changes in percent water content were related to $\Delta\Psi_{xylem}$ with information from pressure-volume relationships.

Chapter V. Quantifying water uptake by wet shoots of *Abies amabilis* and *Tsuga heterophylla* with shoot drying curves.

Introduction and objectives

The forest canopy in the Findley Lake Basin was wet for about one-third of all hours during the 1995 growing season (Figure II.1). Approximately 375 mm of precipitation fell upon the forest canopy during the 1995 growing season, but much of this water did not arrive at the forest floor. Throughfall was roughly 30% less than above-canopy precipitation at Findley Lake, and 10-15% less than precipitation at the midslope and summit of Findley Ridge (Figure II.2). The "black box" approach used in forest hydrology assumes that the difference between precipitation and throughfall is due to evaporation (McNaughton and Jarvis 1986). This assumption is well founded, since models of canopy evaporation, which are based on meteorological variables, often closely approximate actual evaporation losses (Gash *et al.* 1995). However, during the extensive hours that the Findley Basin canopy was wet, other water-consuming processes may have occurred in addition to evaporation. Specifically, some of the water may have been directly absorbed by the canopy. The uptake of water by plant shoots has been qualitatively demonstrated with isotopic tracers (Vaadia and Waisel 1963; Leyton and Armitage 1967), fluorescent dyes (Katz *et al.* 1989; Grammatikopolous and Manetas 1994; Munné-Bosch *et al.* 1999) and other techniques (Stone 1957). Nonetheless, the importance of the contribution of direct water uptake to apparent evaporation losses from wet forest canopies is not known.

Water uptake by wet shoots of *Tsuga heterophylla* and *Abies amabilis* will be examined in this chapter. In comparison to the large-scale measurements of canopy structure and throughfall described in chapters I and II, this chapter examines a phenomenon at the level of shoots and needles. The chapter is organized around two objectives. The first is to develop a technique to estimate the uptake of water by wet conifer shoots. The xylem water potential-based technique described in chapter IV provided evidence that wet shoots of *Abies* and *Tsuga* absorb water, but appeared vulnerable to error. Estimates from a more rigorous method are needed to quantify the acquisition of water by the wet foliage, and ultimately, the canopy of the Findley Lake Basin.

The second objective of the chapter is to estimate the conductance of *Abies amabilis* and *Tsuga heterophylla* shoots to both the outward and inward movement of water. It is postulated that *Abies amabilis* and *Tsuga heterophylla* shoots absorb water when wet. Next, it is postulated that conductance of shoots to water loss or water uptake will be a function of species and of shoot morphology. The movement of water through the surfaces of shoots will be assumed to occur by passive diffusion through relatively uniform, non-porous and non-living membranes (Schreiber and Riederer 1996a). Therefore, it is postulated that the conductance of *Abies amabilis* and *Tsuga heterophylla* cuticles to water efflux will be similar to the conductance of *Abies amabilis* and *Tsuga heterophylla* cuticles to water influx.

Materials and methods

A drying curve method for the estimation of water uptake by wet shoots: concepts

Chapter IV described experiments with the Scholander pressure bomb where the amount of water absorbed by shoots of *Abies amabilis* and *Tsuga heterophylla* was estimated from measured changes in xylem water potential. The technique appeared to have two sources of error: errors stemming from the interpretation of pressure:volume curves, and error from the possible injection of water into the tissues of shoots while inside the pressure chamber. This portion of the dissertation introduces a technique to estimate water uptake by wet, detached conifer shoots. The technique is based on the on the interpretation of shoot drying curves. It is important to emphasize that these experiments were conducted in a nearly light-free room to reduce the problem of water loss through stomata.

Cape and Percy (1996) developed a method to study the water relations of conifer needles. They carefully recorded the change in needle mass over time, and fitted the data with the expression:

$$R_t = R_\infty + (R'_0 - R_\infty)e^{-kt} \qquad (5.1)$$

where R_t is the leaf relative water content (RWC) at time t, R_∞ is the RWC of the sample in equilibrium with the surrounding air, R'_0 is the extrapolated RWC of the needles at time at time zero, and k is a rate constant. They used their technique to estimate the cuticular conductance (g_c), solute content, and relative water content of excised *Picea rubens* and *Picea abies* needles.

In this study, an approach similar to the Cape and Percy method was used to estimate changes in the mass of shoots that had been moistened with deionized water. However, instead of using relative water content, total shoot weight was used. As discussed in the next section, this different approach provided an estimate of the quantity of water absorbed through the surfaces of moistened shoots.

Envision a wet conifer shoot as having two storage pools of water: one pool is held by shoot tissues, and the other is moisture that adheres to the shoot's surface. The external pool loses water rapidly by evaporation, while the internal pool loses water slowly, primarily by diffusion across barrier membranes (Figure V.1). The amount of water that is held by the wet shoot is the sum of the two storage pools:

$$W_{total} = W_{shoot} + W_{surface} \qquad (5.2)$$

where W_{total} is the total quantity of water held by the shoot, W_{shoot} is the water held in the tissues of the shoot, and $W_{surface}$ is the water present on the shoot surface. The water content of either storage pool at time t is given by:

$$W_t = W_0 e^{-kt} \qquad (5.3)$$

where W_0 = the initial amount of water in a given storage pool, W_t is the quantity of water remaining in the storage pool time t, and k is a rate constant determining the rate of loss from the storage pool. Since the amount of water retained by a shoot is equal to the sum of the two storage pools, the quantity of water held by a shoot at time t is estimated by:

$$W_{total(t)} = W_{shoot(0)} e^{-k_{shoot}t} + W_{surface(0)} e^{-k_{surface}t} \qquad (5.4)$$

Equation 5.4 is the basis of the weight loss model used in my study. Tyree *et al.* (1992) used an equation of this form to model the diffusion of KCl across isolated cuticles of *Citrus aurantium.* Equation 5.4 models a composite process of additive, non-interacting fast and slow rates. Tyree *et al.* interpreted the fast rate to be movement of KCl through dynamic polar pores in the cuticle, and speculated that the slow rate was diffusion through wax. $W_{surface} = 0$ when the shoot is surface-dry, and equation 5.4 simplifies to:

$$W_{total(t)} = W_{shoot(0)} e^{-k_{shoot}t} \qquad (5.5)$$

The value of the rate constant $k_{surface}$ is much greater than the value of k_{shoot}, since $k_{surface}$ is primarily determined by the boundary layer conductance of the shoot, while k_{shoot} integrates the conductances of highly effective moisture barriers in the periderm and cuticle. The derivative of the two halves of equation 5.4 provides estimates for the moisture fluxes from the two storage pools, W_{shoot} and $W_{surface}$:

$$J_{shoot} = W_{shoot}(-k_{shoot} e^{-k_{shoot}t}) \qquad (5.6)$$

$$J_{surface} = W_{surface}(-k_{surface}e^{-k_{surface}t}) \qquad (5.7)$$

Each flux is associated with a conductance. The first, $g_{surface}$, is a composite of the conductances that control the rapid rate of water loss, $J_{surface}$. It is largely determined by the conductance of the boundary layer of air that surrounds the shoot. The second conductance, $g_{shoot}^{\min}$, corresponds to the conductance of the shoot after the stomatal closure. The minimum shoot conductance is often used as a proxy for the more difficult-to-measure (because of the difficulty associated with isolating stomata and their uncertain contribution towards water loss) cuticular conductance, g_c (Kersteins 1996). In addition to the loss of water through the cuticle, shoots may also lose water through the periderm. However, for a typical shoot the total surface area of the cuticle is substantially greater than the surface area of the periderm. Since the conductances of the cuticle and the periderm are approximately equal in trees (Garrec and Barrois, 1992; Schönherr and Ziegler, 1980), $g_{shoot}^{\min}$ should approximate g_c.

Cuticular conductance will be expressed in two ways in this chapter. The conductance of the cuticle to the outward flux of water, or cuticular transpiration, will be denoted by $g_{c_{out}}$. The conductance of the cuticle to the inward flux of water will be symbolized by $g_{c_{in}}$. It is first postulated that $g_{c_{out}}$ and $g_{c_{in}}$ are approximately equal.

Even under conditions that induce maximal stomatal closure, an unknown amount of water will inevitably escape through damaged or partially closed stomata (Kersteins 1996). Water loss through open stomata may contribute to either J_{shoot} or $J_{surface}$. Given that typical stomatal conductances are twenty to one hundred times or more greater than cuticular conductances (Nobel 1999), water flux through open stomata will most likely be partitioned to the fast rate, $J_{surface}$. The drying curve method being presented here, therefore, has a bias toward under estimating the amount of water absorbed by wet shoots.

Fick's first law is used to calculate the conductances:

$$g = \frac{flux}{a\Delta c_i} \qquad (5.8)$$

where a is the effective surface area across which the flux occurs, and Δc_i is the difference in concentration of water on either side of the relevant barrier (Nobel 1999). To facilitate scaling the results of these experiments with shoots to the canopy, a will be assumed to equal two times the shadow area of the needles flattened against a smooth surface. The concentration gradient, Δc_i, will be derived by assuming that the Ψ of free water on the surface of the shoots = 0, and the Ψ of water in the xylem of the shoots is –1.3 MPa. The latter estimate is based from the experiments with the Scholander pressure bomb.

With the method that is being presented here, the contribution of W_{shoot} to the total shoot mass is inferred, rather than obtained through direct measurement. When a shoot is wet, water from both internal and external pools contributes towards its mass. The water content of a wet shoot then is most accurately termed as the apparent water content (*AWC*). If W_{shoot} and $W_{surface}$ are expressed on a mass basis, the apparent water content is given by:

$$AWC = \frac{(W_{shoot} + W_{surface} + m_d) - m_d}{m_d} \bullet 100 \qquad (5.9)$$

where m_d is the oven-dry mass of the shoot. Note that when the shoot is dry and $W_{surface} = 0$, the shoot AWC is the same as the shoot water content.

A drying curve method for the estimation of water uptake by wet shoots: comparison with the Cape and Percy model (1996).

Cape and Percy (1996) used equation 5.1 to fit the drying curves of excised, fully hydrated conifer needles for time periods following the initiation of evaporation between 3 and 55 hours (Cape and Percy 1996). The model did not fit the rapid loss in needle mass from t=0 to t=3 hours; this phase was attributed to transpiration through stomata. Their method cannot be used to model the drying curves of wet shoots. Cape and Percy (1996) used the initial fresh weight of fully hydrated needles to determine their relative water content. The fresh weight of a wet shoot cannot be known due to the uncertain mass of water adhering to its surfaces.

The Cape and Percy model has advantages in comparison to the weight loss model used in this dissertation (equation 5.4). The Cape and Percy model has an asymptote, R_∞, which is an estimate of the equilibrium needle (or shoot) water content. Equation 5.4 does not have an asymptote, and as a consequence predicts incorrectly that the water content of a wet shoot trends toward zero over large time scales. The Cape and Percy model is the appropriate model to use for surface dry needles or shoots, especially when information such as solute content is desired. However, the Cape and Percy model does not fit the initial period of change in needle or shoot mass, when essentially all of the water on the surface of a wet shoot would be lost. In comparison, equation 5.4 smoothly fits the initial (t < seven hours) drying curves of wet shoots, when the rate of water loss is governed by a combination of both fast and slow conductances. Equation 5.4 partitions water content into internal and external pools, and is therefore useful for determining the initial water content of a wet shoot or needle.

A drying curve method for the estimation of water uptake by wet shoots: experimental procedures

Sun- and shade-adapted shoots were collected during morning hours from the crowns of mature *Tsuga heterophylla* and *Abies amabilis* trees at Findley Lake, WA (elevation = 1140 m) and near Talapus Lake (elevation = 1000 m) 8 km west of Snoqualmie Pass, WA. The shoots were placed in a ziplock bag as they were clipped from the crown, and were then rapidly transferred to an ice chest for immediate transport to the Tree Physiology Lab at the University of Washington. The shoots were stored overnight in a refrigerator at 3°C.

The following day the ends of the shoots were re-cut and sealed with melted paraffin. The surface of the shoots was typically moist with water that had condensed during storage. The shoots were randomly assigned to either the control (n=10) or treatment (n=10) group, and suspended from a wire in a well-ventilated, darkened room. During the shoot uptake experiments, the temperature of the room varied between 19.5 and 21°C, and the relative humidity was approximately 35 ± 5%. Each shoot was weighed at approximately ten to fifteen minute intervals on an analog balance. Shoots that lost needles during the experiment were eliminated from the data. After the shoots were surface-dry (Δshoot mass/Δt $\approx$ constant) and had been weighed at least six times during an approximate period of 100 minutes, each shoot in the treatment group was sprayed for one minute with deionized water, and then sealed in a ziplock bag. The Ψ_{xylem} of the shoots after the initial period of desiccation and before treatment with deionized water was assumed to be –1.3 MPa. Treated shoots were re-irrigated with deionized water at 30-minute intervals. After ± 120 minutes, the treated shoots were removed from their ziplock bags, blotted dry with paper toweling, weighed, and re-suspended from the wire to dry for at least 200 minutes. Shoots in the control group were also sprayed for one minute with deionized water, but were then immediately blotted dry with paper toweling, weighed, and then hung from the wire to dry for 200 minutes. During the 200 minute drying period, both the control and treatment groups of shoots were re-weighed at ten to fifteen minute intervals.

For each shoot, the shadow area of a sub-sample of needles pressed flat under a pane of glass was measured with the Optimas Digital Analyzer. The stems and needles from each shoot were dried separately for 48 hours at 70°C. The fresh weights recorded during the experiment were converted to apparent water content (AWC). ΔAWC/time for each shoot was fitted to equation 5.4 using least squares non-linear regression (SYSTAT v. 6) to obtain estimates of W_{shoot}, $W_{surface}$, g_{shoot}, and $g_{surface}$ for each of the two drying curves.

The shoot drying curves were analyzed as follows (refer to Figure V.2). Assume that the pool of water held by the tissues of a well-hydrated *Tsuga heterophylla* shoot (W_{shoot}) is at its maximum value at the start of the experiment. The shoot is probably coated with a thin film of moisture ($W_{surface}$) that condensed during overnight storage, adding to the shoot AWC. A ± 100-minute period of slow desiccation is initiated, during which the shoot is repeatedly weighed. During this period of desiccation, the AWC of the shoot declines as $W_{surface}$ is depleted at a rapid rate by evaporation. After $W_{surface}$ has been lost to evaporation, AWC ≈ true water content, and the water remaining in the shoot is held in the pool W_{shoot}. The slow rate of moisture loss from W_{shoot} is determined by the combined conductances of the needle cuticle and twig periderm. Equation 5.4 is used to fit the drying curve to the change in shoot mass ($\Delta W_{shoot} + \Delta W_{surface}$) over time, while equation 5.6 describes the flux of water from the shoot per unit time. The value of W_{shoot} at t = 0 is an estimate of the initial shoot AWC.

After the initial period of desiccation, the shoot is moistened with deionized water for ± 120 minutes. A second drying curve is needed to estimate the quantity of water that may have been absorbed content by the shoot. Initially, the shoot is wet. $W_{surface}$ contains an ample quantity of water, and W_{shoot} may have changed during treatment due to moisture uptake through the periderm or cuticle. As before, $W_{surface}$ is rapidly depleted by evaporation, and the slope of the drying curve flattens once $W_{surface}$ is exhausted. Equation 5.4 is used to find $W_{shoot(0)}$, an estimate of the shoot water content following treatment. The difference between the pre-treatment and post-treatment values of W_{shoot} is an estimate of the change in shoot water content due to moisture uptake.

The water fluxes from the drying shoots estimated with equation 5.6 were used to derive apparent values of $g_{c_{out}}$. The flux used to estimate $g_{c_{in}}$ was determined by dividing the change in apparent water content due to treatment, by the duration of treatment with deionized water. Cuticular conductances were expressed in terms of area (units m s^{-1}) and mass (units $m^3g^{-1}s^{-1}$). Area-based conductance is a convenient scaling variable, and is the standard method for expressing cuticular conductance (Kersteins 1996). However, mass-based expressions of conductance can sometimes reveal more useful information than area-based estimates (Tipton and White 1995). To estimate conductances, the shoot-to-air water vapor pressure gradient was estimated using measured values for air temperature and relative humidity, but Ψ_{xylem} was assumed to be –1.3 MPa.

The driving force for $g_{c_{out}}$ was assumed to be the shoot-to-air gradient in water vapor concentration. Using Fick's First Law (equation 5.8), $g_{c_{out}}$ was estimated by:

$$area - based\ g_{c_{out}} = \frac{\frac{\Delta AWC}{2a}}{c^*_{wv} e^{\frac{\bar{V}_w \Psi_{xylem}}{RT}} - c^*_{wv} \frac{rH}{100}} \qquad (5.10)$$

where ΔAWC is the change in apparent water content over time, a is the shadow needle area of the shoot (cm^2), c^*_{wv} is the saturation concentration of water vapor (gm^{-3}), $\overline{V}_w$ is the partial molal volume of water (1.807×10^{-5} m^3 mol^{-1}), Ψ_{xylem} is the xylem water potential of the shoot (assumed to be –1.3 MPa), R is the real gas constant (8.314×10^{-6} m^3 MPa mol^{-1} K^{-1}), T is the ambient temperature (°K), and rH is the ambient relative humidity. Using measured values for water loss by an *Abies amabilis* sun-adapted shoot (leaf area = 402.77 cm^2) at 25°C and 42% relative humidity, equation 5.10 becomes:

$$g_{c_{out}} = \frac{0.000335\, gH_2O\, m^{-2} s^{-1}}{22.85\, gH_2O\, m^{-3} - 9.69\, gH_2O\, m^{-3}} = 2.55\, x 10^{-5}\, ms^{-1}$$

Mass-based $g_{c_{out}}$ was estimated by substituting *m*, the oven-dry mass of the treated shoot for *a* in equation 5.10.

Area-based $g_{c_{in}}$ was obtained in a similar manner. In the case of $g_{c_{in}}$, however, the driving force was assumed to be the water vapor concentration gradient between water coating the surfaces of the shoot, and the internal shoot tissues:

$$area\text{-}based\ g_{c_{in}} = \frac{\dfrac{\Delta AWC}{2a}}{c^*_{wv} - c^*_{wv} e^{\frac{\bar{v}_w \Psi_{xylem}}{RT}}} \qquad (5.11)$$

As before, *m* was substituted for *a* in equation 5.11 to express $g_{c_{in}}$ in terms of mass. When actual values of the parameters used to estimate $g_{c_{in}}$ for the same *Abies* amabilis sun-adapted shoot referred to above are inserted in equation 5.11, the expression becomes:

$$\textit{area-based } g_{c_{in}} = \frac{0.000137\, gH_2O\, m^{-2} s^{-1}}{23.07\, gH_2O\, m^{-3} - 22.85\, gH_2O\, m^{-3}} = 6.30 x 10^{-4}\, ms^{-1}$$

The treatment period in the majority of the drying curve experiments was standardized to 110 – 130 minutes. A slightly different experiment was conducted to test the effect of the duration of treatment on ΔAWC and $g_{c_{in}}$. In this experiment, sun-adapted *Abies amabilis* shoots were misted for either one minute (n=10), 130 minutes (n=10) or 370 minutes (n=10). The resulting changes in AWC were estimated with drying curves.

Results

Change in shoot water content

Surface wetting increased the water content of shade-adapted shoots of *Abies amabilis* and *Tsuga heterophylla* (Figure V.3). The AWC of treated, shade-adapted shoots of *Abies amabilis* increased nearly five times that of control shoots (0.80 vs. 0.17%). For treated shade-adapted *Tsuga heterophylla* shoots, the difference was almost seven-fold (1.33 vs. 0.19%). For both species, the increases in the water content of shade-adapted shoots moistened with water were significantly greater than the mean increases in the water content of the controls ($p<0.01$, Student's t-test).

Treatment with deionized water also enhanced the AWC of sun-adapted shoots of *Abies* and *Tsuga* (Figure V.3). For *Abies amabilis,* treatment increased the mean AWC 0.39 ± 0.09 percent (n=20), while the AWC of treated *Tsuga heterophylla* sun-adapted shoots rose 0.25 ± 0.16 percent (n=14). In comparison, the AWC of the control group of *Abies amabilis* sun shoots increased 0.19 ± 0.06 percent, while the AWC of the *Tsuga heterophylla* sun shoot control group fell -0.05 ± 0.06 percent (n=19). The contrast between the means of the treatment and control groups of sun-adapted shoots of both species was less robust ($p<0.10$, Student's t-test) than the contrast between the mean increases in AWC of the treated and untreated shade-adapted shoots. The data for the change in percent AWC of *Tsuga heterophylla* shoots were more variable (coefficient of variation = 2.1) than the same data for *Abies amabilis* shoots (coefficient of variation = 1.1). The difference may have been due to the occasional loss of delicately attached *T. heterophylla* needles during the experiments.

Treatment with deionized water accounted for 23% of the variance in the water content of the detached shoots (Table V.1), supporting the hypothesis that shoots of *Tsuga heterophylla* and *Abies amabilis* can absorb water when wet. Shoot morphology was also an important factor, accounting for 16% of the variance in the change in water content. The treatment*shoot morphology effect was strongly significant (F-ratio 32.2, $p<0.01$), reflecting the greater sensitivity of shade-adapted shoots to treatment with de-ionized water. As a single factor, species was not significantly related to the change in shoot AWC. However, the species*shoot type interaction was significant (F-ratio 18.4, $p<0.01$), explaining 5% of the variance in the data. *Tsuga heterophylla* shade-adapted shoots were apparently more absorptive than *Abies amabilis* shade-adapted shoots. The large amount of variance attributable to error (44%) had many potential sources, including needle drop, epiphyllic debris, measurement inaccuracy, error in interpreting the drying curves, month-to-month physiological changes in the shoots, and other factors.

Another way to examine the ability of wet conifer shoots to absorb water is to examine the relationship between uptake and time. If moistened shoots are able to absorb water, then the change in water content should be related to the duration of wetness. A long-duration experiment provided additional evidence that wet shoots can acquire water. The mean change in the AWC of a group of *Abies amabilis* sun-adapted shoots treated for 370 minutes was nearly three times greater than the change of the water content of a group of *Abies* shoots treated for 130 minutes (Figure V.4A). The slope of ΔAWC between two and six hours was apparently linear.

Shoot conductance to water influx ($g_{c_{in}}$)

Shoot morphology was the only factor that significantly affected area-based estimates of $g_{c_{in}}$, (F-ratio = 11.1, p<0.01) accounting for about 15% of the variability in the data (Table V.2). The area-based $g_{c_{in}}$ of shade-adapted shoots was generally greater than the area-based $g_{c_{in}}$ of sun-adapted shoots (Figure V.5A). However, the factors species and species*shoot morphology were not significant (Table V.2), and there were no significant differences between the group means (Table V.3).

The estimates of mass-based $g_{c_{in}}$ were less variable than the area-based estimates, and the trends were more robust (Figure V.5B). The influence of shoot morphology on mass-based estimates of $g_{c_{in}}$ was highly significant (F-ratio = 69.2, p<0.01), explaining about 40% of the variability in the data. Species significantly influenced mass-based $g_{c_{in}}$ (F-ratio = 18.6, p<0.01), as did the interaction factor species*shoot morphology (F-ratio 15.3, p<0.01). The mean mass-based $g_{c_{in}}$ of shade-adapted shoots of the two species was greater than the mean $g_{c_{in}}$ of sun-adapted shoots (Table V.3). The high mass-based $g_{c_{in}}$ of the *Tsuga heterophylla* shade-adapted shoots, substantially greater than the $g_{c_{in}}$ of *Abies* shade-adapted shoots (Table V.3), accounted for most of the significance of the species effect.

The duration of treatment did not have a discernable effect on $g_{c_{in}}$ over the time intervals that were tested. In the long-duration trial, the mean $g_{c_{in}}$ of shoots kept moist for 130 minutes was not significantly different than the mean $g_{c_{in}}$ of shoots moistened for 370 minutes (Figure V.4B).

Shoot conductance to water loss ($g_{c_{out}}$ *)*

The microenvironments of the upper and lower canopy at Findley Lake differ in net solar insolation, maximum leaf temperature, atmospheric turbulence, and evaporative stress. Since the shoots of the upper canopy develop in an environment where moisture stress is high, it was expected that the $g_{c_{out}}$ of sun-adapted shoots would be less than the $g_{c_{out}}$ of shade-adapted shoots. It was also expected that *Tsuga heterophylla*, considered to be more drought-resistant than *Abies amabilis* (Kotar 1972), would develop water-conserving cuticles with lower conductances to water vapor than *Abies amabilis*.

The area-based estimates of $g_{c_{out}}$ revealed few differences between the conductances of the detached *Abies* and *Tsuga* shoots (Figure V.6A and Table V.4). Within each species, there were no significant differences between the $g_{c_{out}}$ of shade- and sun-adapted shoots (Table V.4). The $g_{c_{out}}$ of *Tsuga heterophylla* shade-adapted shoots was slightly but significantly lower than the $g_{c_{out}}$ of both the shade- and the sun-adapted *Abies amabilis* shoots ($p<0.05$, Bonferroni's multiple comparison). An ANOVA analysis (Table V.5) also showed that the effect of species was significant, with the area-based $g_{c_{out}}$ of *Tsuga heterophylla* tending to be lower than the area-based $g_{c_{out}}$ of *Abies amabilis*.

A different pattern of relationships between shoot morphology, species, and cuticular conductance emerges when $g_{c_{out}}$ is derived from the flux of water vapor per gram of dry needle mass (Figure V.6B and Table V.4). In contrast to the area-based data, the effects of shoot morphology and species were both significant influences on the mass-based estimates of $g_{c_{out}}$, and accounted for the most of the variability in the data (Table V.5). Shoot morphology more strongly influenced the estimates of $g_{c_{out}}$ than did the effect of species. For both *Abies amabilis* and *Tsuga heterophylla*, the mean mass-based $g_{c_{out}}$ of shade-adapted shoots was significantly greater than the mean mass-based $g_{c_{out}}$ of sun-adapted shoots ($p<0.01$, Bonferroni's multiple comparison). The significance of the species effect was due to the high mass-based $g_{c_{out}}$ of the *Tsuga heterophylla* shade-adapted shoots (7.3×10^{-7} m^3 g^{-1} s^{-1}) in comparison to the $g_{c_{out}}$ of shade-adapted *Abies amabilis* shoots (5.2×10^{-7} m^{-3} g^{-1} s^{-1}). The mean mass-based estimates of $g_{c_{out}}$ for the sun-adapted shoots of the two species were not significantly different (Table V.4).

The patterns observed for the change in AWC of the detached shoots (Figure V.3) paralleled the trends found for mass-based $g_{c_{out}}$, but not for area-based $g_{c_{out}}$. Shade-adapted shoots showed both: 1) greater mean mass-based values of $g_{c_{out}}$ and 2) larger increases in water content after treatment with deionized water than did sun-adapted shoots. The effect of shoot morphology accounted for most of the variability in ΔAWC of detached shoots after treatment with deionized water (Table V.1). As shown, species was the most important variable influencing area-based $g_{c_{out}}$ (Table V.5). However, species could not be strongly linked to changes in the AWC of surface-wetted detached shoots (Table V.1), nor was species the most significant predictor of the variation in mass-based $g_{c_{out}}$ (Table V.5).

Comparison of $g_{c_{out}}$ *and* $g_{c_{in}}$.

Schreiber and Riederer (1996) presented compelling evidence that the loss and uptake of water through the cuticle occurs by diffusion and through a common pathway. Their study gave strong support to the simple diffusional model of transcuticular water flux. If the simple diffusional model accurately describes water flux through cuticles, then $g_{c_{in}}$ should be simply the reverse of $g_{c_{out}}$, and—since water either entering or exiting a cuticular membrane would diffuse through a similar domain—the two conductances should be of approximately equal value.

The estimates of mass-based $g_{c_{out}}$ and mass-based $g_{c_{in}}$ indicated that irrespective of the direction of water flux, the conductivity of the cuticles of shade-adapted shoots was greater than the conductivity of the cuticles of sun-adapted shoots. The results for mass-based $g_{c_{out}}$ and mass-based $g_{c_{in}}$ also suggest that *Tsuga heterophylla* shade-adapted shoots were markedly more conductive to water than the three other shoot types. However, the values of $g_{c_{in}}$ estimated for detached *Tsuga heterophylla* and *Abies amabilis* shoots were greater than the corresponding estimates of $g_{c_{out}}$ (Figure V.7). The $g_{c_{in}}$ of the sun-adapted shoots of the two species was about ten times greater than $g_{c_{out}}$. The gap between $g_{c_{in}}$ and $g_{c_{out}}$ was even greater for shade-adapted shoots of *Abies amabilis* and *Tsuga heterophylla*: for these shoots, $g_{c_{in}}$ was approximately twenty times greater than $g_{c_{out}}$. The influence of the direction of water flux on the conductance of the detached shoots was highly significant (F-ratio = 513.1, $p<0.01$). Contrasts of similar significance were seen between the area-based estimates of $g_{c_{in}}$ and $g_{c_{out}}$.

Although there was at least an order of magnitude difference between $g_{c_{in}}$ and $g_{c_{out}}$ for the same species and shoot type, there was a link between the two conductances. The mass-based estimates of $g_{c_{out}}$ and $g_{c_{in}}$ (Figure V.8B) were significantly related (F-ratio 43.5, $p<0.01$). A similar relationship, however, was not found between area-based $g_{c_{in}}$ and area-based $g_{c_{out}}$ (Figure V.8A).

Discussion

Water uptake by wet Abies amabilis and Tsuga heterophylla shoots.

Water on the foliage of conifers has been shown to increase the survival (Stone 1957a, 1957b) and promote the growth (Boucher *et al.* 1995) of seedlings, or improve the water status of desiccated shoots or whole trees (Johnston 1964; Katz *et al.* 1989; Boucher *et al.* 1995). These findings suggest that conifers may be able to acquire water directly from wet needles or stems. Motivated by these earlier works, a hypothesis examined in this dissertation was that wet *Abies amabilis* and *Tsuga heterophylla* shoots absorb water from their moistened surfaces. Indeed, the experiments with the Scholander pressure bomb provided qualitative evidence that wet shoots are capable of water uptake (Chapter IV).

Multiple lines of evidence suggested that the moistened shoots absorbed water. Deionized water increased the apparent water content of treated shoots (Table V.1 and Figure V.3). The water content of shoots treated with mist for 120 minutes increased more than the water content of the controls (Student's t-test, $t=8.77$, $p<0.001$). In the experiments with the Scholander pressure bomb, moistening shoots with deionized water increased the Ψ_{xylem} of the treated shoots (Table IV.1). The changes in Ψ_{xylem} suggested that the tissues of the treated shoots absorbed water. A dose response was observed in the pressure bomb experiments: the longer that the shoots were moistened, the more Ψ_{xylem} increased (Figure IV.3). Similarly, the water content of *Abies amabilis* sun-adapted shoots also increased in proportion to the time of exposure to deionized water (Figure V.4A). Interestingly, the relationship between ΔAWC and time appeared to be linear, suggesting that the gradients that drive water uptake by wet shoots were maintained for at least the first six hours that shoots are moistened with water. As a whole, these findings support the hypothesis that *Abies amabilis* and *Tsuga heterophylla* shoots absorb water when moistened with water.

The control group of shoots was included in these experiments to test the possibility that changes in apparent water content may have been due to hydration of shoot surfaces, or to water absorption by hygroscopic substances adhering to the shoot. The control shoots were moistened with water for one minute. Their increase in apparent water content was small, but significantly different from zero (Student's t-test, $t=2.97$, $p<0.01$), indicating either: 1) water retention by lichens, molds, dust, aphid secretions or other substances coating shoot surfaces; 2) hydration of the cuticle or the periderm; or 3) a small amount of water penetration into the shoot.

The amount of water absorbed by the wet shoots was small. There are few published values to place the results of this study into context. Katz *et al.* (1989), who used the $\Delta\Psi_{xylem}/\Delta t$ technique to study water absorption by wet *Picea abies* shoots, provided the single literature value for water uptake by conifers: a 6 to 8% increase in AWC after 200 minutes of misting with deionized water. Their estimate was more than 3.5 times greater than the apparent rate of uptake by *Tsuga heterophylla* shade-adapted shoots, the most absorptive of the four types of shoots examined in this study.

The estimates for water uptake by detached *Tsuga* and *Abies* shoots determined by the drying curve technique are also lower than the estimates provided by the $\Delta\Psi_{xylem}/\Delta t$ method for the same species (Chapter IV). The $\Delta\Psi_{xylem}/\Delta t$ method yielded estimated increases in shoot AWC of 3.7 and 8.0% for sun-adapted and shade-adapted shoots, respectively (duration of treatment =

120 minutes). These estimates scaled up to 6930 liters of water absorbed per hectare for a precipitation event of average duration (20.4 hours) at Findley Lake. Using similar assumptions (SLA sun shoot ≈ 40 cm^2 g^{-1}, SLA shade shoot ≈ 100 cm^2 g^{-1}, Findley Lake LAI = 8.5 m^2 m^{-2}) canopy water uptake at Findley Lake would be in the range of 540 to 1060 l ha^{-1} when based on the results from the drying curve experiments.

The influence of shoot morphology on conductance to water loss and water gain.

There are at least three types of influences on within-species variation in cuticle properties: 1) environmental limits on carbon fixation, 2) physiological stress, and 3) factors that interfere with plant metabolism. These factors relate to the quantity of carbon fixed by leaves, and to carbon allocation in developing leaves. Once cuticles have formed, a fourth factor, age, becomes important due to the inevitable breakdown in cuticle integrity over time (see review in chapter III).

Sun- and-shade-adapted *Abies amabilis* and *Tsuga heterophylla* shoots develop in environments that vary significantly with respect to light and height. Height certainly relates to light availability and carbon fixation, but also to water stress: Ψ_{xylem} decreases 0.03 MPa per meter due to the combined influences of gravity and friction (Nobel 1999). Additionally, the exchange of humidified air entrained in the canopy with drier air from the bulk atmosphere tends to increase with height in a forest canopy, resulting in larger leaf to air water vapor gradients, and greater potential rates of transpiration. This greater potential, in turn, affects both internal and external needle morphology. For these reasons, it was postulated that in addition to height related differences in the morphology of shoots and needles (Figure I.4), the properties of cuticles that control water movement would also vary with canopy position.

Elsewhere in this dissertation, it was shown that the cuticles of shade-adapted needles were more wettable than the cuticles of sun-adapted needles (Table II.3, Figure II.9). Whereas the functional relationship between surface wettability and cuticular conductance is not known, the wettability results suggested that a suite of cuticular properties also varies with shoot morphology. In the experiments with the Scholander pressure bomb, it was found that shoot morphology was significantly related to changes in the Ψ_{xylem} of shoots treated with deionized water (Table IV.1): the Ψ_{xylem} of shade-adapted shoots was more sensitive to wetting than the Ψ_{xylem} of sun-adapted shoots (Figure IV.3). The mass-based estimates of $g_{c_{out}}$ and $g_{c_{in}}$ showed a significant effect of shoot morphology, as did the area-based estimates of $g_{c_{in}}$.

The experiments based on shoot drying curves demonstrated that shoot morphology was not significantly related to the area-based $g_{c_{out}}$ of *Abies amabilis* and *Tsuga heterophylla* (Table V.1). Similar results were noted by Heinsoo and Koppel (1999), who found that the area-based $g_{c_{out}}$ of *Picea abies* varied little with depth in the canopy. The mass-based $g_{c_{out}}$ of the two species, however, was strongly linked to shoot morphology (Table V.5), with the $g_{c_{out}}$ of shade-adapted shoots greater than the $g_{c_{out}}$ of sun-adapted shoots (Table V.4). Shoot morphology was a significant influence on the area- and mass-based $g_{c_{in}}$ of *Abies* and *Tsuga* (Table V.2). The trend was more robust for mass-based $g_{c_{in}}$. From either area- or mass-based perspectives, the $g_{c_{in}}$ of shade-adapted shoots was greater than the $g_{c_{in}}$ of sun-adapted shoots (Table V.3). The majority of the data presented in this dissertation support the hypothesis that the cuticular conductances of sun- and shade-adapted shoots are different.

The effect of species on conductance to water loss and water gain

Cuticular conductance varies significantly among plant species (Schönherr and Schmidt 1979; Lendzian *et al.* 1986; Hadley and Smith 1990a; Baur *et al.* 1996; Becker *et al.* 1996; Kersteins 1996; Schreiber *et al.* 1996; Schreiber and Riederer 1996a; Schreiber and Riederer 1996b; Baur *et al.* 1997; Niederl *et al.* 1998; Riederer and Schreiber 2001; Anfodillo *et al.* 2002). The large variation in inter-specific cuticular conductance reflects basic differences in the structure of plant cuticles (Holloway 1982a), as well as finer-scale differences in the arrangement of the lipid layer (Schreiber *et al.* 1996; Baur *et al.* 1997). Cuticular conductance has been shown to reflect plant adaptation to broadly defined climatic zones (Schreiber and Riederer 1996b). Within a region, cuticular conductance has been linked to species tolerances to desiccation (Hadley and Smith 1990a). Given the importance of species with respect to cuticular conductance, it was anticipated that the cuticular conductances of *Abies amabilis* and *Tsuga heterophylla* would be different.

Species significantly influenced the mass- and area-based $g_{c_{out}}$ (Table V.5), and mass-based $g_{c_{in}}$ (Table V.2). The associations between species and cuticular conductance were less robust than the relationships between shoot morphology and conductance (Tables V.2 and V.5). While species was less important than shoot morphology, the effect of species still accounted for some of the variability in the estimated $g_{c_{out}}$ and $g_{c_{in}}$ of *Tsuga* and *Abies* shoots. The hypothesis that cuticular conductance is a function of species was moderately supported by these data.

Although the effect of species on $g_{c_{in}}$ and $g_{c_{out}}$ was significant, it is not clear how much of the species effect can be explained by intrinsic differences between the properties of *Abies amabilis* and *Tsuga heterophylla* cuticles, and how much is due to other species-dependent variables. Irrespective of how conductance was expressed (area- or mass-based) or the direction of water flux, the cuticular conductances of sun-adapted shoots were mostly similar (Figures V.5 and V.6). The significance of the species effect appeared to be rooted in variability of the mass-based conductances of shade-adapted shoots (Figures V.5B and V.6B). A portion of this relationship can be explained by species-related variation in specific leaf area (SLA).

In the Findley Lake Basin, mature *Tsuga heterophylla* were more plastic with respect to SLA than *Abies amabilis*. The mean specific leaf area (SLA) of the *Tsuga heterophylla* shade-adapted shoots used in these experiments (119.5 cm^2g^{-1}) was nearly three times greater than the SLA of *Tsuga* sun-adapted shoots (45.0 cm^2g^{-1}). In contrast, the mean SLA of the *Abies amabilis* shade-adapted (SLA=63.4 cm^2g^{-1}) and sun-adapted shoots (SLA=34.7 cm^2g^{-1}) varied by roughly two (see the discussion on canopy architecture in chapter II). The mass-based estimates of g_c tracked the trends in SLA, and the two variables were significantly correlated (Pearson's correlation coefficient = 0.80, $p<0.01$). Nonetheless, the effect of species was significant even when SLA was added to the ANOVA models for conductance.

The relationship between species and cuticular conductance must include effects beyond species-related differences in morphological plasticity. Differences in needle retention may be one of these. *Abies amabilis* retains ten or more age classes of needles; *Tsuga heterophylla* keeps needles 4-7 years. Coniferous cuticles erode over time (Reicosky and Hanover 1976; Günthardt 1984; Riederer 1989; Van Gardigen *et al.* 1991) and cuticular conductance has been shown to increase as needles age (Reiners and Olson 1984; Hadley and Smith 1990a; Schreiber 1994; Heinsoo and Koppel 1999), or to remain constant for several years (Anfodillo *et al.* 2002). Species-related differences in needle retention may explain a portion of the significance of the species effect with respect to $g_{c_{in}}$ and $g_{c_{out}}$.

Comparison of estimates of $g_{c_{out}}$ *for Tsuga heterophylla and Abies amabilis with estimates for other species.*

The estimates of $g_{c_{out}}$ for *Abies amabilis* and *Tsuga heterophylla* from this study are within the range of values for the $g_{c_{out}}$ of other conifer species (Table V.6). Compared to the $g_{c_{out}}$ of *Abies grandis*, *Abies lasiocarpa*, *Pseudotsuga menziesii* and other co-occurring species, the $g_{c_{out}}$ of *Abies amabilis* and *Tsuga heterophylla* are in the same range, but higher (Table V.6). Given that *Abies amabilis* and *Tsuga heterophylla* are among the least drought tolerant of Northwest conifers (Minore1979), the values of $g_{c_{out}}$ reported for the two species in this study fit within their broader ecophysiological context.

The variability of the data that has been reported by different investigators (Table V.6) is striking. Kersteins (1996) reviewed cuticular conductance in plants. With regard to factors that influence estimates of cuticular conductance, he found that the effect of experimental technique was comparable to the effect of species. Vapor fluxes from leaves with exposed stomata-bearing surfaces under conditions of assumed or factual closure produced the highest mean estimates of $g_{c_{out}}$. Experiments with isolated cuticles produced the lowest values of $g_{c_{out}}$. Kersteins found that the mean estimates from the two techniques were different by an order of magnitude. Bias due to technique is the first reason why estimates of cuticular conductance are not strictly comparable.

The second reason why estimates of $g_{c_{out}}$ are difficult to compare involves the assumptions that are made regarding the movement of water through plant cuticles. According to the simple diffusion hypothesis, conductance is a constant property of a cuticle. In this study, the cuticular conductances of the two species were greater when wet ($g_{c_{in}}$), and lower when dry

($g_{c_{out}}$). This is important with respect to the influence of experimental technique on estimates of $g_{c_{out}}$, and especially the $g_{c_{out}}$ of conifers. With few exceptions, the $g_{c_{out}}$ of conifers has been measured by gaseous flux, or by recording the $\Delta m/\Delta t$ of severed needles or shoots. The $g_{c_{out}}$ of a drying shoot typically declines sharply, and then decreases at a constant rate 3-10 hours after the initiation of the experiment (Cape and Percy 1996; Anfodillo *et al.* 2002). Unusually high values of $g_{c_{out}}$, such as those often yielded by the minimum vapor flux method, are often attributed to either incompletely closed stomata or cuticular damage (Kersteins 1996). However, if the conductance of cuticles is affected by hydration (Seymour 1980; Hoad *et al.* 1996; Boyer 1997; Hoad *et al.* 1997; Schreiber *et al.* 2001), then values of $g_{c_{out}}$ obtained by gravimetric methods over very long time intervals (50 hours +) and under conditions of extreme dehydration may not reflect physiologically normal values for living plants.

Area-based versus mass-based measures of conductance

Cuticular conductance is usually reported on a per unit area basis, with units m s^{-1} (review by Kersteins 1996). Perhaps one reason for this convention is that stomatal conductance is also reported on a per unit area basis. Area-based conductances are also convenient to expand from small scales (needle, shoot) to larger scales (branch, canopy). However, a leaf-level strategy is to maximize carbon gain and minimize water loss per unit of biomass. Measures of gas exchange per unit area may overlook functionally significant aspects of leaf design (Figure V.9). For example, Tipton and White (1995) examined cuticular transpiration by three varieties of *Cercis* from both area- and mass-based perspectives. They reported that area-based $g_{c_{out}}$ did not relate to the drought hardiness of the three varieties, whereas mass-based $g_{c_{out}}$ did.

In this study, the two measures of conductance provided conflicting information. For example, the area-based $g_{c_{out}}$ of *Abies amabilis* was greater than the area-based $g_{c_{out}}$ of *Tsuga heterophylla* (Table V.4). The mass-based estimates of $g_{c_{out}}$ told a different story: the mass-based $g_{c_{out}}$ of *Tsuga* was greater than the mass-based $g_{c_{out}}$ of *Abies*. Similarly, the area-based $g_{c_{out}}$ of the sun-adapted shoots was greater than the $g_{c_{out}}$ of shade-adapted shoots, while the $g_{c_{out}}$ of shade-adapted shoots was greater than the $g_{c_{out}}$ of sun-adapted shoots (Table V.4). The same paradox held for estimates of $g_{c_{in}}$: the mean area-based $g_{c_{in}}$ of *Abies amabilis* was greater than the area-based $g_{c_{in}}$ of *Tsuga heterophylla* ($p=0.44$, Student's t-test), while the mass-based $g_{c_{in}}$ of *Tsuga* was greater than mass-based $g_{c_{in}}$ of *Abies* ($p<0.001$, Student's t-test). It is clear that $g_{c_{in}}$ and $g_{c_{out}}$ vary with shoot morphology and species. The interpretation of these relationships, however, hinges upon how cuticular conductance is expressed.

The mass-based expressions of g_c appeared to be more meaningful than area-based estimates, for at least two reasons. First, the mass-based estimates of $g_{c_{out}}$ and $g_{c_{in}}$ explained more of the variability in the data, and yielded stronger relationships between dependent and independent variables than did the area-based measures (Tables V.2 and V.5). Second, in *Abies amabilis* and *Tsuga heterophylla*, the mass of needles changes more plastically in response to the light environment than the needle shadow area. The projected area of two needles from different light environments can be similar, even when their total mass differs greatly (Figure V.9). Therefore, area-based measures of conductance may capture less of the adaptive significance of variation in leaf morphology than mass-based measures.

$g_{c_{out}}$ *versus* $g_{c_{out}}$.

With both *Abies amabilis* and *Tsuga heterophylla*, the $g_{c_{in}}$ of sun-adapted shoots was approximately ten times greater than $g_{c_{out}}$, while for shade-adapted shoots, $g_{c_{in}}$ was about twenty times higher than $g_{c_{out}}$ (Figure V.7). Interestingly, insect cuticles, which are structurally similar to plant cuticles (Hadley 1981), are also known to behave in this way. Insect cuticles are more permeable to water in the outside to inside direction versus the inside to outside direction (Hurst 1948).

Water conservation is the most important function of plant cuticles, and low conductivity to water is one of their most intrinsic properties. According to the passive diffusion hypothesis (Lieb and Stein 1969), water flux through a non-living membrane depends solely upon the concentration of gradient of water and the conductance of the membrane. The conductance is an innate membrane property, unaffected by the direction of the concentration gradient. The expected equivalence of $g_{c_{out}}$ and $g_{c_{in}}$ accrues from this hypothesis. The large gap between $g_{c_{out}}$ and $g_{c_{in}}$ is evidence that the passive diffusion hypothesis may not fully explain water movement through plant cuticles. On the other hand, while $g_{c_{out}}$ was much smaller than $g_{c_{in}}$, the two quantities were significantly related (Figure 8B). This suggests that there is either a common mechanism or a common pathway for water movement through the cuticles of *Abies amabilis* and *Tsuga heterophylla*. Potential mechanisms of water movement through cuticles of *Abies amabilis* and *Tsuga heterophylla* will be explored in the next chapter.

Evaluation of the $\Delta m/\Delta t$ technique

The technique developed in this study appears capable of quantifying water uptake by detached conifer shoots. Nonetheless, the method is not a direct measure. Potentially significant sources of error may result in disparities between apparent uptake and actual water gain by the detached shoots. Some of the potential sources of error include (summary in Table V.7):

1. Needle drop. Weakly attached needles were susceptible to loss each time that the shoots were handled. This was especially true for *Tsuga heterophylla* needles, which are connected to stems with slender petioles. Since the experiments were conducted in a darkened room, it was difficult to know when needle drop occurred, or when dehisced needles were discovered, to which shoots they belonged. For the average-sized shoot used in the uptake experiments, the loss of a single *Tsuga* needle would reduce fresh mass by 0.10%, while the loss of a more massive *Abies* needle would diminish the mass of a fresh fir shoot by 0.15%. Clearly, needle dehiscence can be a major source of error, and introduces an experimental bias towards the underestimation of water uptake.

2. Epiphyllic coatings. Dust, bacteria, fungi, lichens and other potentially hydrophilic substances often abound on the needles and stems of conifer shoots. Manipulable fragments were gleaned from the shoots; however, all surface debris could not be removed without risking damage to the cuticle. Furthermore, the needles of *Abies amabilis* shade shoots collected at Findley Lake were often sticky with aphid secretions that encouraged the growth of a dark, felt-like mold. This problem was so ubiquitous in the Findley old-growth stands that an alternative sample site was chosen near Talapus Lake, northwest of Snoqualmie Pass.

The influence of surface contaminants on estimates of moisture uptake would be a function of their respective conductances. If the conductance of the epiphyllic coatings is much greater than the conductance of the cuticle, then the rate of evaporation from the hydrated materials would be rapid. Equation 5.4 would attribute water loss from the surface debris to evaporation from the fast pool, $W_{surface}$, and the bias on estimates of water uptake by moistened shoots would be minor. However, if the conductance of epiphyllic debris is comparable to the conductances of the cuticle or periderm, then equation 5.4 would assign this water loss to evaporation from the slow pool, W_{shoot}. In this case, the bias would be to over-estimate water uptake.

Epiphyllic substances lack the functional equivalent of a waxy plant cuticle. The outer cortex of lichens is primarily a shield against physical damage and sunlight, rather than a barrier to water loss (Raven *et al.* 1999). Since the conductances of coniferous cuticles to water are almost certainly lower than the conductances of debris on shoot surfaces, the experimental bias due to epiphyllic coatings was probably low.

3. Open stomata. Darkness stimulates, but does not guarantee, stomatal closure. If stomatal transpiration was high following treatment, then the resulting water loss would likely be interpreted as evaporation from $W_{surface}$, and water uptake would be underestimated. Very low post-treatment stomatal transpiration would be interpreted as water loss from W_{shoot}, without affecting estimates of absorption. Therefore, the experimental bias due to water loss through open stomata would be a small to potentially significant underestimate of shoot water uptake.

4. Xylem water potential. Ideally, all of the shoots would have been tested at the same initial xylem water potential, and at similar ambient temperature and relative humidity. They were not. This was especially true for the xylem water potential of the shoots, which was initially a function of the soil moisture content, the atmospheric vapor pressure deficit, the time of year and the time

of day; and then would become even more variable depending on how long it would take to collect a branch, clip the shoots, conduct the experiment, and so on.

As inferred from pressure-volume curves (Figure IV.4), Ψ_{xylem} varied between -1.0 and -2.0 MPa during the uptake experiments. Ψ_{xylem} has an almost negligible influence on estimates of minimum shoot conductance, since the water content of the atmosphere largely controls the leaf-to-air water vapor gradient. However, the influence of the xylem water potential on estimates of $g_{c_{in}}$ is critical, especially at values of Ψ_{xylem} greater than -1.0 MPa. The experiments with the Scholander pressure bomb showed that the Ψ_{xylem} of detached shoots treated with a mist of deionized water increased in proportion to the amount of time that the shoots were wet (Figure IV.3A). Increases in Ψ_{xylem} diminish the driving force for uptake. If uptake proceeds at a constant rate and Ψ_{xylem} is assumed to be static, the bias would be to underestimate the $g_{c_{in}}$ of a hydrating shoot. Note that the $g_{c_{in}}$ of *Abies amabilis* sun-adapted shoots was apparently constant for at least the first six hours that the shoots were moistened with water (Figure V.4B), indicating that either Ψ_{xylem} did not change, or that $g_{c_{in}}$ was directly proportional to Ψ_{xylem}.

5. Boundary layer conductance. If the shoot boundary layer conductance is lower than the minimum conductance of a desiccating shoot, then the estimation of minimum shoot conductance could become problematic. A low boundary layer conductance would establish an envelope of humidified air surrounding the shoot, and would invalidate the key assumption that the ambient water vapor concentration determines the driving force for water loss from the shoot. Martin et al. (1999) found that the boundary layer conductance of *Abies amabilis* at low wind speed (0.2 m s^{-1}) was approximately 0.02 m s^{-1}, or about 400 times greater than the $g_{c_{out}}$ measured for *Abies* in

this study. In light of their finding, the ambient humidity can be used to calculate the driving force for $g_{c_{out}}$ (F. Meinzer, personal communication). The boundary layer conductance was probably not an important source of error in estimating the $g_{c_{out}}$ of desiccating *Abies* and *Tsuga* shoots.

6. Shoot size. There was a limit on the size of the shoot that could be manipulated on the laboratory balance. The limit on maximum shoot size introduced a bias towards younger age classes of needles. The bias was greatest for sun shoots, which have more newly formed growth than shade shoots; and for *Abies*, which has a strongly determinate pattern of growth and retains more age classes of needles than *Tsuga*. Conifer needles tend to become more wettable and more permeable to water as they age (Schreiber 1994). A bias towards younger needle age classes is a potential scaling problem: shoot-level estimates of water exchange could have been less than the actual values for the whole canopy. The likely influence of this bias on estimates of conductances, however, was minor.

7. Water uptake via the cut stem. In the water uptake experiments, the cut ends of the detached shoots were sealed with paraffin wax. However, if the temperature of the melted wax was too low, it solidified upon contact with the stem before the wound was thoroughly sealed. Water uptake through the cut stem would have resulted in an overestimate of water absorption through the cuticle. Since cavitation had occurred, this source of error would likely have been small.

8. Outliers. Often, of the ten shoots in the treatment group, one to three shoots would have: 1) drying curves that were too erratic to fit with equation 5.4; 2) unusually large changes apparent in

water content; or 3) a large decrease in mass, probably resulting from the loss of needles. Obvious outliers were not included in the data, which may have introduced a subjective bias.

9. Stems and needles. The analytical focus of the experiments was on water exchange by needles, because for a conifer shoot, needle surface area is greater than stem surface area. The relationship between stem age, stem diameter, and stem surface area was quantified for shade- and sun-adapted *Tsuga heterophylla* branches collected at Findley Lake. For a given age, stem diameters from the sun-adapted branch were greater. Although the ratio of needle to stem dry weight was lower for shade-adapted shoots (2.7) vs. sun-adapted shoots (3.5), the ratio of the projected area of needles to the projected area of stems was lower for sun-adapted shoots (24.2) as compared to shade-adapted shoots (30.8).

Nonetheless, Katz et al. (1989) proposed that the stems are the primary sites of water exchange. If their hypothesis were true, then the fluxes of water through the periderm would have to be almost an order of magnitude higher than comparable fluxes through the cuticle to yield similar estimates for shoot-level exchange. This scenario seems unlikely, given that Garrec and Barrois (1992) and Schönherr and Ziegler (1980) found that the permeabilities of the cuticle and bark to water are comparable. Schaefer and Reiners (1990) added that in stems, dead cells limit contact between water and the apoplast. This, combined with their low surface area, implies that stems are minor sites for water uptake when compared to leaves. Any water absorption that may have occurred through the periderm would have resulted in a small overestimate of $g_{c_{in}}$.

10. Differences between detached shoots and canopies. As discussed, the Ψ_{xylem} of a wet, detached shoot should increase as the shoot absorbs water. In a tree canopy, water absorbed by

wet foliage may be exported elsewhere, in accordance with internal gradients of Ψ_{xylem}. Thus, in a whole canopy, Ψ_{xylem} at the sites of absorption may be more buffered against change than in small detached shoots. For this reason, water uptake experiments with detached shoots may underestimate the quantity of water that can be absorbed by a forest canopy. The rate of water uptake by detached shoots appeared to remain constant for periods up to six hours (Figure V.4A). Nonetheless, water uptake studies with detached shoots may prove problematic over longer time scales.

Summary

Assume that a shoot is wet and its stomata are closed. When the shoots begins to dry, water will be lost from its surface more rapidly than water can escape through the cuticle. This simple principle underlies the drying curve technique used in this chapter to assess the uptake of water by wet *Abies* and *Tsuga* shoots. The new technique appears to be sufficiently accurate to quantify the changes in shoot mass that would be caused by the absorption of small amounts of water.

The results obtained by the drying curve technique indicated that wet *Tsuga* and *Abies* shoots absorb water. The morphology of shoots was more strongly related to water uptake than species, although both factors were significant. Water absorption was quantified as the change in shoot weight per unit projected leaf area, and as the change in shoot weight per unit of dry shoot mass. The mass-based results were more robust and more physiologically meaningful than the uptake data expressed in terms of area. Unexpectedly, the conductances of the detached *Abies* and *Tsuga* shoots to water influx were at least an order of magnitude greater than their conductances to water efflux.

The drying curve technique has important limitations. First, the xylem water potential and other parameters of a detached shoot are assumed to remain constant during the experiment. Second, the technique requires each shoot to be manipulated many times, compounding the probability of experimental error. Third, water uptake by detached shoots may not scale directly to water uptake whole plants. Finally, the drying curve technique is an indirect method that needs validation by more direct measures.

Table V.1. ANOVA analysis of the effects of treatment (misting: wet for 180 minutes, then blot dry; or control: wet for one minute, then blot dry), species (*Tsuga heterophylla* or *Abies amabilis*) and shoot morphology (sun- or shade-adapted) on the change in percent water content after wetting detached conifer shoots with deionized water. The changes in water content were interpreted from drying curves, based on repeated gravimetric measurements of detached shoots in a darkened laboratory at t = 20°C and rH = 40%. The shoots were collected from mature, co-dominant trees at Findley Lake, WA (elevation = 1170 m), and west of Snoqualmie Pass, WA (elevation = 1000m).

Effect	ss	DF	F-ratio	P
Treatment	11.49	1	81.01	0.000
Shoot morphology	7.93	1	55.91	0.000
Species	0.01	1	0.08	0.780
Treatment*shoot morphology	4.57	1	32.24	0.000
Shoot morphology *species	2.61	1	18.38	0.000
Treatment*species	0.62	1	4.38	0.038
Treatment*shoot morphology *species	0.65	1	4.61	0.033
Error	21.99	155		

Table V.2. ANOVA analysis of the influences of species (*Tsuga heterophylla* or *Abies amabilis*) and shoot morphology (sun- or shade-adapted) on area-based and mass-based estimates of $g_{c_{in}}$. The data were collected from repeated gravimetric measurements of detached shoots in a darkened laboratory at t = 20°C and rH = 40%. The shoots were moistened for approximately 120 minutes with deionized water. The shoots were collected from mature, co-dominant trees at Findley Lake, WA (elevation = 1170 m), and near Talapus Lake west of Snoqualmie Pass, WA (elevation = 1000 m).

Method	Effect	ss	DF	F-ratio	P
Area-based	species	7.98	1	0.74	0.392
	shoot morphology	119.71	1	11.13	0.001
	species*shoot morphology	3.18	1	0.30	0.589
	error	677.42	63		
Mass-based	species	278.57	1	18.58	0.000
	shoot morphology	1037.38	1	69.21	0.000
	species*shoot morphology	229.99	1	15.34	0.000
	error	944.33	63		

Table V.3. Area- and mass-based cuticular conductances of detached *Abies amabilis* and *Tsuga heterophylla* shoots to water influx ($g_{c_{in}}$). The shoots were moistened with deionized water for approximately 120 minutes. The data were obtained from repeated gravimetric measurements of detached shoots in a darkened laboratory at t = 20°C and rH = 40%. The shoots were collected from mature, co-dominant trees at Findley Lake, WA (elevation = 1170 m), and near Talapus Lake west of Snoqualmie Pass, WA (elevation = 1000 m). The data are the means ± SE. Means followed by the same letter are not significantly different (p≤0.05, Bonferroni's multiple comparison).

species	sun-adapted shoots	shade-adapted shoots
area-based $g_{c_{in}}$ x 10^4 m s^{-1}		
Abies amabilis	4.7 ± 0.5 ^{a,b}	7.2 ± 0.8 ^{b}
Tsuga heterophylla	3.5 ± 0.6 ^{a}	6.9 ± 0.8 ^{a,b}
mass-based $g_{c_{in}}$ x 10^6 m^3 g^{-1} s^{-1}		
Abies amabilis	2.9 ± 0.3 ^{a}	7.4 ± 0.6
Tsuga heterophylla	3.3 ± 0.5 ^{a}	15.9 ± 1.5

Table V.4. Area- and mass-based cuticular conductances of detached *Abies amabilis* and *Tsuga heterophylla* shoots to water efflux ($g_{c_{out}}$). The data were obtained from repeated gravimetric measurements of slowly desiccating, detached shoots in a darkened laboratory at t = 20°C and rH = 40%. The shoots were collected from mature, co-dominant trees at Findley Lake, WA (elevation = 1170 m), and near Talapus Lake northwest of Snoqualmie Pass, WA (elevation = 1000 m). The data are the means ± SE. Means followed by the same letter are not significantly different (p≤0.05, Bonferroni's multiple comparison).

species	sun-adapted shoots	shade-adapted shoots
area-based $g_{c_{out}}$ x 10^5 m s^{-1}		
Abies amabilis	3.9 ± 0.3 ^{a,b}	3.8 ± 0.1 ^{a}
Tsuga heterophylla	3.3 ± 0.2 ^{a}	3.0 ± 0.1 ^{b}
mass-based $g_{c_{out}}$ x 10^7 $m^3g^{-1}s^{-1}$		
Abies amabilis	2.7 ± 0.2 ^{a}	4.7 ± 0.2
Tsuga heterophylla	3.0 ± 0.1 ^{a}	7.1 ± 0.2

Table V.5. ANOVA analysis of the effects of species (*Tsuga heterophylla* or *Abies amabilis*) and shoot morphology (sun- or shade-adapted) on area-based and mass-based estimates of $g_{c_{out}}$. The data were collected from repeated gravimetric measurements of detached shoots in a darkened laboratory at t = 20°C and rH = 40%. The shoots were collected from mature, co-dominant trees at Findley Lake, WA (elevation = 1170 m), and near Talapus Lake west of Snoqualmie Pass, WA (elevation = 1000 m).

Method	Effect	ss	DF	F-ratio	P
Area-based	species	17.64	1	14.85	0.000
	shoot morphology	2.32	1	1.96	0.164
	species*shoot morphology	0.74	1	0.62	0.433
	error	212.61	179		
Mass-based	species	72.10	1	36.08	0.000
	shoot morphology	378.12	1	189.20	0.000
	species*shoot morphology	46.54	1	23.18	0.000
	error	357.73	179		

Table V.6. Estimates of cuticular conductance for conifers. Four methods were used to estimates these conductances: 1) the water vapor flux through enzymatically removed and cuticles; 2) the change in mass over time of detached needles; 3) the change in mass over time of detached shoots; and 4) porometer-measured vapor fluxes from intact shoots under conditions of minimal stomatal conductance (no illumination + drought stress). See Kersteins (1996) for a review of the methods used to measure cuticular conductance, as well as the original table with stomatal conductances from other types of plants.

Species	$g_{c_{out}}$ ($\mathrm{m\ s^{-1} \times 10^5}$ two-sided projected LA)	Method	Reference
Abies alba	14.0	isolated cuticles	Lendzian *et al.* 1986
Abies amabilis	**3.8-3.9**	Δm/Δt detached shoots	**this study**
Abies grandis	3.0	min. water vapor flux	Running 1976
Abies lasiocarpa	1.2	Δm/Δt detached shoots	Hadley and Smith 1990a
Abies magnifica	7.0	min. water vapor flux	Running 1976
Abies procera	32.0	min. water vapor flux	Running 1976
Picea abies	2.4-3.5 [a]	Δm/Δt excised needles	Anfodillo *et al.* 2002
Picea abies	0.3	Δm/Δt detached shoots	Baig and Tranquillini
Picea abies	4.3-8.7	Δm/Δt excised needles	Barnes and Davison 1988
Picea abies	37.0-49.0	min. water vapor flux	Barnes *et al.* 1990
Picea abies	3.5-6.0	Δm/Δt detached shoots	Heinsoo and Koppel 1999
Picea abies	1.4	Δm/Δt detached shoots	Tranquillini 1974
Picea engelmannii	0.8	Δm/Δt detached shoots	Hadley and Smith 1990a
Picea engelmannii	2.0	min. water vapor flux	Kaufmann 1976
Picea glauca	44.0	Δm/Δt excised needles	Cowling and Kedrowski
Picea rubens	1.3	Δm/Δt detached shoots	Boyce *et al.* 1992
Picea rubens	1.1	Δm/Δt detached shoots	Herrick and Friedland
Picea sitchensis	47.0	min. water vapor flux	Running 1976
Picea sitchensis	1.4	Δm/Δt detached needles	van Gardingen *et al.* 1991
Pinus albicaulis	0.6	Δm/Δt detached needles	Sowell *et al.* 1982
Pinus banksiana	<1.0	Δm/Δt detached needles	Cowling and Kedrowski
Pinus cembra	1.0-1.9 [a]	Δm/Δt detached needles	Anfodillo *et al.* 2002
Pinus cembra	1.3-2.2	Δm/Δt detached needles	Körner ex Kersteins 1996
Pinus contorta	<1.0	Δm/Δt detached needles	Cowling and Kedrowski
Pinus contorta	0.8	Δm/Δt detached shoots	Hadley and Smith 1990a
Pinus flexilis	0.8	Δm/Δt detached shoots	Hadley and Smith 1990a
Pinus ponderosa	0.6	Δm/Δt detached shoots	Hadley and Smith 1990a
Pinus ponderosa	5.0	min. water vapor flux	Running 1976
Pinus sylvestris	2.6-8.3	Δm/Δt detached needles	Cape and Fowler 1981
Pinus sylvestris	1.0	Δm/Δt detached needles	Grace 1990
Pinus sylvestris	2.1-3.8	Δm/Δt detached needles	Körner ex Kersteins 1996
Pinus sylvestris	1.1	Δm/Δt detached needles	van Gardingen *et al.* 1991
Pseudotsuga	0.3	Δm/Δt detached shoots	Hadley and Smith 1990a

Table V.6 continued

Pseudotsuga	3.0	min. water vapor flux	Running 1976
Tsuga heterophylla	7.0	min. water vapor flux	Running 1976
Tsuga heterophylla	**3.0-3.3**	$\Delta m/\Delta t$ detached shoots	**this study**

[a] multiplied by the approximate correction factor of 1.6 to convert from total to projected area.

Table V.7. Potential experimental biases of drying curve technique to estimate the uptake of water by moistened shoots.

potential sources of error	nature of the error
decrease in shoot mass due to needle drop	small to large underestimate of $g_{c_{in}}$
water absorption by epiphyllic coatings	small overestimate of $g_{c_{in}}$
transpiration through stomata	small to large underestimate of $g_{c_{in}}$
change in Ψ_{xylem}	small to large underestimate of $g_{c_{in}}$
low boundary layer conductance	small underestimate of $g_{c_{out}}$
shoot size	small underestimate of $g_{c_{in}}$ and $g_{c_{out}}$
water infiltration through wounds	small overestimate of $g_{c_{out}}$
identification of outlying data points	under- or overestimates of $g_{c_{in}}$ and $g_{c_{out}}$
water uptake through the periderm	small overestimate of $g_{c_{in}}$
detached shoots as the experimental system	trend to underestimate $g_{c_{in}}$

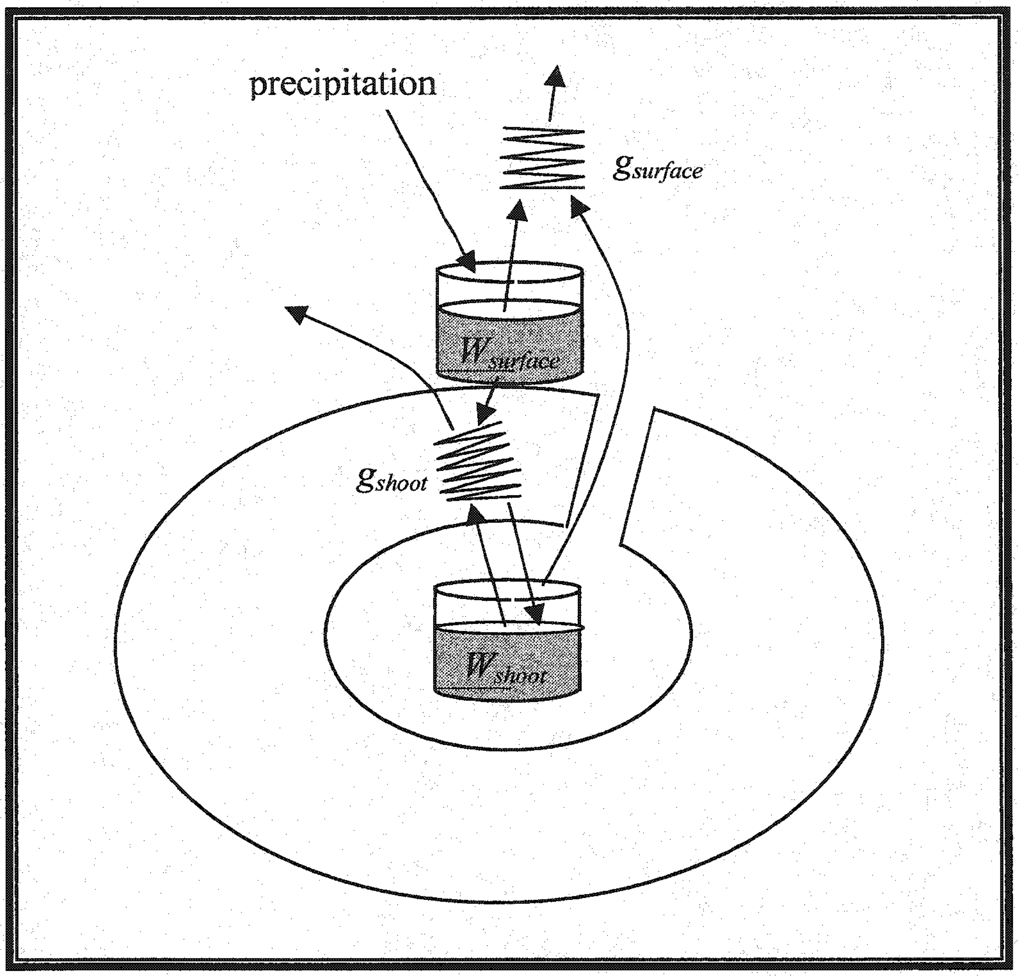


Figure V.1. Conceptual model of a wet conifer shoot. $W_{surface}$ is the surface pool of moisture that clings to the cuticle. $W_{surface}$ is replenished by precipitation, and is depleted by evaporation or uptake by the shoot. W_{shoot} is the internal pool of water held by shoot tissues. The slow rate of water movement from $W_{surface}$ to W_{shoot} through the cuticle is controlled by a conductance, g_{shoot} ($\approx g_c$). Loss from W_{shoot} through the cuticle or periderm is regulated by the slow conductance, g_{shoot}. Loss from W_{shoot} through stomatal pores is regulated by the fast conductance, $g_{surface}$. Loss from $W_{surface}$ is also assumed to be regulated by $g_{surface}$.

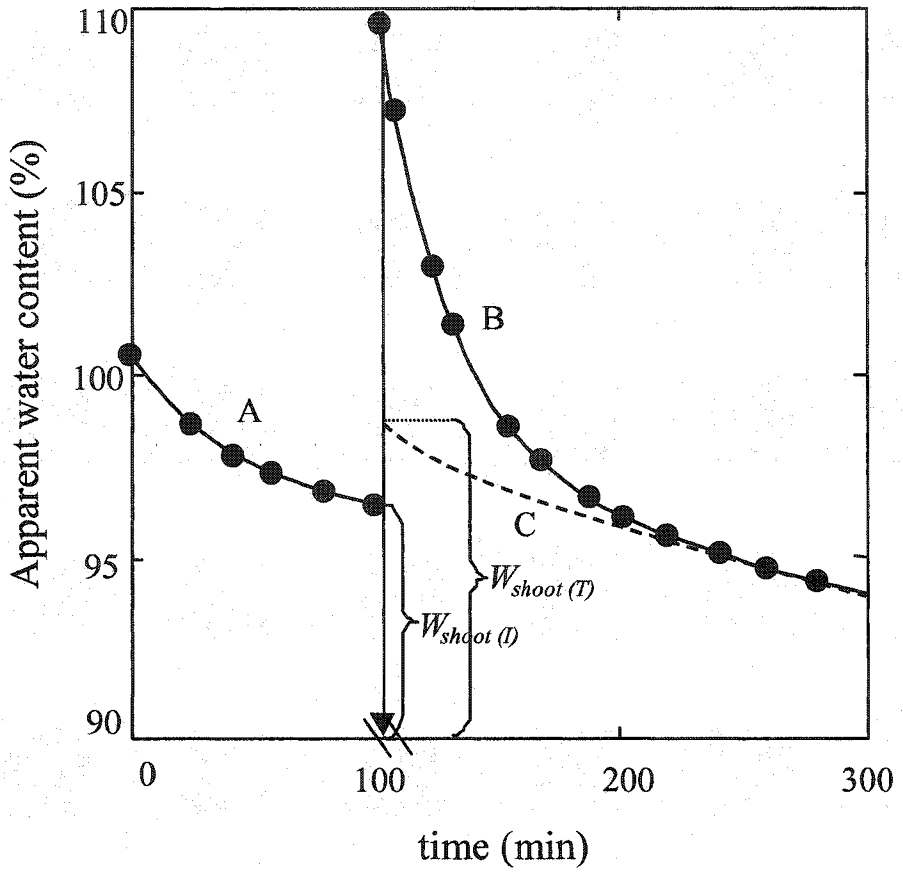


Figure V.2. The use of the drying curve technique to estimate water uptake by a wet *Tsuga heterophylla* shoot. During the first period of desiccation (0-100 minutes), a *Tsuga heterophylla* shoot is hung from a wire in a darkened room. The solid line (A) is the plot of equation 5.4, fitted to the first six data points. At the end of the first period of desiccation, the shoot is surface-dry and its $AWC = W_{shoot(I)}$. The shoot is then moistened for 120 minutes with deionized water (time interval not shown). The second period of desiccation begins at time = 100 minutes. The solid line (B) is the plot of equation 5.4, fitted to the last 12 data points. The dashed line (C) is the plot of equation 5.4 with the value of W_{suface} set to 0. The apparent water content of the shoot after treatment ($W_{shoot\ (T)}$) is estimated by the y-value of line C at the start of the second desiccation period (time ≈ 100 minutes). The difference between $W_{shoot\ (T)}$ and $W_{shoot\ (I)}$ is an estimate of the amount of water absorbed by the shoot.

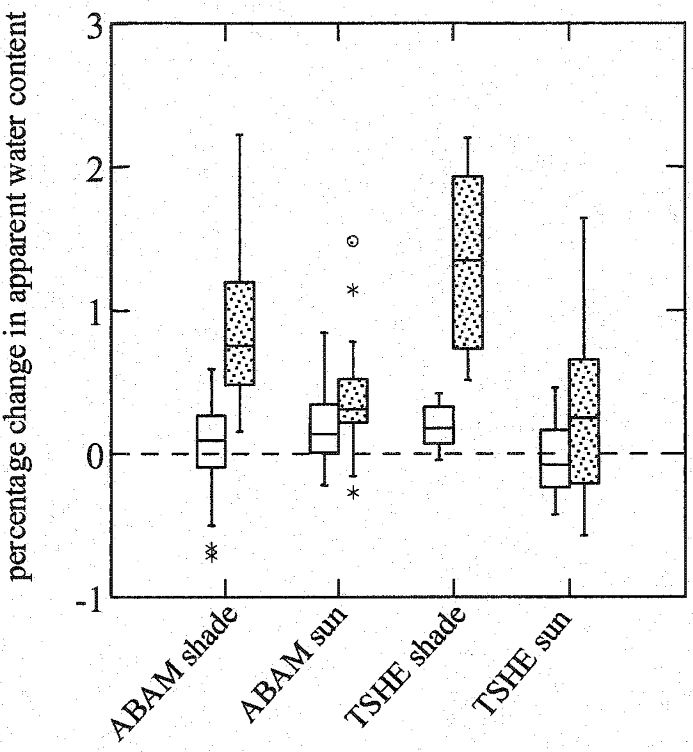


Figure V.3. The change in percent water content of detached *Abies amabilis* and *Tsuga heterophylla* shoots moistened for either one minute (control group, white boxes) or 120 minutes (treatment group, stippled boxes) with deionized water. The central 50% of the data points falls within the range of the boxes. The shoots were collected at Findley Lake, WA (elevation = 1170 m), and at a site northwest of Snoqualmie Pass, WA (elevation = 1000 m).

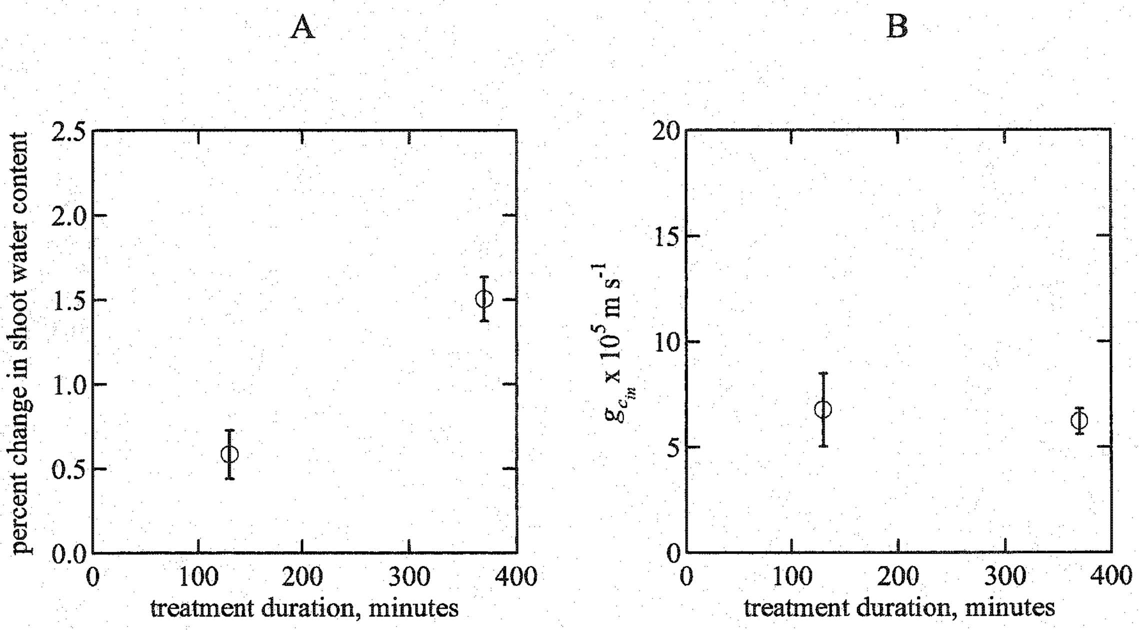


Figure V.4. A) The percent change in the apparent water content of *Abies amabilis* sun-adapted shoots moistened with deionized water for either 130 minutes (n=10) or 370 minutes (n=10). The change in water content was estimated from the analysis of shoot drying curves. The data are the means ± SE. The means are significantly different ($p<0.01$, Student's t-test). B) Area-based shoot conductance to water influx of the same two groups of shoots. The data are the means ± SE. The mean conductances of the two treatment intervals were not significantly different ($p>0.20$, Student's t-test). The shoots were collected on 12 June 1999 near Talapus Lake, WA.

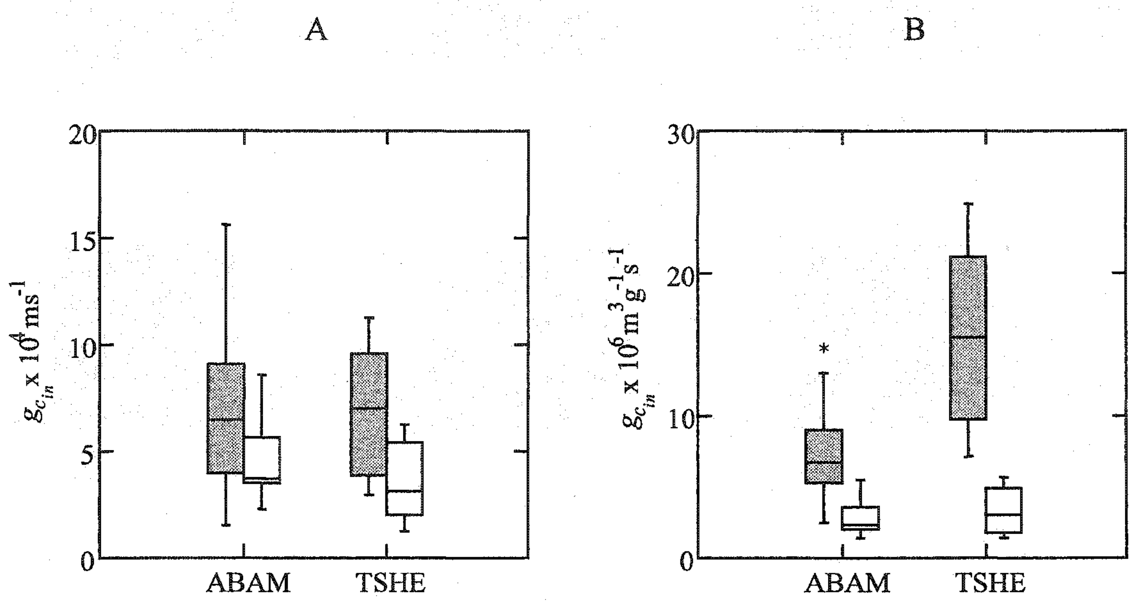


Figure V.5. Area-based (A) and mass-based (B) estimates of $g_{c_{in}}$ for shade- (gray boxes) and sun-adapted (unfilled boxes) detached *Abies amabilis* (ABAM) and *Tsuga heterophylla* (TSHE) shoots moistened with deionized water for 120 minutes. Prior to treatment, the xylem water potential of the shoots was assumed to be -1.3 MPa. The shoots were collected from the upper and lower canopies of mature, co-dominant trees at Findley Lake (elevation = 1170 m) and near Talapus Lake west of Snoqualmie Pass, WA (elevation = 1000 m).

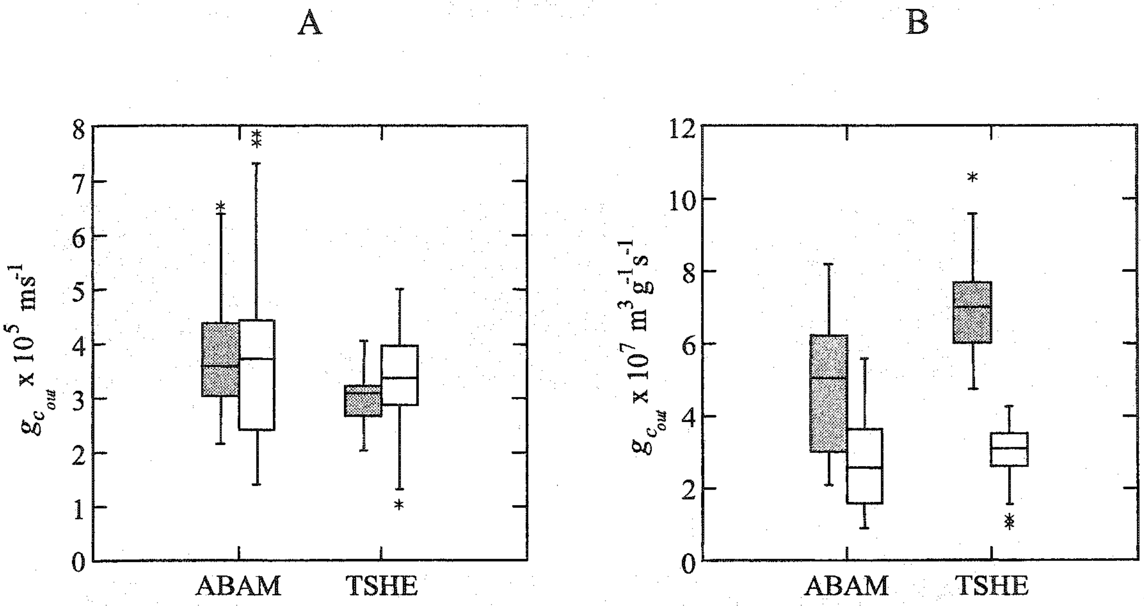


Figure V.6. Area-based (A) and mass-based (B) estimates of $g_{c_{out}}$ for shade- (gray boxes) and sun-adapted (unfilled boxes) detached *Abies amabilis* (ABAM) and *Tsuga heterophylla* (TSHE) shoots. The central 50% of the data points falls within the boxes. Shoot conductance was determined from the analysis of drying curves of slowly desiccating shoots in a darkened lab at t = 20°C and rH = 40%. The shoots were collected at Findley Lake, WA (elevation = 1170 m) and near Talapus Lake west of Snoqualmie Pass, WA (elevation = 1000 m).

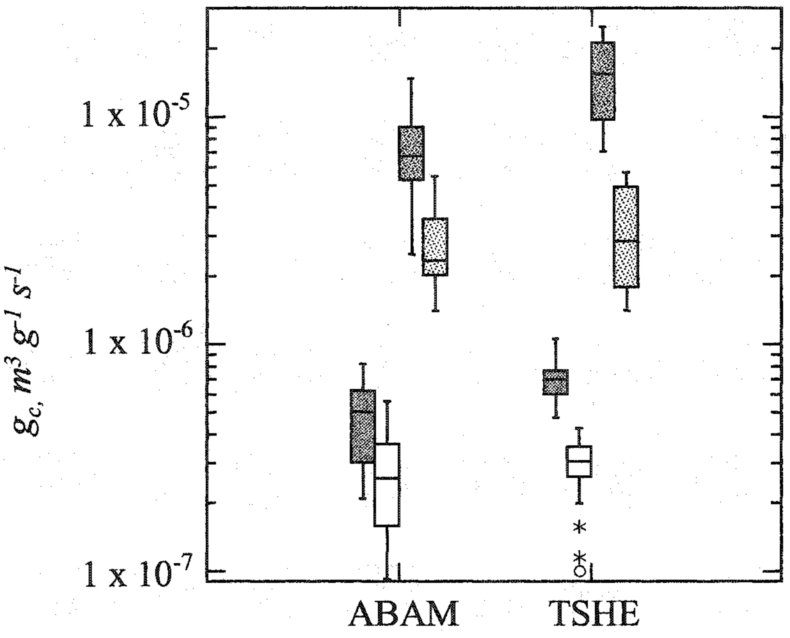


Figure V.7. Mass-based estimates of $g_{c_{out}}$ (no stipples) and $g_{c_{in}}$ (stippled) for detached *Abies amabilis* (ABAM) and *Tsuga heterophylla* (TSHE) shade- (gray boxes) and sun-adapted (white boxes) shoots. The shoots were repeatedly weighed for 100 minutes while desiccating in a darkened room. Then, the shoots were treated with deionized water for 120 minutes, and again weighed repeatedly while slowly drying. Prior to treatment with deionized water, the xylem water potential of the shoots was assumed to be -1.3 MPa. The conductances are based upon the analysis of the shoot drying curves. The shoots were collected from the upper and lower canopies of mature, co-dominant trees at Findley Lake (elevation = 1170 m) and near Talapus Lake (elevation = 1000 m) northwest of Snoqualmie Pass, WA.

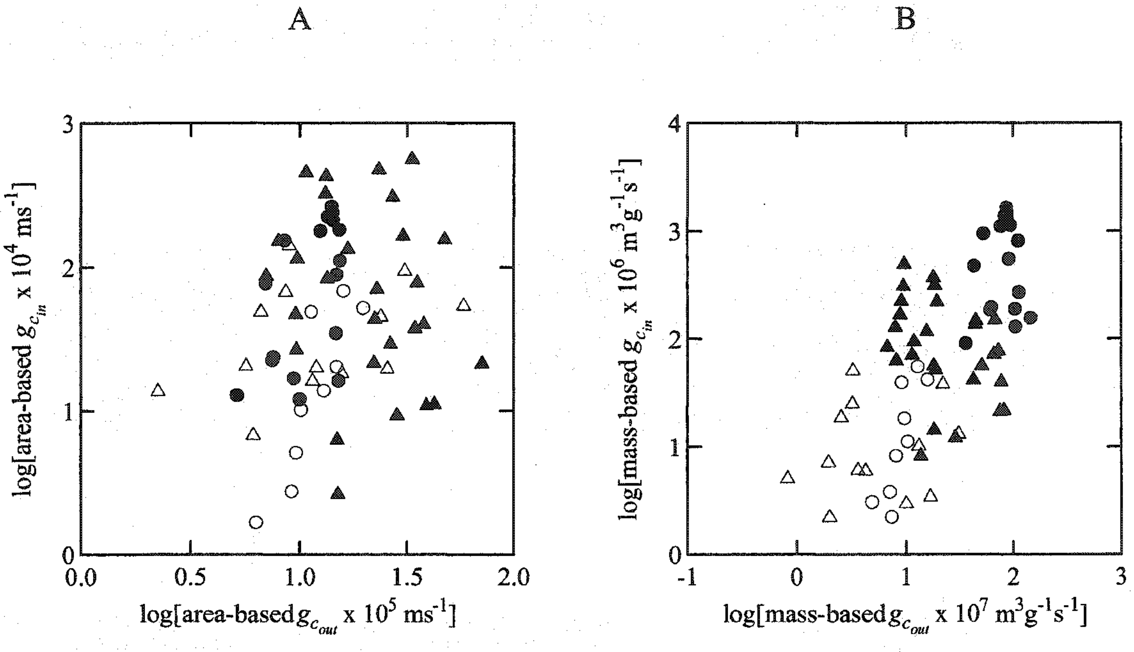


Figure V.8. A) The relationship between the log of area-based $g_{c_{in}}$ and the log of area-based $g_{c_{out}}$. The best-fit regression equation is: log(area-based $g_{c_{in}}$) = 1.19 + .39 log (area-based $g_{c_{out}}$), F-ratio = 2.3, $r^2 = 0.02$. B) The relationship between the log of mass-based $g_{c_{in}}$ and the log of mass-based $g_{c_{out}}$. The best-fit regression equation is: log(mass-based $g_{c_{in}}$ = 0.54 + .43 log (mass-based $g_{c_{out}}$), $r^2 = 0.40$, F-ratio = 43.5. *Tsuga heterophylla*, circles; *Abies amabilis*, triangles; sun-adapted shoot, open symbols; shade-adapted shoots, filled symbols. The data were from detached shoots of mature, co-dominant trees collected at Findley Lake, WA (elevation = 1170 m), and a site northwest of Snoqualmie Pass, WA (elevation = 1000 m).

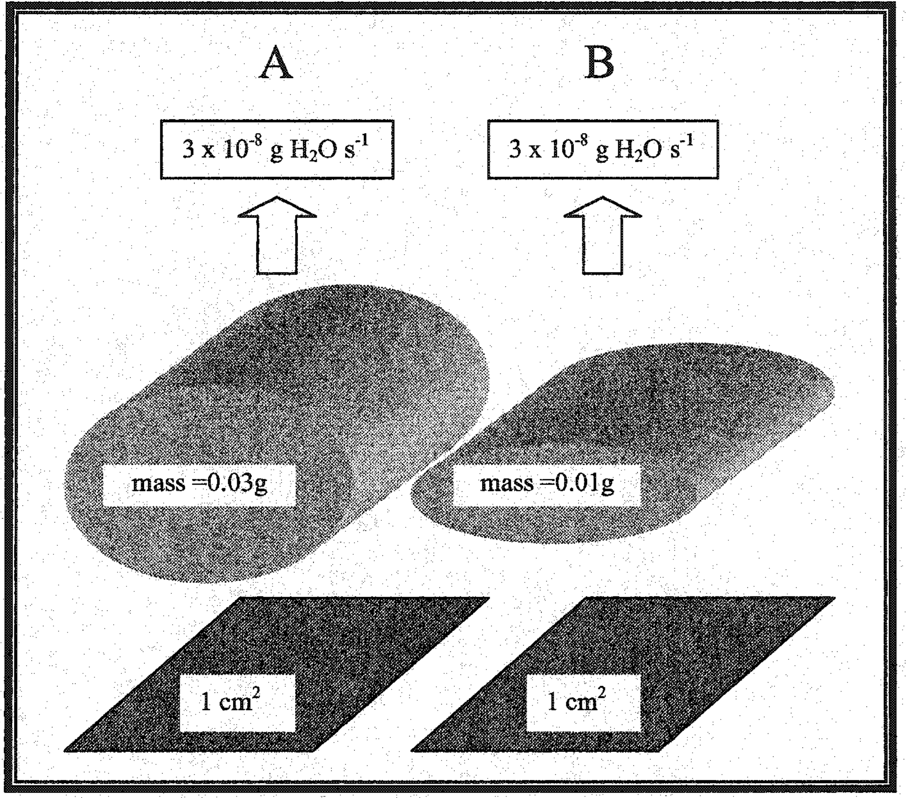


Figure V.9. Differences between area- and mass-based cuticular conductance. A representative sun-adapted needle (A) and shade-adapted needle (B) have the same projected area (1.0 cm^2) and rate of water loss (5 x 10^{-6} g H_2O). Assuming a relative humidity of 40% and an ambient temperature of 20 °C, the area-based cuticular conductances of the two needles are the same (1.47 x 10^{-5} m s^{-1}). The mass-based estimates of cuticular conductance for the two needles, however, are quite different. Sun-adapted needle A would have a mass-based cuticular conductance of 9.8 x 10^{-8} m^3 g^{-1} s^{-1}. The mass-based cuticular conductance of shade-adapted needle B would be 2.9 x 10^{-7} m^3 g^{-1} s^{-1}. Area-based conductance does not reveal that the thicker sun-adapted needle minimizes water loss per unit of needle biomass—an important adaptation to microenvironments with high evaporative stress.

Chapter VI. A model for transcuticular water movement in shoots of conifers.

Introduction and objectives

In Chapter V, the cuticular conductance (g_c) of sun-acclimated shoots of *Abies amabilis* and *Tsuga heterophylla* differed greatly depending upon the direction of water movement: $g_{c_{in}} \cong 10\ g_{c_{out}}$. The disparity was even greater for the shade-acclimated shoots, for which $g_{c_{in}}$ was about twenty times greater than $g_{c_{out}}$ (Figure V.8). The substantial difference between values of $g_{c_{out}}$ and $g_{c_{in}}$ is intriguing, because a large discrepancy between the two conductances would not have been predicted by the passive diffusion hypothesis, the most widely accepted explanation of how water moves through plant cuticles (Schreiber and Riederer 1996a).

The objective of this chapter is to explore why cuticular conductances were different; that is why dry and moist foliage of *Abies* and *Tsuga* shoots behaved unequally. To approach this problem, a process-based model will be proposed to describe the movement of water across coniferous cuticles. The two conditions under which cuticular conductance was measured in this thesis—shoots wet and shoots dry—will be considered separately, since the predictions that accrue from each case are quite different. As will be seen, a model based on the dynamic micropore hypothesis can predict the cuticular conductance of wet shoots ($g_{c_{in}}$), but would over-predict the cuticular conductance of dry shoots ($g_{c_{out}}$). In contrast, the free-volume or passive diffusion hypothesis (Schreiber and Riederer 1996a) can explain how water is lost from dry shoots ($g_{c_{out}}$), but lacks a mechanism to explain why cuticular conductance is apparently increased when the shoots are moistened with water ($g_{c_{in}}$). The two-part model for water movement through the cuticles of *Abies* and *Tsuga* shoots that will be proposed here is not unlike the behavior of another wettable porous medium, soil (Nobel 1999).

Wet cuticles: water influx through dynamic micropores

As discussed in chapter III, the abundant hydrophilic fibers in the polymer matrix of Holloway type IV cuticles may provide micropores for water movement. These micropores would not be ducts or other tube-like structures that communicate across the width of the cuticle (Franke 1967; Lyshede 1978; Hoch 1980; Miller 1985; Miller 1986a; Miller 1986b). Rather, micropores would be veins of wettable materials that can support continuous threads of water when the cuticle is fully hydrated. In comparison to diffusion, the passage of water through micropores would be by laminar flow, and the driving force would be the gradient in water potential (Schönherr 1976a). An important assumption will be made: the putative micropore system is ephemeral, establishing when the cuticle surface is wet or the atmosphere surrounding the cuticle is saturated, and losing continuity when the cuticle surface becomes dry. This assumption is supported by the work of researchers who have found that g_c can be dynamically related to a_{wv} (Schönherr and Schmidt 1979; Seymour 1980; Sowell *et al.* 1982; Becker *et al.* 1986; Hadley and Smith 1990; Boyer *et al.* 1997; Hoad *et al.* 1997; de Ruiter and Meinen 1998; Schreiber *et al.* 2001). See chapter III for a review of this literature.

Laminar water movement through dynamic micropores may be the mechanism that explains why the estimates of $g_{c_{in}}$ were greater than the corresponding estimates of $g_{c_{out}}$ for shoots of *Abies amabilis* and *Tsuga heterophylla* (Figure V.8). A Holloway type IV cuticle is analogous in important ways to a soil: a semi-solid matrix interlaced with a network of very small pores. Accordingly, the approach that will be taken here parallels that used by Nobel (1999) to analyze the conductance of a fine-textured soil. *Abies alba*—a species similar in growth form and physiological tolerances to *Abies amabilis*—will be used as an archetype, since more is known about the cuticle transport properties of this species than for the cuticles of North American Pinaceae. Parameters from other species of conifers will be used where data for *Abies alba* are not available, or from angiosperm cuticles where critical parameters for conifers have not been estimated.

The conductance of a porous cuticle will be a function of the diameter, number, and length of micropores. The micropores will be assumed cylindrical. The conductivity of a cylindrical tube is given by Poiseuille's Law (Nobel 1999), which states:

$$L^{pore} = \frac{r^2}{8\eta} \qquad (6.1)$$

where L^{pore} is the hydraulic conductivity of a pore, r is the radius of the pore, and η is the viscosity of water (Nobel 1999). Schönherr (1976a) estimated that the radius of hydrated cuticular micropores in *Citrus aurantium* is approximately 0.46 nm. Inserting the values for η at 20°C (1.002 x 10^{-3} Pa s, Nobel 1999) and for the radius of a hydrated pore provides an estimate for the hydraulic conductivity of a single micropore, or 2.6 x 10^{-11} m^2 s^{-1} MPa^{-1}.

Solids occupy most of the porous polymer matrix. The fraction occupied by pores has to be calculated to estimate the number of micropores in the cuticle. By extrapolation from Chamel *et al.* 1992, an *Abies alba* cuticle in contact with water contains 23% water by weight. Assuming that the specific weight of a dry *Abies alba* cuticle is 1.37 g cm^3 (value given for *Picea abies* cuticles by Schreiber and Schönherr 1990), and that thickness of an *Abies alba* cuticle is 6μm (figure for the width of *Picea abies* cuticles taken from Baig and Tranquillini 1976, and consistent with the range of widths reported for coniferous cuticles by Miranda and Chapekar 1980), the water content of one cm^2 of hydrated *Abies alba* cuticle is 1.9 x 10^{-4} g. All of this water is assumed to be located in micropores. The number of micropores in the hydrated cuticle is given by:

$$n\ pores = \frac{volume\ of\ water\ in\ the\ cuticle}{\pi r^2 \Delta x \vartheta} \qquad (6.2)$$

where Δx is the width of the cuticle, and ϑ is the tortuosity factor. The tortuosity factor is needed because due to deflection by imbedded waxes and strands of cutin, the actual path length of the micropores is many times greater than the width of the cuticle.

Tortuosity factors for coniferous cuticles are not available from the literature. Baur *et al.* (1999) demonstrated that waxes are largely responsible for the tortuosity of the diffusion path in plant cuticles. The diffusibility of octadecanoic acid in reconstituted *Abies alba* wax is intermediate in comparison to similar estimates of D for the waxes of fifteen other species (Schreiber and Riederer 1996). Values of ϑ due to wax for Holloway type IV cuticles varied from 28 for *Camellia assamica*, to 370 for *Citrus aurantium* (Baur *et al.* 1999). octadecanoic acid has an intermediate diffusibility in reconstituted *Abies alba* cuticle wax in comparison to cuticular waxes of fifteen other plant species, suggesting an intermediate level of tortuosity (Schreiber and Riederer 1996a). Therefore, ϑ in *Abies alba* cuticles will be assumed to have a value of 100. When these values are inserted into equation 6.2, a hydrated *Abies alba* cuticle is estimated to have 4-5 x 10^{11} micropores cm^{-2}.

Now that the number of micropores has been estimated, the hydraulic conductivity of the cuticle ($L^{cuticle}$) is found by:

$$L^{cuticle} = L^{pore} \pi r^2 n_{pores} \qquad (6.3)$$

Each of these micropores is assumed to communicate with the surface of the cuticle. The estimated value of $L^{cuticle}$ is 8.2 x 10^{-14} $m^2\ s^{-1}\ MPa^{-1}$, a value similar to what might be expected for a moist, porous soil (Nobel 1999). The anticipated water flux through the hydrated *Abies alba* cuticle is given by an analogy with Darcy's Law (Nobel 1999):

$$J_w = -L^{cuticle} \frac{\Delta\Psi}{\Delta x\,\vartheta} \qquad (6.4)$$

$\Delta\Psi$ is obtained from equation 3.1, where a^o/a^i is assumed to equal 1.0, and $\Psi_{epidermis}$ is assumed to be –1.0 MPa. When doubled to account for both sides of the leaf, J_w would be 1.0 g H_2O absorbed m^{-2} projected leaf area hr^{-1}. This value is in the same range as the J_w estimated for wet *Abies amabilis* shade-acclimated shoots in the present study (0.69 g m^{-2} hr^{-1}.

Several key assumptions were made in these calculations. The first is that Poiseuille's Law is valid for very small pores. Poiseuille's Law assumes that the flow of liquid through a circular tube is laminar. In laminar flow, rates may approach zero along the walls of the tube, but proceed at increasingly higher velocities towards the center (Nobel 1999). Since a pore 0.92 nm in diameter is wide enough to accommodate three water molecules side-by-side, the interaction between the pore walls and water may imply that that the resulting flow is non-laminar, in which case Poiseuille's Law would not correctly estimate the pore's hydraulic conductivity. However, Paganelli and Solomon (1957) used Poiseuille's Law to derive apparently correct estimates for micropore radii in blood cell membranes, and their approach has been successfully applied to estimate the size of micropores in artificial porous membranes (Schönherr 1976a). The assumption that the putative micropores are circular is also reasonable, since a circular pore would have the lowest surface area to volume ratio and therefore most stable configuration of any plausible pore geometry. Thus, in lieu of another method to derive the hydraulic conductivity of a micropore, the use of Poiseuille's Law appears to be valid for well-hydrated cuticles.

While the width of a cuticle is not difficult to measure, it is an over-simplification to assume that cuticle width is uniform. The opposite is true: cuticle width is highly variable at scales ranging from ecotype, shoot position, year of formation, and whether the cuticle is from the abaxial or adaxial side of the leaf. Cuticle width is variable even at the cellular level. The polymer matrix tends to deepen above the juncture of epidermal cells, to the point of partially separating the underlying cells (Norris and Bukovac 1968; Chabot and Chabot 1977). An important implication of the patterns of cuticle thickness is that the bulk of trans-cuticular water movement may be concentrated at localized regions of the leaf surface, and should be anticipated to vary with some of the larger-scale factors mentioned above.

The most sensitive assumption used in these calculations is that the effective width of an *Abies alba* cuticle is one hundred times greater than its actual width. The sinuous route followed by penetrating molecules is almost certainly many times greater than the actual width of the cuticles that they traverse (Baur *et al.* 1996; Baur *et al.* 1999; Schreiber *et al.* 1996). Estimates of tortuosity are based on studies of extracted waxes. Measuring the tortuosity of a coniferous cuticle is a more difficult task, since the path length in thick-cuticled plants is probably due to deflection by both imbedded waxes and polymerized cutins (Baur *et al.* 1999). Perhaps for this reason, tortuosity estimates for the cuticles of conifers have not been published. The value used for tortuosity in the above calculations could be in error by as much as a factor of three. An error of this magnitude would increase or decrease estimates of J_w by a factor of ten.

The micropores in the cuticle were assumed to behave as tiny discrete channels, each communicating with the surface of the cuticle. The assumption that the pores are continuous through the thickness of the cuticle to the surface is tenuous. Micrographs of *Abies balsamea* cuticles show that the density of polysaccharide fibers decreases dramatically towards the surface (Chabot and Chabot 1977). Experiments with salts of heavy metals have demonstrated that polysaccharide fibrils penetrate to the surface of *Malus pumila* (Hoch 1980), and that the polar sites are present on the cuticle surface *Allium cepa* (Schönherr and Bukovac 1970). In the case of both *Malus* and *Allium*, swabbing the cuticle with a wax solvent increased the number of sites where the polar metals precipitated on the cuticle surface. It is not known if this same effect—an increase in the number of polar sites on the surface of a cuticle—would also be induced by hydration. A factor that accounts for the skewed distribution of polar channels in a cuticle would be an improvement to the model that has been described here, but there is no basis to decide what an appropriate value of this factor should be.

Finally, the radius of a micropore in a conifer cuticle was assumed to be 0.46 nm. This is another sensitive assumption, since Poiseuille's Law states that the conductance of a tubular pore varies with the fourth power of its radius (Nobel 1999). No estimates for the size of micropores in conifer cuticles are available. Schönherr (1976b) demonstrated that isolated cuticles of *Citrus aurantium* and *Allium cepa* had similar micropore radii. Good evidence that the maximum pore radius in a cutinized polymer is near 0.46 nm is provided by size limitations on penetrating molecules. Cuticles behave like molecular sieves. Molecules as large as glucose (radius = 0.44 nm) can pass through isolated cuticles, while slightly larger molecules such as sucrose are almost completely reflected (Schönherr 1976a). The size limit of micropore radii in cutin is due to cross-links between polymer chains (Luque *et al.* 1995). Leaf turgidity may affect the size of cuticular micropores: Price (1982) observed that the radii of pores stretched over turgid leaves might be greater than the pore radii of isolated cuticles. While the available evidence is not extensive, a value near 0.46 nm is the best available approximation for the micropore radius through polymerized cutin in a coniferous cuticle. A ± 0.05 nm error in the pore radius would produce an estimate of J_w 1.2 to 1.8 times greater than the J_w measured for shade-acclimated *Abies amabilis* shoots.

Dry cuticles: water efflux by diffusion

As will be seen, the presence or absence of liquid water on the surface of a leaf dictates much of the dynamic behavior of putative cuticular micropores. As soon as the surface of a wet needle dries, a matric potential would be created by water-wall adhesive forces at the mouths of the micropores, and is given by:

$$\Psi_{matric} = \frac{-2\sigma\cos\alpha}{r} \qquad (6.5)$$

where Ψ_{matric} is the matric pressure in MPa, σ is the surface tension of water in MPa m^{-1}, and α is the contact angle of the meniscus with the pore wall (Nobel 1999). Ψ_{matric} is sensitive to water loss from the leaf. At 20°C, the surface tension of water is 7.28 x 10^{-8} (Nobel 1999). When $\alpha =$ 89.7°—as might be expected for the meniscus of a micropore well-replenished with water from the leaf—and $r = 0.46$ nm, Ψ_{matric} is –1.66 MPa. However, when the rate of micropore replenishment is less than the rate of evaporation from mouth of the pore, tension would depress the meniscus and intensify the hydrostatic pressure on the column of water. Under these conditions, α becomes acute and may approach 0° (Nobel 1999). For values of α of 89°, 85° and 70°, Ψ_{matric} would be –5 MPa, -28 MPa, and –108 MPa, respectively.

The flux density of water moving through the cuticle from the epidermis towards the dry surface is given by the analogy with Darcy's law (equation 6.4). The driving force, $\Delta\Psi$, is the difference between Ψ_{leaf} and Ψ_{matric}. As discussed above, Ψ_{matric} becomes small when the rate of evaporation from the micropores is greater than the rate of replenishment. The driving force for water movement through the cuticle, then, is regulated by the flux density of water vapor escaping from the mouths of the micropores:

$$J_{wv} = n_{pores}\pi r^2 g_{wv}^{bl}\left(N_{wv}^{pore} - N_{wv}^{bl}\right) \qquad (6.6)$$

where g_{wv}^{bl} is the boundary layer conductance to water vapor, and N_{wv}^{pore} and N_{wv}^{bl} are the mole fractions of water vapor in the micropores and boundary layer, respectively. Martin *et al.* (1999) reported that the g_{wv}^{bl} of *Abies amabilis* shoots ranged from 400 – 6000 mmol m^{-2} s^{-1}, with an average g_{wv}^{bl} in the field of approximately 2000 mmol m^{-2} s^{-1}. These values are twenty to fifty times greater than the stomatal conductances of the same shoots. N_{wv}^{pore} and N_{wv}^{bl} are given by:

$$N_{wv}^{pore} = N_{wv}^{*} e^{\frac{\Psi_{matric}\overline{V_w}}{RT}} \qquad (6.7)$$

$$N_{wv}^{bl} = N_{wv}^{*} \frac{a^o}{a^i} \qquad (6.8)$$

where N_{wv}^{*} is the saturation mole fraction of water vapor, $\overline{V_w}$ is partial molal volume of water, and a^o/a^i is the water activity of the boundary layer. Since *Abies amabilis* shoots are usually well-coupled with the atmosphere (Martin *et al.* 1999), the water activity of the boundary layer will be similar to the ambient water activity.

The boundary layer conductance, g_{wv}^{bl}, is much greater than the conductance of the cuticle, $L^{cuticle}$. This implies that rate of evaporation from the mouths of the micropores will always exceed the rate of replenishment from the epidermis to the cuticle. As water escapes from the cuticle, α will become small and Ψ_{matric} will decrease. Ψ_{matric} can attain equilibrium with Ψ_{bl} when the air is humid (Figure VI.1). When Ψ_{matric} is –48 MPa, for example, liquid water in the micropores would be in equilibrium with an atmosphere with seventy percent relative humidity at 20°C. At equilibrium the gradient N_{wv}^{pore} - N_{wv}^{bl} would be zero, but the gradient Ψ_{leaf} - Ψ_{matric} would be quite large. Under these conditions, water would move from the epidermis into the cuticular micropores and increase α. The increase in α would augment Ψ_{matric} and reduce the driving force for flux through the cuticle, while increasing the driving force for evaporation. Thus in *humid* atmospheres, a^o/a^i would control Ψ_{matric}, and the conductivity of the cuticle would control evaporation.

Water columns in micropores cannot be sustained in a drier atmosphere (Figure VI.1). Water in very small capillaries in contact with hydrophilic materials can possibly withstand a negative tension of up to 100 MPa (Nobel 1999). Although micropores could not exist if their linings were not hydrophilic, there is probably less attraction between water molecules and the linings of a cuticular micropore than between water and the substances that line the interstices of plant cell walls. Therefore, the threshold for tension on water columns in micropores would likely be substantially less than 100 MPa—perhaps 70 MPa, which would correspond to an atmospheric a^o/a^i of 0.6. The threads of water in the micropores would be ruptured once this threshold is exceeded. Interestingly, an a^o/a^i of 0.6 is the value at which multiple layers of water are re-established in hydrating *Lycopersicon esculentum* (Luque *et al.* 1995) and *Araucaria bidwillii* (Reina *et al.* 2001) cuticles, and at which Van Hove and Adema (1996) postulated that an aqueous continuum is formed between the surface and interior of leaves of *Phaeseoulus vulgaris* and *Populus spp.*

Laminar flow through the cuticle would cease once the continuity of water in the micropores is lost (Figure VI.1). Clusters of water molecules would be left clinging to stronger bonding sites within the polymer matrix. Water molecules would penetrate the cuticle by diffusion, for which the driving force would be $N_{wv}^{leaf} - N_{wv}^{bl}$. Water molecules diffusing through the cuticle would condense on the many small meniscii until the Ψ_{matric} generated at each meniscus was near the value of Ψ_{leaf}. Only at the surface of the cuticle would Ψ_{matric} remain in equilibrium with Ψ_{bl}.

The conductance of the micropore system would be dynamic. Between full hydration, and the point at which Ψ_{matric} exceeds the tension threshold of water in the micropores, g_c should decrease. The walls of the polymer matrix are flexible. The tension exerted at the meniscus of a cuticular micropore would pull on the walls of the polymer matrix and cause the pores to shrink (Figure VI.1). Thus, it cannot be assumed that r would remain constant in a desiccating cuticle. If r becomes smaller as Ψ_{matric} becomes more negative, then equation 6.3 predicts that $L^{cuticle}$ would diminish as well. Laminar flow would be constrained when the pore radius is reduced to a size around 0.3 nm, and would be impossible through pores with a radius less than 0.2 nm. Under these conditions, Poiseuille's Law is no longer valid for describing water movement through the cuticle. If it were possible to monitor water movement through a single micropore of a drying needle, it would not be unreasonable to expect a slow decline and then a rapid drop in the rate of water molecule passage as the pore diameter steadily diminished. Water in collapsed micropores would be nearly immobile, since the attraction between the polar pore walls and the bound molecules would tend to counter the forces for movement. Diffusion would be the only mechanism for water movement through desiccated cuticles.

Implications of the proposed two-part model for water movement in cuticles of conifers.

In the present study, the mean values of $g_{c_{in}}$ that were determined for shoots of *Tsuga heterophylla* and *Abies amabilis* were greater than the mean values of $g_{c_{out}}$ (Figure V.7). However, the two conductances were related. The natural log of mass-based $g_{c_{in}}$ was significantly correlated with the natural log of $g_{c_{out}}$ (Figure V.8B). The combined data of Schreiber and Riederer (1996a, 1996b), Niederl *et al.* (1998) and the present study show a somewhat similar relationship. The natural log of $g_{c_{in}}$ is significantly correlated ($r^2 = 0.55$, $p<0.01$) with $g_{c_{out}}$ (Figure VI.2). The data from the present study agree with the trend of the transformed data of Schreiber and Riederer (1996a, 1996b) and Niederl *et al.* (1998), suggesting that the measured differences between the $g_{c_{in}}$ and $g_{c_{out}}$ of *Abies amabilis* and *Tsuga heterophylla* are not inconsistent with the literature. The ratio between $g_{c_{in}}$ and $g_{c_{out}}$ is exponentially greater as the value of $g_{c_{out}}$ increases. The relationship between $g_{c_{in}}$ and $g_{c_{out}}$ may be stratified by cuticle structure (Figure VI.2). Additional data from cuticles of known Holloway type would be required to explore this last possibility.

The movement of water movement through cuticles of *Abies* and *Tsuga* has been approached by using the analogy of a porous medium, such as soil, or for the cuticle, a dynamic micropore. This analysis can explain the behaviors of dry and wet cuticles that were detailed in Chapter V. If the proposed model indeed holds, then there should be two consequences. The first is that while the mechanisms by which water enters and exits the cuticle may be different, the route is the same. As shown by Schrieber and Riederer (1996a) and Niederl *et al.* (1998), all molecules that penetrate the cuticle follow this route, regardless of their polarity. The reason for this is that the architecture of the transcuticular pathway is determined by the arrangement of crystalline waxes in the cuticle (Baur *et al.* 1999). In a Holloway type IV cuticle, cuticular waxes are organized as thin plates, nearly impenetrable to all molecules, embedded in a cutinized matrix. The wax plates increase the tortuosity and thereby the length of the diffusion path. All penetrating molecules are relegated to the thin zones of amorphous materials between the moats of wax (Riederer and Schneider 1990). The effect of specific kinds of cuticle structures on cuticular permeability shown by Gouret *et al.* (1993) indicates that elements of the polymer matrix may also affect the architecture of the diffusion pathway. Since the route for movement is similar regardless of the direction or the mechanism of movement, $g_{c_{in}}$ and $g_{c_{out}}$ are intrinsically related.

Second, the hydration of the cuticle increases the conductance of the pathway for diffusion, not just for water, but for all diffusing polar molecules. Evidence for this is provided by Niederl *et al.* (1998) and is further supported by de Ruiter and Meinen (1998), who showed that hydration increased the diffusion of 2,4-D through the cuticle of *Solanum nigrum.* Substances such as tributyl phosphate and alcohol ethoxylate are known to increase the mobility of diffusants by plasticizing cuticular waxes (Burghardt 1998; Buchholz and Schönherr 2000). Kersteins (1994) hypothesized that water may be a plasticizer of plant cuticles. Luque *et al.* (1995), however, found that water does not affect cuticles in this way. Nor would such an effect be expected, since it is difficult to conceive of a mechanism by which water could interact with the crystalline waxes that determine ϑ. Therefore, the effect of water must be to increase the conductivity of diffusion paths through the amorphous domains between the wax platelets. The hydration of polar functional groups in the amorphous zone would establish realms within the cuticle with properties increasingly dominated by water as hydration continues. The hydrated realms could correspond to the dynamic micropores postulated by Schönherr (1976a).

Alternatively, the water-filled regions in hydrated cuticles may establish a volume with lower resistance to diffusion than the materials of a dry cuticle. If this is true, the low D of amorphous wax and polymerized cutin would be replaced by a value closer to the diffusivity of water in water, or 2.44 x 10^{-5} m s^{-1}, as proposed by Schreiber *et al.* (2001). These two alternatives for water movement through cuticles are not incompatible with the experiments of Schreiber and Reiderer (1996), Niederl *et al.* (1998), and Baur *et al.* (1999), since the route and the path length of the hydrated regions would be largely dictated by the arrangement of waxes in the cuticle. The conductance of cuticles in a water:cuticle:water system, however, should be proportionately greater for polar versus non-polar permeands.

Thus, dry and wet Holloway type IV cuticles may have distinctly different behaviors. Their dissimilar transport properties are reflected by the differences in $g_{c_{in}}$ and $g_{c_{out}}$ that were found in this study and by the findings of other investigators that have been discussed elsewhere in this dissertation.

Identifying the mechanism responsible for water uptake by wet shoots of Abies amabilis and Tsuga heterophylla.

The model proposed here, based on the dynamic micropore hypothesis, suggests that when needles are wet, continuous albeit highly tortuous columns of water are established in the cuticle. Under these conditions, the driving force for the movement of water molecules is the difference in water potential between the needle surface and the epidermis. When needles are dry, water molecules move in the opposite direction and follow the same routes through the cuticle, but move by passive diffusion. The driving force is the concentration gradient of water vapor on either side of the cuticle. The dynamic micropore hypothesis could explain: 1) how water enters needles; 2) the large differences between $g_{c_{out}}$ and $g_{c_{in}}$ that were seen in this study; 3) the dynamic response of $g_{c_{out}}$ to humidity (Schönherr and Schmidt 1979; Seymour 1980; Sowell *et al.* 1982; Becker *et al.* 1986; Hoad *et al.* 1997; Schreiber *et al.* 2001); and 4) the dynamic relationship between $g_{c_{out}}$ and leaf water status (Hadley and Smith 1990; Sowell *et al.* 1996; Boyer *et al.* 1997; De Ruiter and Meinen 1998). This concept is analogous to water movement through soils, valid for both wet and dry cuticles. Water enters soil by laminar flow, yet must exit soil by vaporous diffusion once the continuity of water-filled pores in the soil has been disrupted by desiccation (Nobel 1999).

The alternative explanation would be that water exits and enters the cuticle by diffusion. Diffusion seems to describe cuticular transpiration, and can predict water uptake if $g_{c_{in}}$ is substituted for $g_{c_{out}}$. However, the diffusion hypothesis does not account for the dynamic nature of g_c.

The problem is how to discern the mechanism responsible for water uptake by wet conifer shoots. A starting point could be that the predicted influences of temperature and shoot water potential are significant and different for each hypothesis (Figure VI.3). For a given shoot water potential, the micropore hypothesis predicts that water uptake will increase linearly with temperature. The temperature sensitivity of the micropore hypothesis is due to the temperature dependence of the viscosity of water, while changes in shoot water potential determine the magnitude of the driving force. In contrast, the diffusional hypothesis predicts that for a given shoot water potential, uptake increases exponentially with temperature. Here, the primary effect of temperature is to increase the value of N_{wv}^{*}, the saturation mole fraction of water vapor. Increases in N_{wv}^{*} augment the concentration gradient that drives the diffusion of water vapor across the cuticle.

The differences between the two hypotheses are most pronounced for the combination of low shoot water potential and warm temperature. The micropore model predicts greater quantities of uptake when the temperature is less than 20°C, while the diffusional model predicts greater amounts of uptake for temperatures warmer than 20°C. By holding shoot water potential constant while varying temperature, it may be possible to identify which mechanism is responsible for water uptake by wet shoots. These data could be obtained by employing the drying curve method developed in this dissertation (see Chapter V).

Summary

In Chapter V, it was found that the conductances of *Abies amabilis* and *Tsuga heterophylla* shoots to water uptake was much greater than their conductances to water loss. A model based on water movement through soil was developed in this chapter to explain these data. The model proposes that desiccating shoots lose water by passive diffusion, but absorb water through dynamic micropores that are established when the surface of the cuticle is wet. In the final chapter of this dissertation, the dynamic micropore model will be used to estimate the quantity of water absorbed by the forest canopies of the Findley Lake Basin during the 1995 growing season.

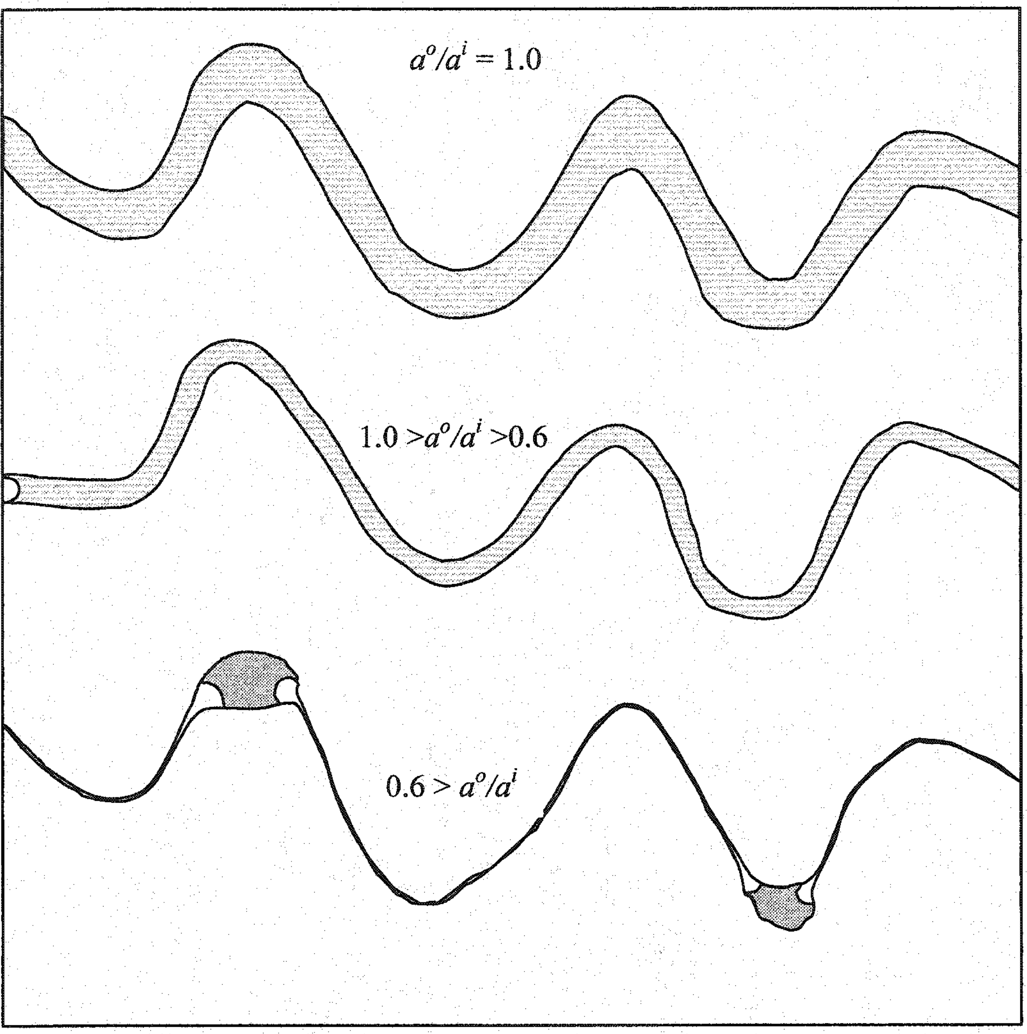


Figure VI.1. Representations of micropores in a desiccating cuticle. In a saturated atmosphere ($a^o/a^i = 1.0$), micropores are completely hydrated with continuous threads of water. The rate of evaporation from the mouth of the pore exceeds the rate of replenishment when a^o/a^i is less than 1.0, but greater than approximately 0.6. Under these circumstances, the increasing tension on the water column causes the meniscus at the air/water interface to retreat and the pore diameter to shrink. The columns of water in hydrated micropores should rupture when a^o/a^i approaches 0.6, leaving clusters of water molecules trapped at discrete sites within the cuticle. Micropores are not tubular structures *per se*, rather, concentrations of polar materials that develop a pore-like structure upon hydration.

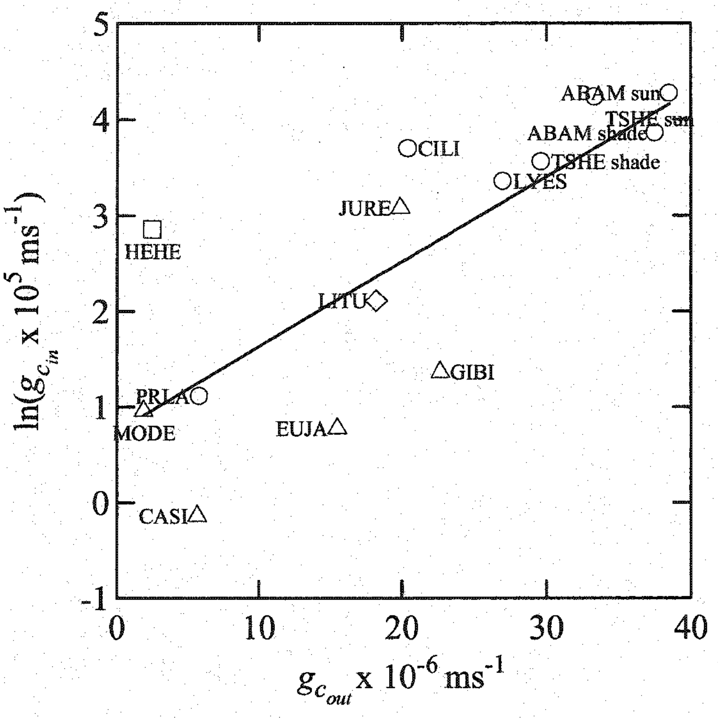


Figure VI.2. The relationship between $g_{c_{in}}$ and $g_{c_{out}}$ for 13 plant species. The symbols refer to Holloway cuticle structures: Δ = type I, + = type II, O = type IV, × = type unknown. The equation of the regression line is: $\ln(g_{c_{in}} \times 10^5 \text{ m s}^{-1}) = 0.743 + 0.089(g_{c_{out}} \times 10^6 \text{ m s}^{-1})$; $r^2=0.55$, $p<0.01$. The data for the cuticles of angiosperm plants are from Schreiber and Riederer (1996a), Schreiber and Riederer (1996b) and Niederl *et al.* (1998). The data for *Abies amabilis* and *Tsuga heterophylla* are the mean values for the area-based conductances of sun- and shade-acclimated shoots taken from the present study.

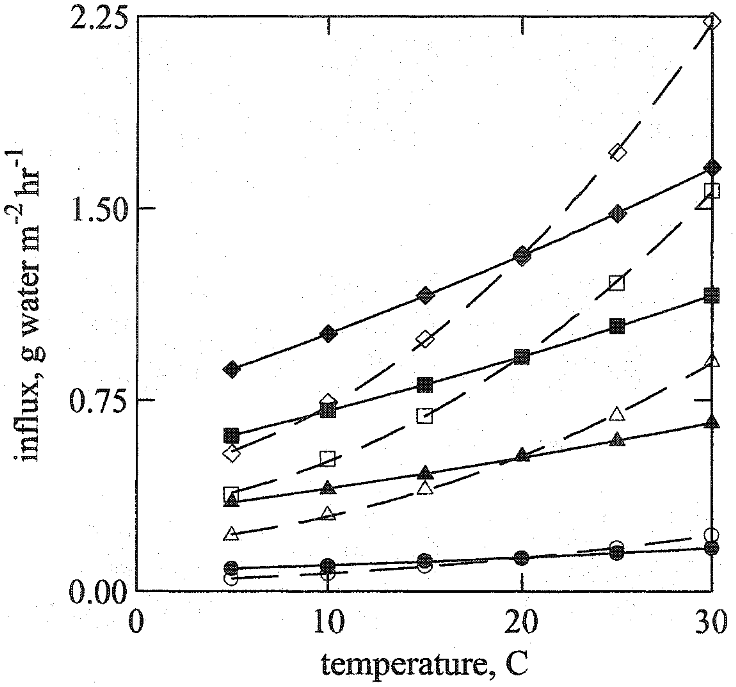


Figure VI.3. Rates of water uptake predicted for shade-acclimated *Abies amabilis* shoots by the dynamic micropore (solid lines and solid symbols) and free-volume (dashed lines and open symbols) hypotheses of cuticle transport as a function of temperature and epidermal water potential. Circles, $\Psi_{epidermis}$ = -0.2 MPa; triangles, $\Psi_{epidermis}$ = -0.8 MPa; squares, $\Psi_{epidermis}$ = -1.4 MPa; diamonds, $\Psi_{epidermis}$ = -2.0 MPa. The models predict that when $\Psi_{epidermis}$ is held constant, water uptake is either a near-linear function of temperature if water enters the cuticle through dynamic micropores, or an exponential function of temperature if water enters the cuticle by diffusion.

Chapter VII. Water fluxes in the forest canopies of the Findley Lake Basin.

Introduction and objectives

The fate of water on tree canopies in the Findley Lake Basin is the subject of this dissertation. The context of the study was established in Chapter I: the variation in stand structure, leaf area and species composition along a gradient of elevation. Chapter I also examined some of the morphological differences between *Abies amabilis*, the most important tree species in the Findley Lake Basin, and *Tsuga heterophylla*, a widespread species in western Washington that nears its elevational limit at Findley Lake. Although both species are shade tolerant, *Tsuga heterophylla* had three times more leaf area per unit basal area than did *Abies amabilis*. Moreover, while *Abies* accounted for more than 75% of the basal area at Findley Lake, *Tsuga* displayed nearly half of the leaf area. *Tsuga* was uncommon at the mid- and high-elevation sites on Findley Ridge. The estimated leaf area indices of these two sites (5.9 and 5.8 $m^2 m^{-2}$, respectively) were lower than the estimated leaf area index (8.5 $m^2 m^{-2}$) at Findley Lake.

Chapter II explored the input and redistribution of precipitation in the Findley Lake Basin. Data on canopy wetness (Figure II.1), throughfall (Figure II.2), fog precipitation (Figures II.3 and II.6) and climate were presented for the growing seasons of 1994, which was drier than normal, and 1995, a summer with near-normal precipitation (Table VII.1). The amount and distribution of throughfall varied sharply along the study elevational gradient, i.e. from 1170 to 1320m. On Findley Ridge, the canopy widely redistributed throughfall to create wet and dry zones on the forest floor (Figure II.7), but the difference between precipitation and throughfall was small (Figure II.2). Throughfall was more evenly distributed under the canopy at Findley Lake (Figure II.7), and there was a large difference between the amount of precipitation and the quantity of throughfall (Figure II.2).

Five hypotheses were developed to explain the differences in interception losses along the elevational gradient: site-driven differences in canopy leaf area; variation in canopy coupling with the atmosphere; occult precipitation from fog; elevation-related differences in evaporation rates; or the direct absorption of precipitation by the canopy. Of these hypotheses, the least was known about the possible importance of foliar uptake. Given that the Findley Lake Basin canopy was wet during 28% (upper canopy at Findley Lake) to 34% (lower canopy on Findley Ridge) of all hours during the 1995 growing season, foliar uptake certainly could have been important.

Accordingly, the next four chapters investigated water absorption by shoots of *Abies amabilis* and *Tsuga heterophylla*. Chapter III reviewed the literature on the synthesis, structure and properties of coniferous cuticles with respect to water movement. Chapter IV described attempts to quantify water uptake by foliated shoots of *Tsuga* and *Abies* with a Scholander pressure bomb. The approach proved flawed, although it provided good qualitative evidence that shoots of the two species may absorb water when wet (Figure IV.3). Chapter V introduced a gravimetric technique to study shoot water uptake. The gravimetric technique produced apparently robust results that indicated wet shoots absorb water (Figure V.3), and that shoot morphology was an important determinant of the amount of water absorbed (Table V.4). Moreover, shoot conductance to water gain was much greater than the conductance of the same shoots to water loss (Figure V.7). Chapter VI presented a process-based model of water movement through cuticles of *Abies* and *Tsuga*. To explain large differences in the conductances of shoots to water influx and efflux, the model assumed that water escapes the leaf by molecular diffusion, but enters through dynamic micropores that form when the cuticle is hydrated.

The canopy processing of precipitation, then, has been explored at an array of scales, with an assortment of techniques, in the field, and in the laboratory. This final chapter will use a model to integrate this information at the level of a forest canopy. The objective is to identify some of the controls and consequences of canopy interactions with precipitation in the Findley Lake Basin.

Materials and methods

Approach

A canopy-level model was developed to analyze fluxes of water in the forest canopy of the Findley Lake Basin during the 1995 growing season. The canopy model had three sub-units: throughfall, transpiration, and uptake. The throughfall component was based on a model by Gash *et al.* (1995). The transpiration sub-unit used an empirical model developed by Martin *et al.* 2001. The uptake model was described in Chapter VI. The three parts of the model were integrated by assuming that the canopy holds two pools of water (Figure VII.1). The external pool of canopy water represents the moisture that clings to needles, stems, lichens and other canopy surfaces. The internal pool is the water held by plant tissues. The external pool of canopy water is replenished by precipitation, and is depleted by evaporation and by shoot water uptake. Although there may be sub-pools that fill at different rates and that have variable access to precipitation, throughfall and stemflow were assumed to commence when the external pool was full. Root and canopy uptake add water to the internal pool. Water is lost from the internal pool by transpiration. Although field data on climate and throughfall were collected in 1994 and 1995, the analysis focuses on the 1995 growing season, because data on canopy wetness—essential for determining water uptake—were not collected in 1994.

Precipitation

Precipitation in 1995 was measured at five-minute intervals from 30 July to 31 October 1995 with a tipping bucket rain gauge at the lower Findley landing (elevation = 1040 meters).

The tipping bucket data are separate from the more accurate records presented in Chapter II. The period of record for these data was 66.2 days.

Evaporation

Evaporation from wet canopies was estimated in two ways: empirically, and with the Penman-Monteith equation. The empirical method calculated evaporation as the difference between precipitation and measured amounts of throughfall. The Penman-Monteith (Monteith 1965) equation was used to estimate evaporation in the canopy model:

$$E = \frac{\left[(s \cdot R_n) + (\rho \cdot c_p \cdot VPD \cdot g_{aM})\right]}{\left[(\lambda \cdot s) + (\lambda \cdot \gamma)\right]} \qquad 7.1$$

where s = the slope of the saturation specific humidity versus temperature relationship at mean surface temperature, R_n = net radiation (W m^{-2}), ρ = the density of air (kg m^{-3}), c_p = the specific heat of air (J kg^{-1} K^{-1}), VPD = the atmospheric water vapor pressure deficit, g_{aM} = the conductance of the canopy, λ = the latent heat of vaporization of water (J kg^{-1}), and γ = the psychometric constant (g kg^{-1} K^{-1}).

Equation 7.1 requires net radiation, which was not monitored in 1995. Instead, the 1994 net radiation data were regressed on 1994 short wave radiation. The regression (net radiation, W m^{-2} = -25.59 + 0.69*short wave radiation, W m^{-2}; r^2 = 0.95, F-ratio = 69654) was then used to derive the estimated net radiation for 1995 from 1995 short-wave radiation, which was monitored in 1995. The vapor pressure deficits at Findley Lake, the midslope of Findley Ridge, and Findley Ridge were based on the temperature and relative humidity at the lower Findley landing, adjusted for elevation. The atmosphere was assumed to cool adiabatically at the rate of 5°C km^{-1}. Canopy conductance (g_{aM}) was assumed to be 0.335 m s^{-1} when the wind speed was greater than 0.1 m s^{-1}

(Martin *et al.* 2001). When the wind speed was less than 0.1 m s^{-1}, g_{aM} was assumed to be 0.035 m s^{-1} (Martin *et al.* 2001).

Canopy capacitance

Canopy capacitance, defined as the amount of water left on the canopy in zero evaporation conditions when rainfall and throughfall have ceased (Gash *et al.* 1995), is a key variable for the estimation of throughfall. To estimate canopy capacitance, the live crown of a mature *Tsuga heterophylla* was partitioned into four strata of equal thickness. One branch was randomly sampled from each stratum. After all lichens were removed, the branches were weighed, immersed briefly in water, suspended horizontally until dripping had ceased, and were then re-weighed. The difference in weight was divided by the projected needle area of each branch to estimate the water retention capacity of foliage in each stratum. It was assumed that the per-unit projected leaf area water retention capacities of *Abies amabilis* and *Tsuga heterophylla* were equal.

The contribution of lichens to canopy capacitance was approached in a similar way. Four branches were harvested from the crown of a mature *Tsuga heterophylla*, one from each stratum. All lichens were then carefully gleaned from the branches, air-dried, and then weighed to determine the dry mass of lichens per unit of projected leaf area, by stratum. The harvested lichens were weighed, hydrated and weighed again to determine their mean water holding capacity. The contribution of lichens to the total canopy capacitance was then estimated by: dry mass lichens m^{-2} LA * 3 g H_2O g^{-1} lichen.

Canopy water uptake

The micropore uptake model presented in Chapter VI was used to estimate water absorption by wet canopies. Briefly, the hydraulic conductivity of a hydrated micropore is estimated by equation 6.1, the number of micropores in a hydrated cuticle by equation 6.2, the hydraulic conductivity of the cuticle by equation 6.3, and the water flux through a hydrated cuticle by equation 6.4. In the micropore model, the variables with the largest influence on water uptake are the radius of a hydrated micropore, r; the cuticle width, Δx; the percent water content of a fully-hydrated cuticle; the tortuosity factor, ϑ; and the xylem water potential, Ψ_{xylem}. Since the model in Chapter VI was developed for *Abies alba*, these parameters required calibration to reflect the transport properties of *Abies amabilis* and *Tsuga heterophylla*.

Of the variables above, there is little disparity in the published estimates for r and the percent water content of a fully hydrated cuticle (Schönherr 1976a; Schönherr and Schmidt 1979; Chamel *et al.* 1992; Reina *et al.* 2001). The values of these variables (0.46 nm and 22.6%) were the same as the values used in the *Abies alba* micropore model in Chapter VI. Given that the values of r and of the water content of a hydrated cuticle were fixed, the differences in the $g_{c_{in}}$ of *Tsuga heterophylla* and *Abies alba* were assumed to be controlled by Δx and ϑ. With respect to Δx, the effect of species is probably less important than the effect of microenvironment. Leaves developing in full sunlight have been shown to allocate more carbon to the cuticle than leaves developing in shade (Juniper 1960; Hallam 1970; Baker 1974; Hunt and Baker 1982). Additionally, vapor pressure deficits (Baker 1974; Hunt and Baker 1982) and drought (Baker 1974; Hunt and Baker 1982; Bondada *et al.* 1996; Dixon *et al.* 1997; Gilly *et al.* 1997; Svenningson and Liljenberg 1986) can be stimuli to shunt more carbon to developing cuticles. Since light is more abundant and water stress more intense in the upper canopy, the cuticles of sun-adapted needles should be thicker than the cuticles of shade-adapted needles. Shade-adapted needles were assumed to have the same Δx as *Abies alba*, or 6 µm. The cuticles of sun-adapted needles were assumed to be thicker, or 8 µm.

The tortuosity of the diffusion path differs widely among plant species, with reported values ranging between 28 and 759 (Baur *et al.* 1999). For *Abies alba*, ϑ was assumed to be 100. Relative values of ϑ for *Abies amabilis* and *Tsuga heterophylla* were found by setting appropriate values for r, Δx, and the percent water content of a fully hydrated cuticle in the micropore uptake model. Then, ϑ was varied until the simulated amount of water uptake was equivalent to the means of actual measurements (Figure V.3). Using this method, ϑ for sun- and shade-adapted *Abies amabilis* shoots was assumed to be 130 and 122, respectively, and 152 and 125 for sun- and shade-adapted *Tsuga heterophylla* shoots.

The xylem water potential determines the driving force for water uptake. Teskey *et al.* (1984a) monitored Ψ_{xylem} of *Abies amabilis* saplings in the Cedar River Watershed at monthly intervals during a growing season. The mean Ψ_{xylem} of the 5-6 m tall trees in their study was -0.66 MPa. The height of the midpoint of the canopy at Findley Lake is about 25 meters. Using the data of Teskey *et al.* as a starting point, and assuming that Ψ_{xylem} decreases -0.01 MPa m^{-1} due to gravity, and –0.02 MPa m^{-1} due to friction within the conducting path (Zimmermann 1983), a typical Ψ_{xylem} for *Abies* at Findley Lake would be about –1.04 MPa. The Ψ_{xylem} of *Tsuga heterophylla* at Findley Lake will also be assumed to be –1.04 MPa.

Water uptake was assumed to occur whenever the canopy was wet. These data were obtained from the canopy wetness sensors described in chapter II (Figure II.1). The upper and lower canopy sensors were assumed to monitor the wetness of sun- and shade-adapted shoots, respectively. Canopy wetness at the midslope of Findley Ridge, where sensors were not installed, was assumed to be the same as wetness in the forest canopy on the spine of Findley Ridge.

Finally, the estimated water fluxes m^{-2} were scaled to the canopies of the Lake, Midslope and Ridge sites by expanding by the LAI estimated for each site, partitioned between *Abies* and *Tsuga* (Table I.2). The rates of foliar uptake by *Tsuga mertensiana* and *Tsuga heterophylla* were assumed to be equal. *Abies amabilis* crowns were assumed to be made of 60 percent sun-adapted and 40 percent shade-adapted foliage, while *Tsuga heterophylla* crowns were assumed to be composed of 30 percent sun- and 70 percent shade-adapted foliage (Figure I.7).

Throughfall

In the canopy water model (Figure VII.1), the external pool of canopy water was considered analogous to a bucket of finite capacity. The capacity of the bucket—the canopy capacitance—was determined by the combined water retention capacities of the foliage and lichens. Water additions to the bucket from precipitation were given every five minutes by the tipping-bucket rain gauge. Water removals by evaporation and uptake were also computed at five-minute intervals. Whenever the external pool of canopy water attained its maximum capacity, additional precipitation was assumed to fall to the forest floor as throughfall.

Transpiration

Martin *et al.* (2001) developed an empirical equation for daily stand transpiration at Findley Lake during the 1994 growing season:

$$E_{T_{\mathrm{stand}}} = 0.056 \cdot R_c + 1.97 \cdot VPD - 0.465 \cdot VPD^2 \qquad (7.2)$$

where R_c is the daily cumulative net radiation (MJ m^{-2}) and *VPD* is the average water vapor pressure deficit (kPa). Their 1994 data were from an automated weather station installed on a tower at Findley Lake. Equation 7.2 was used to estimate transpiration in the Findley Lake Basin during the 1995 growing season, based on meteorological data collected at lower Findley Landing. As previously mentioned, 1995 net radiation was regressed from pyranometer radiation. Estimates of *VPD* for Findley Lake, the midslope of Findley Ridge, and Findley Ridge were adjusted for elevation-related effects of temperature and atmospheric pressure. Transpiration was assumed to be zero when the canopy was wet.

Results

Canopy capacitance

The water-holding capacity of *Tsuga heterophylla* branches increased with height in the canopy at Findley Lake (Table VII.2). The surfaces of sun-adapted shoots stored more water than shade-adapted shoots. Similarly, the mass of lichens per m^2 of branch leaf area appeared to decrease in concert with the diminution of light with depth in the canopy. As a result, the water storage capacity due to lichens ($\approx$ 300% of dry weight) on *T. heterophylla* branches decreased from the top to the bottom of the canopy. The canopy capacitance of the Findley Lake canopy was estimated to be 1.7 mm when the water holding capacities of foliage and lichens were combined. The capacitances of the canopies at the midslope and summit of Findley Ridge were reduced in proportion to their lower leaf areas, and were estimated to be 1.2 mm.

Canopy water uptake

The canopy model indicated that, on a per unit area basis, the rate of uptake by shade-adapted shoots was greater than the rate of sun-adapted shoots, and *Abies* was more absorptive than *Tsuga* (Table VII.3). At the level of whole canopies at each of the sites, the mean estimated rate of uptake at Findley Lake (3.22 g m^{-2} hr^{-1}) exceeded the mean rates of uptake at the midslope (2.21 g m^{-2} hr^{-1}) and summit (2.18 g m^{-2} hr^{-1}) of Findley Ridge. The higher mean rate of uptake at Findley Lake was due to: 1) greater canopy leaf area, 2) a larger percentage of relatively absorptive, shade-adapted foliage, and 3) slightly warmer temperatures that reduced the viscosity of water. These factors more than offset the longer duration of canopy wetness at the upper sites (Figure II.1). The importance of *Tsuga* was another factor that contributed to the higher rate of uptake at Findley Lake. *Tsuga* accounted for roughly one third of the basal area at Findley Lake, yet absorbed nearly half of the water taken in by the canopy. The water-capturing ability of *Tsuga* was due to the massive quantity of leaf area displayed by individual trees, of which more than two-thirds was absorptive, shade-adapted foliage. *Tsuga* was scarce at the midslope and summit of Findley Ridge (Table I.2).

At each site, the bulk of the foliar absorption occurred in the lower stratum of the canopy. The higher percentage of total uptake in the lower canopy was due to the greater conductance of shade-adapted foliage (Figure V.5). Furthermore, the lower canopy dried more slowly after precipitation events, and as a consequence, absorbed more water. Over the course of the 1995 growing season, the Findley Lake canopy absorbed the equivalent of 2.6 mm of precipitation, while 2.2 mm were absorbed at the two Findley Ridge sites.

The micropore uptake model was built on a series of assumptions. The first was that the uptake of water by shoots of *Abies* and *Tsuga* occurs primarily through hydrated micropores, and not by diffusion. A diffusion-based model for canopy uptake in the Findley Lake Basin, strongly influenced by the temperature-dependence of N_{wv}, indicated that cumulative water uptake during the 1995 growing season would have been 1.6 mm at Findley Lake, and 1.3 mm at the midslope and summit of Findley Ridge. The mechanism by which water absorption occurs, then, is important, and strongly affects the predictions that accrue from it. Other key assumptions include the xylem water potential in the canopy; the thickness, hydrated water content, specific weight and tortuosity of *Abies* and *Tsuga* cuticles; and the radius of a hydrated micropore (Figure VII.2). A ± 50% error in the estimated versus actual value of any one of these variables would have resulted in a mean range of the estimated seasonal quantity of uptake in the Findley Lake canopy of 1.8 to 3.5 mm.

Throughfall

The amount of throughfall for the 1995 growing season predicted by the canopy water model was close to the measured amount of throughfall at Findley Lake (Table VII.4). Similarly, the amount of predicted throughfall at Findley Lake for the 1994 growing season (97 mm) was comparable to the measured total (104 mm). While the canopy model successfully used the Penman-Monteith equation to predict throughfall at Findley Lake, the model under-predicted the quantity of throughfall at the midslope and summit of Findley Ridge by approximately 40 mm (Table VII.4). Two important canopy-level variables—canopy conductance and canopy capacitance—were assessed in the canopy model to determine their influences on throughfall (Figure VII.3). The model was relatively insensitive to canopy conductance, irrespective of the value used, yet strongly influenced by canopy capacitance.

Transpiration

During 124 days of the 1995 growing season, the Findley Lake canopy transpired 124.8 mm H_2O, or 0.97 ± 0.10 mm per day. The mean estimated transpiration rates from the two upper sites were lower: 0.66 ± 0.07 mm day^{-1} and 0.65 ± 0.07 mm day^{-1} at the midslope and top of Findley Ridge, respectively (Table VII.4). Most of this transpiration occurred on clear days with high net radiation and strong vapor pressure deficits (Figure VII.4). These clear, sunny days (47% of all days in 1995) accounted for 87% of total canopy transpiration and 81% of net solar radiation during the 1995 growing season at the Findley Lake Basin.

Discussion

A water budget for the Findley Lake Basin

During the period 16 June-18 October 1995, 375.5 mm of precipitation fell on the forest canopy at Findley Lake. Of this precipitation, 102.2 mm evaporated from the canopy, 2.6 mm were absorbed into needles and stems, and 271 mm descended to the forest floor as throughfall and stemflow. The Findley Lake canopy transpired 124.8 mm of water during this time, or about 46 percent of the throughfall that fell to the forest floor.

The forest canopy on Findley Ridge received the same apparent amount of precipitation. Of that total, 30-35 mm evaporated, 2.2 mm were absorbed, and 338 to 343 mm dripped from the canopy to the forest floor. Transpiration from the forests on Findley Ridge was about 83 mm, or 24% of throughfall. Thus, along a horizontal distance of 580 meters and a modest gain in elevation of 150 meters, throughfall increased 25%, transpiration decreased 33%, and the apparent evaporation of rain intercepted by the canopy declined by a factor of three.

The slopes above Findley Lake yielded most of the Basin's water during the 1995 growing season. The forested areas (108 ha) on the slopes above the basin floor (58 forested ha) yielded an estimated 278 megaliters of water, more than three-quarters of the estimated water yield from the Findley Lake Basin during the summer of 1995. The enhanced water production from the upper slopes was due to modestly low rates of transpiration, and more importantly, to very low rates of canopy evaporation. The measured 1995 interception loss from the canopy at Findley Lake (27.9%; Figure II.2) was within the expected range for a coniferous forest with moderately high leaf area in an environment of low-intensity, high frequency precipitation (Table II.1). The interception losses from the stands on Findley Ridge were much lower. Only 8.6% (Figure II.2) of the above-canopy precipitation that fell on the midslope of Findley Ridge did not fall to the forest floor. The measured 1995 interception loss on the summit of Findley Ridge (10.0%; Figure II.2) was also small. The interception losses from the mid- and upper elevations of the Findley Lake Basin were unexpectedly low for mature coniferous forests, and were incongruous with results from other studies (Table II.1).

As first discussed in Chapter II, the direct uptake of water by the canopy was one of the explanations proposed to account for the differences between precipitation and throughfall in the Findley Lake Basin. This hypothesis can be rejected with confidence. The estimated quantities of canopy uptake (Table VII.4) were small in comparison to precipitation. Furthermore, of the 2.6 mm of precipitation directly absorbed by the forest canopy at Findley Lake, only 1.4 mm could have potentially dripped to the forest floor as throughfall (1.0 mm at the two upper sites). The remainder of the water absorbed by the canopy was either deposited by rains not intense enough to be recorded by the tipping bucket rain gauge, or by fog.

Elevation-related effects—principally temperature and atmospheric pressure—were also postulated in Chapter II to account for the contrasting interception losses between the three sites.

Temperature and pressure influenced many of the variables used to estimate evaporation in the canopy model, such as N_{wv}, VPD, s, ρ, c_p, λ and γ. When the elevations of the three sites were set to 1170 m in the canopy model (the altitude of Findley Lake), the simulated amounts of throughfall at the two sites on Findley Ridge decreased by about 18 mm. Roughly 12 mm of the predicted decrease were due to greater atmospheric pressure, while 6 mm were related to slightly warmer temperatures. Similarly, reasonable variation in the assumed adiabatic rate (5 ± 1.0 °C) used in the model resulted in a 10 – 15 mm difference in predicted throughfall (Figure VII.3A). Temperature and pressure certainly changed enough over the 150-meter rise in elevation from Findley Lake to Findley Ridge to influence estimated quantities of throughfall, but not enough to account for the ± 70 mm difference in throughfall between Findley Lake and the sites on Findley Ridge.

Variation in canopy conductance (g_{aM}) may have led to different rates of canopy evaporation along the gradient from Findley Lake to Findley Ridge. Two values for g_{aM} were used in the canopy model: one for wind speeds greater than 0.1 m s^{-1} (0.335 m s^{-1}), and another for wind speeds less than 0.1 m s^{-1} (0.034 m s^{-1}). The canopy model was unaffected by values of g_{aM} when wind speeds were less than 0.1 m s^{-1}. When g_{aM} was varied for higher wind speeds between 0.1 and 0.6 m s^{-1} in the canopy model, the predicted amount of throughfall from the Findley Lake canopy varied by approximately four percent of precipitation, or 15 mm (Figure VII.3B). Evaporation from wet canopies in the Findley Lake Basin was surprisingly insensitive to a five-fold variation in g_{aM}, given that g_{aM} regulates the transfer of energy from the atmosphere to the canopy environment (Equation 7.1). R_n and VPD were low during precipitation events in the Findley Lake Basin. The insensitivity of the canopy model to g_{aM} was likely due to the low amount of transferable energy that could cause evaporation during precipitation events.

Reasonable variation in the levels of canopy conductance is not a strong explanation to account for the variability in throughfall along the Findley Lake-to-Findley Ridge gradient.

Given that the forest canopy is the surface that intercepts precipitation, and from which the intercepted precipitation then evaporates, variation in canopy leaf area is another potential explanation for the observed differences throughfall. Indeed, tentative relationships between leaf area and interception losses were observed in the Findley Lake Basin. Findley Lake, the site with the highest leaf area, was the site with the greatest interception loss. Furthermore, the estimated leaf areas of the two sites on Findley Ridge were similar, as were the respective amounts of precipitation lost from the canopy.

There is no term for canopy leaf area in the Penman-Monteith equation (Equation 7.1), which is a "big leaf" model of evaporation. Instead, leaf area relates to canopy storage of precipitation, or canopy capacitance. Of the variables assessed for their influence on predicted quantities of throughfall, the canopy model indicated that canopy capacitance was the most important (Figure VII.3C). Water held in the canopy is available for evaporation, irrespective of the rate of evaporation. By increasing the vulnerability of precipitation to evaporation, an increase in canopy capacitance has the same effect as a decrease in the intensity of precipitation.

The measured capacitance of canopy foliage at Findley Lake was 1.04 mm (Table VII.2). This value agrees with reported canopy capacitances for western forests, which typically range between 0.5 and 2.5mm, depending on the age and leaf area of the stand (review by Zinke 1967), and is nearly the same as the canopy capacitance (1.09 mm) of a closely-spaced, 18 year-old *Picea sitchensis* stand (Teklehaimanot and Jarvis 1991). The additional contribution of lichens to water retention in the canopy at Findley Lake (0.66 mm, Table VII.2) may reflect the influence of stand age on canopy capacitance.

Canopy capacitance was not rigorously quantified for the forest canopy at Findley Lake and was not measured at the other two sites, where it was assumed that capacitance was a direct function of leaf area. Errors in these measurements and assumptions were likely. Even so, even if the canopy capacitance of the two sites on Findley Ridge were over-estimated by a factor of three, no more than half of the approximate 70 mm difference between the quantity of throughfall at these sites versus Findley Lake could be accounted for (Figure VII.3C). An error of this size would require a dramatically incorrect assessment of the LAI of the stands on Findley Ridge, together with near-zero epiphyte loading due to some unknown factor related to elevation.

The canopy model suggested that quantities of 1995 throughfall in the Findley Lake Basin were essentially unaffected by direct absorption in the canopy, fairly insensitive to reasonable ranges of canopy conductance and adiabatic cooling, and strongly influenced by canopy capacitance. Variation in these factors is not sufficient to explain the measured differences between throughfall at the three sites in the Findley Lake Basin during the 1995 growing season. Therefore, another possibility needs to be considered. Moisture from fog or dew not detectable by a rain gauge could have reduced the effective capacitance of the canopy without directly contributing to throughfall. Reducing the capacitance of the canopy has the same influence on throughfall as increasing the intensity of rainfall. In either case, precipitation would reside less time in the canopy, and would be less vulnerable to evaporation. If true, this hypothesis could resolve the minor paradox that fog could not be significantly linked to the total amount of throughfall (Figure II.4), but was significantly related to the percentage difference between precipitation and throughfall (Figure II.6). The difference between the actual and modeled interception losses on Findley Ridge, then, could be a rough estimate of the quantity of fog precipitation. For the 1995 growing season, these estimates would be 46 mm of occult precipitation on the forest canopy at the midslope of Findley Ridge, and 34 mm of occult precipitation at the summit of Findley Ridge.

Water uptake in the canopy

The canopy model indicated that during the 1995 growing season, water uptake by foliage was 2.1% of the estimated quantity of transpiration from the Findley Lake canopy, and 2.6% of the transpiration from the canopies at the two Findley Ridge sites. Essentially all of the water absorbed by the canopy was acquired during cloudy days with lower than normal rates of transpiration (Figure VII.4). If only these days are considered, canopy uptake could have been equivalent to 19% of the cloudy day water use at Findley Lake, and 28% of the cloudy day transpiration from the sites on Findley Ridge.

Cloudy days in the Findley Lake Basin are characterized by cool temperatures, low values of *VPD*, and often rain or fog. The canopy is illuminated by diffuse light of moderate intensity, typically ranging from 25 to 250 W m^{-2}. In 1995, the canopy was wet slightly more than half of all hours during overcast periods in the Findley Lake Basin. Transpiration is low, proceeding at 10 to 13% of the rate that would be anticipated during a clear day.

Under these circumstances, the small quantities of water absorbed by a wet crown could become physiologically significant. Small amounts of water can induce significant change in Ψ_{xylem} when cell walls are inflexible (Boyer 1995; Walters and Reich 1999). The equations of the pressure-volume relationships found for *Abies amabilis* and *Tsuga heterophylla* (Table IV.3) can be used to estimate $\Delta\Psi_{xylem}$ given expected rates of uptake (Table VII.3). Using this approach, water uptake by a wet canopy could result in rates of xylem water potential increase of between +0.007 MPa hr^{-1} for sun-adapted *Abies amabilis* shoots, to +0.026 MPa hr^{-1} for shade-adapted

Tsuga heterophylla shoots. Assuming that the canopy is wet 20.4 hours (the mean duration of wetness in the canopy during the 1995 growing season), the Ψ_{xylem} of shoots in the canopy could increase as much as +0.14 to +0.5 MPa.

It is not clear what the actual magnitude of $\Delta\Psi_{xylem}$ would be. There is a tradeoff between a measurable response to Ψ_{xylem}, and the quantity of water gained by a crown via foliar uptake. The response of Ψ_{xylem} to canopy water uptake would depend on the destination of the absorbed water within a shoot. If the absorbed water were to accumulate near the site of uptake, then the driving force for absorption should decrease as Ψ_{xylem} increases. If the absorbed water were to be redistributed elsewhere in the shoot, or exported to another organ of the plant, then the rate of water absorption may be maintained over time. Alternatively, uptake and transpiration could occur simultaneously in the same shoot. In this case, the water potential gradient may be maintained or even intensified as the shoot gains water.

Uptake-related change in Ψ_{xylem} could be especially significant in the upper stratum of the old growth forest canopy in the Findley Lake Basin. First, the soil is cooler than 2°C—cool enough to inhibit water absorption by roots of *Abies*—during much of the year (Teskey *et al.* 1984b). Second, Ryan and Yoder (1997) proposed that as trees increase in height and age, the resistance of the water transport pathway increasingly limits carbon fixation by foliage at the top of the crown. As tree height increases and the xylem transport pathway becomes less efficient, the potential rate of water loss from transpiration becomes greater than the rate of water re-supply through the stem. Accordingly, stomatal conductance is reduced to avoid dangerously high water tensions in the xylem. The reduction in stomatal conductance results in less carbon dioxide uptake, and unavoidably lower rates of carbon fixation. Height- and age-related reductions in stomatal conductance have been described in *Pinus ponderosa* (Ryan *et al.* 2000), *Pinus* *sylvestris* (Mencuccini and Grace 1996) and *Pseudotsuga menziesii* (Bauerle *et al.* 1999; Phillips *et al.* 2002).

In *Abies amabilis*, Martin (1997) found that the mean stomatal conductance of current-year needles in the old growth stand at Findley Lake (mean height = 32 m) was 51 mmol m^{-2} s^{-1}, compared to 89 mmol m^{-2} s^{-1} in a younger, 15 m-tall stand. Buchmann *et al.* (1998) related that the concentration of canopy CO_2 in the old growth stand at Findley Lake was rarely depleted below atmospheric levels, while CO_2 was depleted as much as 17 ppm in air entrained in the canopies of two younger *A. amabilis*-dominated stands. The differences in CO_2 drawdown reported by Buchmann *et al.* (1998) again suggest that g_s and A are limited by height and age in the Findley Lake old growth. While at least a portion of these reported differences in the g_s of old and young *Abies* were likely related to differences in site quality and specifically, soil nitrogen, the greater resistance to water transport in the stems of the taller old-growth trees may have been equally important in reducing g_s, if not more so.

At least two mechanisms have been identified that enable old and tall trees to partially compensate for the hydraulic limitations of large size. McDowell *et al.* (2002) found that the ratio of leaf area to sapwood area declines in *Pseudotsuga menziesii* as tree height increases. Thus, as trees gain height, servicing a lower area of needles with a greater area of less efficient sapwood minimizes declines in leaf-specific conductance. McDowell *et al.* (2002) found that six other species showed a similar pattern, although *Picea abies* and *Abies balsamea* did not.

The second mechanism that has been shown to compensate for the reduced transport capacity of the sapwood of tall trees is an increase in stem capacitance (Phillips *et al.* 2003). Large trees can store substantial quantities of water in their sapwood. Phillips *et al.* (2003) showed that water stored overnight in stems of large *Pseudotsuga menziesii*, *Quercus garryana* and *Pinus ponderosa* augmented the slow transport of water from the root system to supply mid-day peaks of transpirational demand. In this way, the trees were able to maintain stomatal conductance during periods of high solar irradiance, when the potential for photosynthesis was highest.

The absorption of water by the canopy may be a third mechanism that enables tall trees to compensate for height-related hydraulic limitations. Water absorbed by foliage is a free subsidy that bypasses the limitations of gravity and friction. Once water arrives in the crown, transpiration through stomata is its probable fate, irrespective of how the water was acquired. The extra carbon gain due to the direct absorption of water in the canopy can be approximated by the relation between water use and carbon fixation in C_3 plants (Table VII.5).

To consider the potential consequences with respect to carbon of water uptake in the canopy, consider two trees at Findley Lake. One is an *Abies amabilis*, and the other is a *Tsuga heterophylla*. Both trees have a diameter of 50 cm DBH. The amount of extra carbon gained due to foliar water uptake by these two trees should be different, largely because the crown architectures of the two species are dissimilar. Assume that: 1) the *Abies* crown has a projected leaf area of 143 m^2, while the projected leaf area of the *Tsuga* is 450 m^2 (see empirical equations in Chapter I); 2) the *Abies* crown is about 60 percent sun-adapted foliage and 40 percent shade-adapted foliage (Sprugel unpublished); 3) the *Tsuga* crown is composed of about 30 percent sun-adapted foliage and 70 percent shade-adapted foliage (Figures I.4 and I.7); 4) the upper canopy at Findley Lake was wet 28.9% of the 1995 growing season versus 32.1% in the lower canopy (Figure II.1); 5) when wet, the crowns absorb water at the average modeled rates for the 1995 growing season (Table VII.3)and 6) the water use efficiency of *Abies amabilis* at Findley Lake was estimated to be 8.3g CO_2 l^{-1} H_2O (Tim Martin, personal communication). With these assumptions in place, the *Abies amabilis* should fix about 418 additional grams of CO_2 per growing season due to water uptake by the crown. The *Tsuga* should benefit more, gaining about 1395 additional grams of CO_2.

These estimates of the potential carbon subsidy from water uptake could be conservative, because during the misty, cloudy days when uptake occurs, transpiration is reduced more than radiation. This would enhance water use efficiency, and possibly augment the carbon gain from water uptake. For example, Chen *et al.* (2002) found that the water use efficiency of an old-growth *Pseudotsuga menziesii* forest in southwestern Washington was 5-12 mg g^{-1} in the early morning, when light was sufficient for photosynthesis but not enough heating had yet occurred to increase the vapor pressure deficit. Later, as temperature increased, water use efficiency dropped to 2-3 mg g^{-1}.

Regardless, the additional gains of carbon are large enough to be physiologically significant. If 30% of the fixed carbon were used for maintenance, 418 grams of CO_2 would be enough for an *Abies amabilis* to manufacture about 2.0 m^2 of additional needle area each year, while a *Tsuga heterophylla* at Findley Lake could add about 7.7 m^2 needle area with 1395 grams of extra CO_2. Alternatively, the extra carbon could be invested in reproduction, the replacement of damaged organs, root growth—whatever the strongest sink for carbon would be within the plant. The impact of canopy water uptake on the carbon balance of trees in the Findley Basin would be similar to extending the growing season by 2.5 to 3 days, potentially important in a harsh, often snow-bound environment. Finally, compounded over many years, the disproportionate carbon gains estimated for *Tsuga* due to water uptake could alter the outcome of competition between *Tsuga heterophylla* and *Abies amabilis* in the Findley Lake Basin

Directions for future research

A current emphasis of ecology is to replace the black box models of earlier studies with more dynamic models based upon mechanisms. This dissertation has attempted to look inside the black box of the forest canopy to better understand how forests regulate water. The assumption that canopies do not absorb water was replaced with a mechanistic model of water uptake by wet shoots. The model showed that water uptake in the canopy is ultimately a small flux. The importance of this process is not known. However, for trees near the limits of their tolerances, whether due to succession, age, disease, climate shifts, or some other agent, small processes such as canopy uptake could help to explain the patterns that would emerge. Mechanisms are the key to a dynamic understanding of ecosystem change.

From this perspective, canopy uptake is a process that deserves study at several levels. First, the techniques and models developed in this dissertation need to be verified. Second, foliar uptake needs to be quantified across a range of species. It would be interesting, for example, to compare uptake by *Pseudotsuga menziesii*, a conifer with Holloway type I cuticle structure (Table III.I), with uptake by *Picea sitchensis*, a species that shows affinity for moist and often fog-bound environments. Third, foliar uptake needs to be studied over scales of size, from micro-level study of hydrated cuticles, to field studies that examine responses by whole organisms. Fourth, foliar uptake should be studied over different scales of time, with variation in the duration and frequency of treatments. Finally, changes in water status due to uptake need to be explicitly linked to physiological responses.

Summary

In this final chapter of the dissertation, an integrative model was used to analyze the fate of canopy water during the 1995 growing season in the Findley Lake Basin. The model indicated that more than three-quarters of the basin's estimated water yield came from forests on the middle and upper slopes. The higher water yield from the upper portions of the basin was due to lower rates of transpiration, and to unexpectedly small losses of precipitation intercepted by the canopy. Occult precipitation from fog was the most likely cause of the reduced interception losses from the slopes above Findley Lake. The model was also used to estimate the total quantity of water absorbed by the canopy. While not significant from a hydrological perspective, canopy uptake could be physiologically and ecologically important in the Findley Lake Basin, and is deserving of additional study.

Table VII.1. Periods of record for data on throughfall, canopy wetness, fog and meteorology collected during the growing seasons of 1994 and 1995 in the Findley Lake Basin, Cedar River Watershed, WA.

component	1994	1995
meteorology	17 July-10 October (84 days)	27 July-31 October (79 days)
throughfall	25 July-18 October (86 days)	16 June-18 October (124 days)
canopy wetness	---	7 July-30 October (115 days)
fog sedimentation	---	30 July-15 September (6 events)

Table VII.2. The canopy capacitance of *Tsuga heterophylla* at Findley Lake, Cedar River Watershed, WA. Assuming 1) that the leaf area of *Tsuga heterophylla* at Findley Lake was 4.0 m^2 LA m^{-2}; 2) the leaf area of *Abies amabilis* at Findley Lake was 4.5 m^2 LA m^{-2}; 3) the water holding capacity and lichen loads of *Abies* and *Tsuga* branches were similar; and 4) the distribution of *Abies amabilis* foliage by canopy stratum was 30, 35, 20 and 15%; the estimated canopy capacitance at Findley Lake was 1.7 mm m^{-2}. The canopy capacitance at the midslope and summit of Findley Ridge was assumed to be proportional to the estimated canopy leaf area at these two sites, or 1.2 mm m^{-2}.

<table>
<tr><td colspan="5">water retention capacity of <i>Tsuga heterophylla</i> foliage</td></tr>
<tr><td>canopy stratum</td><td>percent of total species leaf area</td><td>branch leaf area m^2 projected LA</td><td>water retention capacity mm m^{-2} LA</td><td>water retention capacity mm m^{-2} LA</td></tr>
<tr><td>1</td><td>10</td><td>0.4</td><td>0.170</td><td>0.07</td></tr>
<tr><td>2</td><td>30</td><td>1.2</td><td>0.137</td><td>0.16</td></tr>
<tr><td>3</td><td>40</td><td>1.6</td><td>0.109</td><td>0.17</td></tr>
<tr><td>4</td><td>20</td><td>0.8</td><td>0.103</td><td>0.08</td></tr>
<tr><td>all</td><td></td><td></td><td></td><td>0.48</td></tr>
<tr><td colspan="5">water retention capacity of lichens on <i>Tsuga heterophylla</i> branches</td></tr>
<tr><td>1</td><td>10</td><td>0.4</td><td>0.085</td><td>0.03</td></tr>
<tr><td>2</td><td>30</td><td>1.2</td><td>0.081</td><td>0.10</td></tr>
<tr><td>3</td><td>40</td><td>1.6</td><td>0.065</td><td>0.10</td></tr>
<tr><td>4</td><td>20</td><td>0.8</td><td>0.009</td><td>0.01</td></tr>
<tr><td>all</td><td></td><td></td><td></td><td>0.24</td></tr>
<tr><td colspan="5">water retention capacity of <i>Abies amabilis</i> branches</td></tr>
<tr><td>1</td><td>30</td><td>1.350</td><td>0.170</td><td>0.23</td></tr>
<tr><td>2</td><td>35</td><td>1.575</td><td>0.137</td><td>0.22</td></tr>
<tr><td>3</td><td>20</td><td>0.900</td><td>0.109</td><td>0.10</td></tr>
<tr><td>4</td><td>15</td><td>0.675</td><td>0.103</td><td>0.07</td></tr>
<tr><td>all</td><td></td><td></td><td></td><td>0.62</td></tr>
<tr><td colspan="5">water retention capacity of lichens on <i>Abies amabilis</i> branches</td></tr>
<tr><td>1</td><td>30</td><td>1.350</td><td>0.085</td><td>0.12</td></tr>
<tr><td>2</td><td>35</td><td>1.575</td><td>0.081</td><td>0.13</td></tr>
<tr><td>3</td><td>20</td><td>0.900</td><td>0.065</td><td>0.06</td></tr>
<tr><td>4</td><td>15</td><td>0.675</td><td>0.009</td><td>0.01</td></tr>
<tr><td>all</td><td></td><td></td><td></td><td>0.32</td></tr>
</table>

Table VII.3. Mean estimated hourly canopy uptake rates for *Tsuga heterophylla* and *Abies amabilis* during the 1995 growing season in the Findley Lake Basin, Cedar River Watershed, WA. Uptake was estimated with a micropore-based model parameterized with measured climatic data. Ψ_{xylem} was assumed to be –1.04 MPa.

site	mean uptake rate, g H_2O m^{-2} LA hr^{-1}			
	Abies amabilis sun-adapted shoots	*Abies amabilis* shade-adapted shoots	*Tsuga heterophylla* sun-adapted shoots	*Tsuga heterophylla* shade-adapted shoots
Findley Lake	0.31	0.47	0.23	0.45
Midslope of Findley Ridge	0.31	0.47	0.23	0.45
Findley Ridge	0.31	0.47	0.22	0.44

Table VII.4. Estimated water fluxes (mm) in the Findley Lake Basin during 124 days of the 1995 growing season. Precipitation and throughfall were measured; canopy uptake was simulated with a micropore-based model driven by gradients of water potential; transpiration was modeled with an empirical equation taken from Martin *et al* (2001); and evaporation was obtained by subtraction.

site	precipitation	evaporation	canopy uptake	measured throughfall (% of precip)	modeled throughfall (% of precip)	transpiration
Findley Lake 1170 m	375.5	102.2	2.6	270.7 (72%)	270 (72%)	124.8
midslope of Findley Ridge 1230 m	375.5	30.3	2.2	343 (91%)	297 (79%)	84.5
Findley Ridge 1320 m	375.5	35.3	2.2	338 (90%)	304 (82%)	83.0

Table VII.5. Water use efficiencies of conifers.

Study	Species	Water use efficiency g C 1000 g^{-1} H_2O
Chen *et al.* 2002	*Pseudotsuga menziesii*	1.7 - 2.7
Jiang *et al.* 1995	*Picea glauca*	3.7 – 4.9
Moore *et al.* 1999	*Juniperus occidentalis*	4.35
	Juniperus osteosperma	4.03
Sullivan 1997	*Pinus banksiana*	6.6 – 12.2
	Picea mariana	6.4 - 13.7
Sala *et al.* 2001	*Abies lasiocarpa*	4.9 - 17.1
	Pinus albicaulis	4.4 - 10.3
Zhang *et al.* 1994	*Larix occidentalis*	3.7 ± 0.1
Zhang *et al.* 1997	*Pinus ponderosa*	9.8 - 19.6

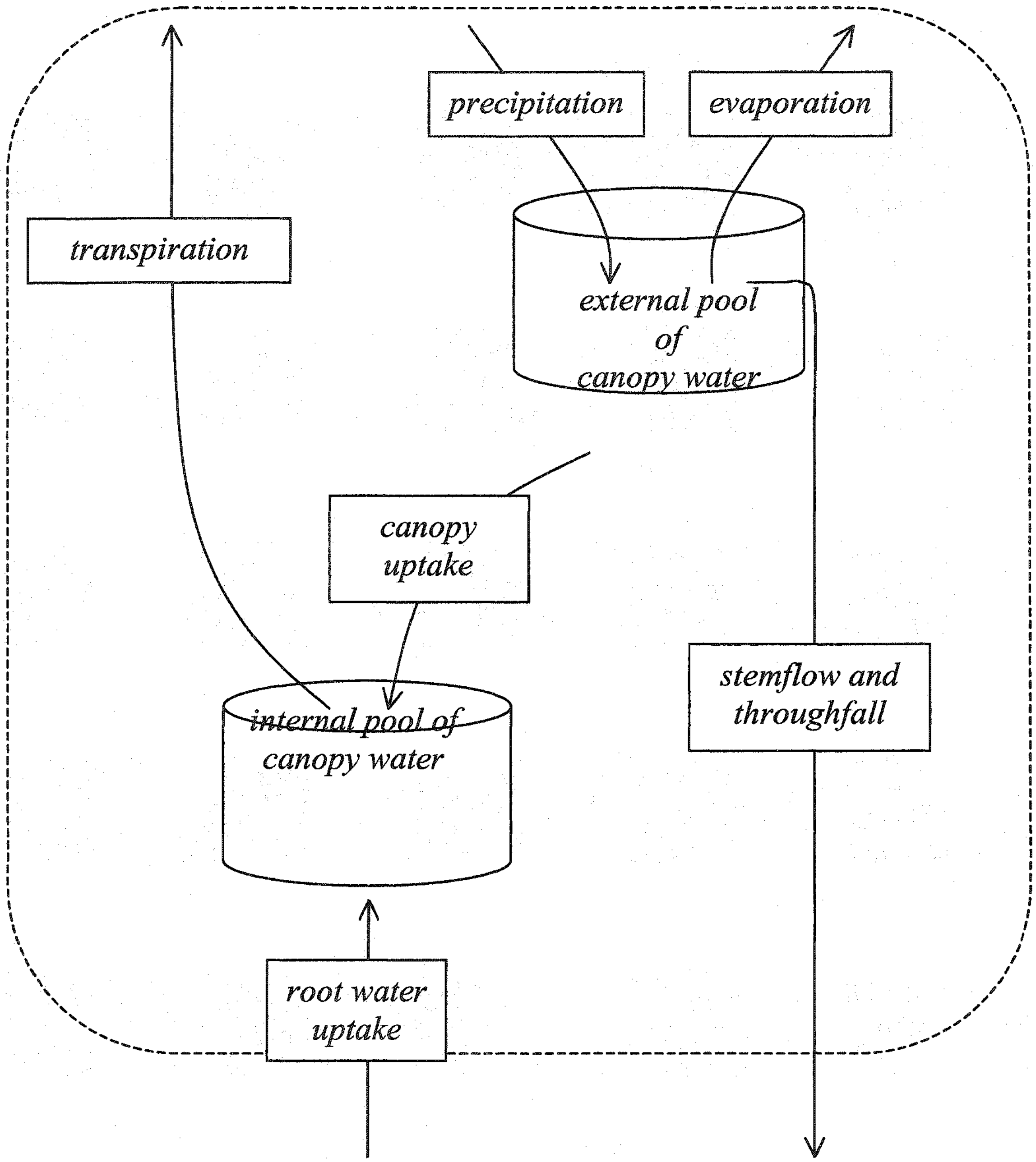


Figure VII.1. Model of water fluxes in a forest canopy. Precipitation fills the external pool of canopy water, which has a finite capacity (the canopy capacitance). Water is lost from the external pool by evaporation, uptake, stemflow and throughfall. Canopy uptake links the external pool to the internal pool of canopy water. The internal pool of canopy water gains moisture by root and canopy uptake, and loses water via transpiration.

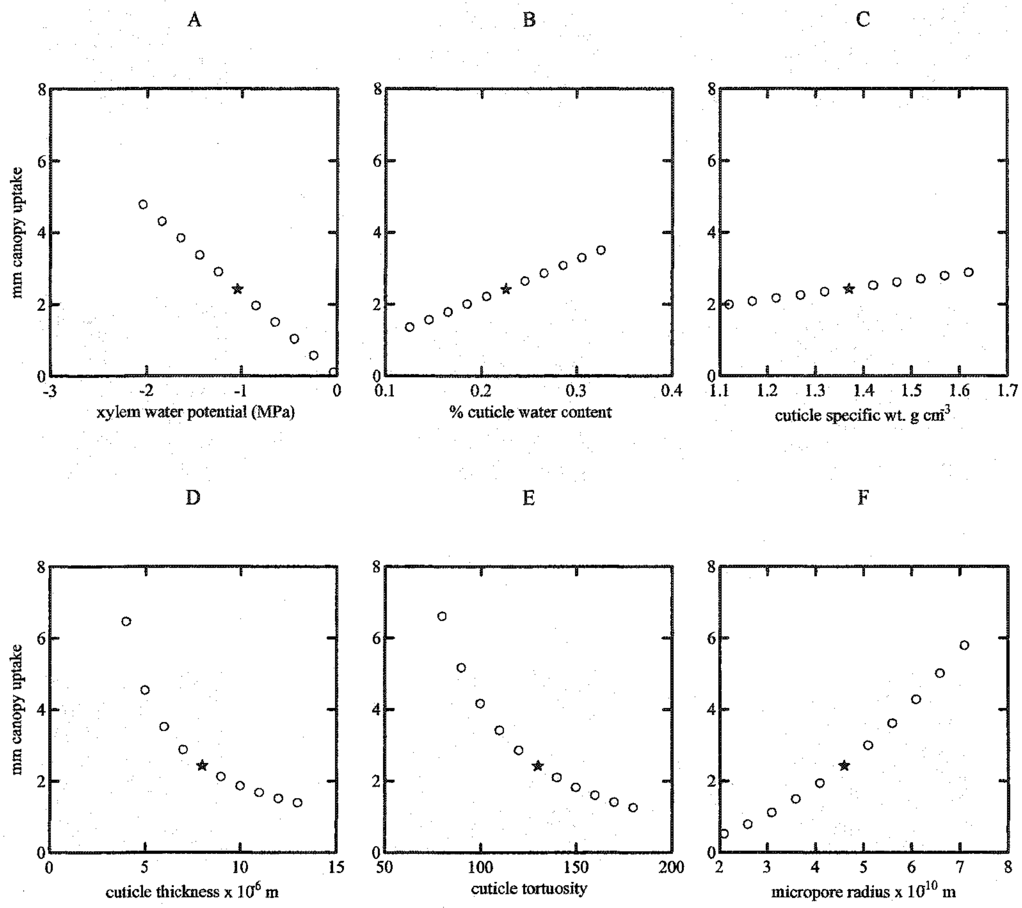


Figure VII.2. The sensitivity of the micropore uptake model to variables that influence the predicted quantity of canopy water absorption for the 1995 growing season at Findley Lake, WA: A) xylem water potential, B) the water content of a hydrated cuticle, C) the specific weight of a coniferous cuticle, D) cuticular thickness, E) the tortuosity of the path for water through across the breadth of a cuticle, and E) the radius of a hydrated micropore. The simulations shown are for a sun-adapted *Abies amabilis* cuticle.

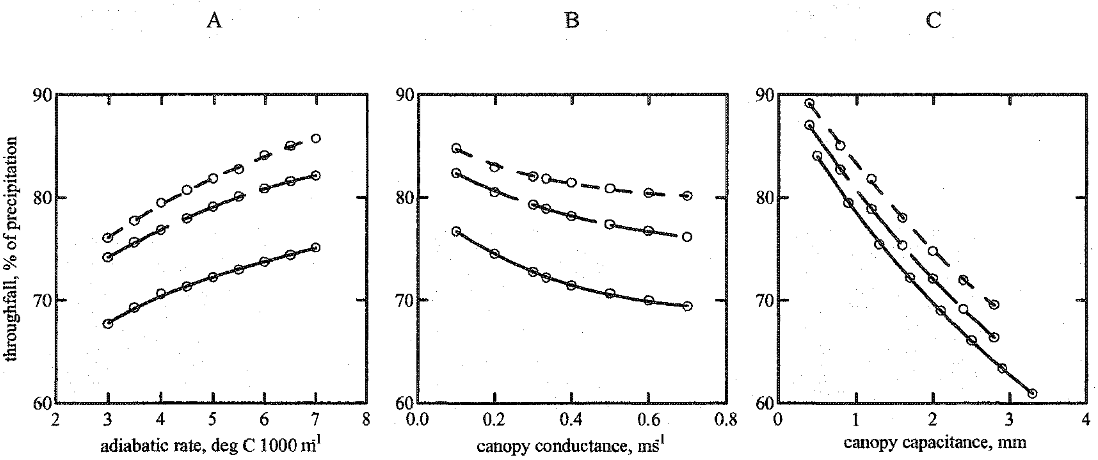


Figure VII.3. The sensitivity of the canopy model to assumptions that influence the predicted quantity of throughfall: A) moist adiabatic cooling rate; B) canopy conductance; C) canopy capacitance. Solid line, modeled throughfall at Findley Lake; long-dashes, modeled throughfall at the midslope of Findley Ridge; short dashes, modeled throughfall at Findley Ridge. Throughfall was modeled as the percent of above-canopy precipitation falling to the forest floor as throughfall plus stemflow (±0.6% of precipitation) during the 1995 growing season at Findley Lake, Cedar River Watershed, WA.

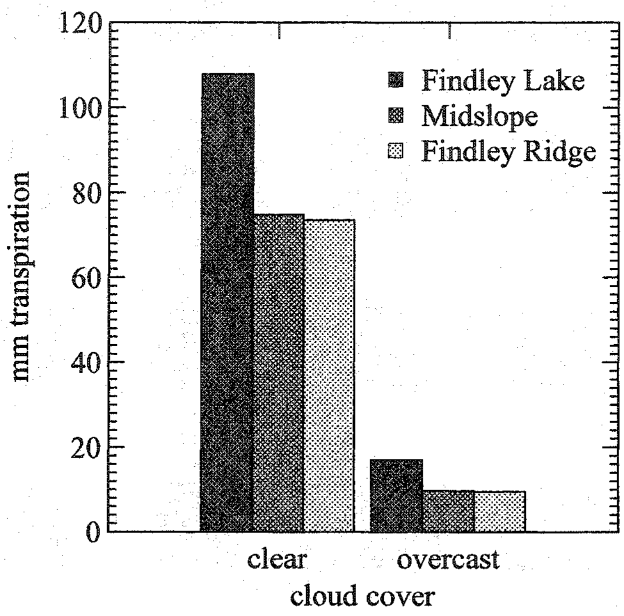


Figure VII.4. Estimated transpiration during 124 days of the 1995 growing season in the Findley Lake Basin, Cedar River Watershed, WA. Although more than half of the days during the summer of 1995 were overcast, nearly 90% of the transpiration in the Basin occurred during clear days.

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Vita

John Rombold was born in Kansas City, Missouri and was raised in Indiana, West Virginia, Virginia and California. He graduated from Jesuit High School in Sacramento, California; received a B.S. in Forestry from the University of California Berkeley and an M.F.S. in Forest Science from Yale University. He served four years as a Peace Corps volunteer in forestry extension in Caagauzú, Paraguay, and later spent four more years participating in a community forestry project in the estuary of the Amazon River near Belém do Pará, Brazil. John lives with his wife Sue and children Cooper and Anna in Bellingham, WA, where he teaches natural resource biology at the Northwest Indian College. He completed his Ph.D. in tree physiology at the University of Washington in 2003.