*Vertical Hydraulic Gradients and the Cause of Foliar Variation in Tall Redwood Trees (Sequoia sempervirens)**

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VERTICAL HYDRAULIC GRADIENTS AND THE CAUSE OF FOLIAR

VARIATION IN TALL REDWOOD TREES (*SEQUOIA SEMPERVIRENS*)

by

Gregory M. Jennings

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We certify that we have read this study and that it conforms to acceptable
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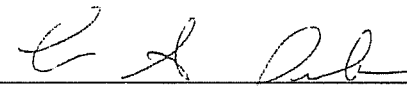

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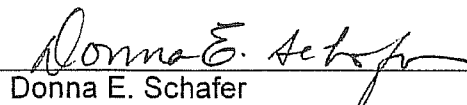   Date

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ABSTRACT

VERTICAL HYDRAULIC GRADIENTS AND THE CAUSE OF FOLIAR

VARIATION IN TALL REDWOOD TREES (*SEQUOIA SEMPERVIRENS*)

Gregory M. Jennings

Recent research on whole-tree water relations has revealed interesting

patterns of hydraulic variation in tall conifers. This work has been accompanied

by an increasing attention to foliar anatomy and the role it plays in a tree's

hydraulic functioning. In spite of broad interest in this topic, redwood, one of the

potentially most interesting species, has been little studied. This thesis addresses

our poor understanding of redwood by examining potential causes of foliar

variation, testing the ability of foliage to directly absorb water, and quantifying

vertical gradients in xylem pressure potential (ψ_x), stable carbon isotope ratio

($\delta^{13}C$), light environment, foliar morphology, and leaf anatomy. On average, from

the lowest to the uppermost foliage (generally 40m to 110m), leaf surface area to

mass ratio (SA:M) decreased from 64.3 cm^2/g to 20.5 cm^2/g, foliar $\delta^{13}C$ increased

from -29.3 ‰ to -25.5 ‰, and vascular bundle cross-sectional area increased

from 0.012 mm^2 to 0.023 mm^2. Height and path length were the best predictors of

foliar SA:M in a step-wise regression model that included wood volume, number

of branch junctions, and light environment. Experimental increases in light environment induced a significant decrease in leaf SA:M and caused significant ^{13}C enrichment relative to reduced light treatments. One hour of experimental misting with water relaxed ψ_x by 20%. There are three major conclusions of this study: (1) photosynthesis in tall redwoods is limited by stomatal conduction, (2) both foliar morphology and $\delta^{13}C$ are affected by light availability, and (3) redwoods are capable of direct foliar absorption of moisture.

ACKNOWLEDGMENTS

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INTRODUCTION

Recent water-relations research on woody plants has revealed interesting patterns of anatomical and physiological variation. Within individual trees, the spatial distribution of properties such as xylem pressure potential, net carbon fixation, stomatal conductance, and xylem anatomy vary systematically with height and crown position (Le Roux et al. 2001). These properties play important roles in a tree's ability to lift water and nutrients from roots to leaves. While much attention has been focused on variations in stem anatomy and its consequences for water transport (reviewed in Gartner 1995), relatively little attention has been given to the hydraulic significance of variations in foliar anatomy (Stenberg et al. 2001). Redwood (*Sequoia sempervirens*), which exhibits marked foliar variation within individual tree crowns, provides an excellent model organism for improving our understanding of the leaf's role in whole-tree water relations.

Lifting water and solutes (xylem sap) against the force of gravity is an essential physiological process for vascular plants. The commonly accepted model for water transport, the cohesion-tension theory (Dixon and Joly 1895), implies certain consequences for plant water relations. First, the force of gravity acting on a standing column of water will generate a constant pressure gradient (the gravitational potential gradient or GPG) of approximately -0.01MPa per 10 meters of height (Bauerle et al. 1999, Holbrook et al. 1996). Second, frictional

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resistance to flow will increase with both rate of transpiration and the total distance water must travel (i.e., path length; Bauerle et al. 1999, Holbrook et al. 1996, Tyree and Ewers 1991). Consequently, at times of rapid transpiration, dramatic increases in xylem tension result, which may induce cavitations and cause permanent xylem dysfunction (Zimmermann 1983). To guard against catastrophic embolisms, many trees reduce transpiration rates through stomatal control. It has been argued that the loss of photosynthetic capacity due to stomatal closure becomes more acute with height, and, thus, hydraulics ultimately limit height growth in conifers (Ryan & Yoder 1997). Recent work, however, suggests that stomatal conductance alone is insufficient to explain lowered photosynthetic rates (Niinemets 2002).

In addition to hydrodynamic factors, both the anatomy and morphology of plant stems and leaves influence a plant's ability to transport water. Variation in the structure of vascular tissue results in differences in stem conductivity (Tyree and Ewers 1991, Ewers & Zimmerman 1984, Tyree et al. 1982). Tracheid diameter, for example, strongly affects conductivity and is correlated with vulnerability to cavitation (Sperry and Tyree 1990). Woody plants make use of this variation. Reduced conductivity at branch junctions allows the main stem, which represents many years of growth, to survive severe water stress while cavitations and embolisms are confined to small, expendable organs

(segmentation hypothesis; Zimmermann 1983). The ability to utilize xylem variation in this way illustrates the interrelated nature of anatomy and hydraulics.

While xylem variation within stems plays a known role in plant water-relations, the hydraulic significance of internal and external leaf variation is not as well understood. Distinct foliar differences exist between "shade" and "sun" leaves in many species (Gutschick 1999, Grassi & Bagnaresi 2001). Sun leaves typically share a suite of distinct characteristics (i.e., thick mesophyll and cuticle, well developed palisade layer on both adaxial and abaxial sides, greater stomatal frequency, and less intercellular air space; Zimmermann 1971, Korstian 1925). These differences have primarily been discussed in terms of photosynthetic efficiency (e.g., Niinemets 1997). For example, it is well known that certain anatomical and physiological adaptations enable shade leaves to make efficient use of low light and sun leaves to avoid photoinhibitory damage at high irradiance (Vallardes & Pearcy 1998). Leaf and shoot orientations also play a role in optimizing photosynthetic efficiency (Stenberg et al. 2001). *Eucalyptus globulus* leaves, for example, are pendulous when grown in full sun, but are held horizontally when developed under 10% sunlight (James and Bell, 2000). While this difference has consequences for light capture, *E. globulus* sun leaves also exhibit increased vascular development. This raises the general question of whether sun leaves are hydraulically modified to function efficiently under

increased transpirational demand as appears to be the case with xylem of other organs (i.e., stems and roots; Sellin 2001).

Tall redwoods offer a unique opportunity to investigate the relationship between leaf variation and whole-plant water relations. Not only do redwoods exhibit a dramatic change in leaf shape and size along the vertical gradient, but their extreme height raises questions about possible hydraulic adaptations unique to redwood. My project addresses three basic questions. First, do correlations exist between foliar morphology and height above ground, path length, or light environment? Second, is the variation in foliar morphology caused by either water stress or light environment? Third, is there evidence that redwood hydraulics function differently than other trees?

METHODS

My study is comprised of two basic components, one descriptive and one experimental. The descriptive portion consists of a sampling regime intended to describe various aspects of redwood hydraulics and leaf morphology along a vertical gradient. The experimental manipulations were designed to discover the cause(s) driving the change in foliar morphology in tall redwoods. I carried out this work at two locations in Humboldt County, California.

Study areas. I selected five tall redwoods in each of two old-growth forests: Prairie Creek Redwoods State Park (PCRSP) and Humboldt Redwoods State Park (HRSP), CA. The ten trees are located on alluvial flats within intact old-growth stands. Trees were not selected randomly but were chosen for height and accessibility. Mean elevation and annual rainfall at the PCRSP sites are 90 meters and 1718 millimeters per year, respectively (reported from Orick, CA), while the HRSP sites have an average elevation of 100 meters and annual precipitation of 1233 mm (reported from Scotia, CA). Both sites experience a coastal-Mediterranean climate, with most precipitation occurring in winter and substantial fog occurring during the summer.

Tree climbing. I accessed tree crowns using standard rope techniques as practiced by the Institute for Canopy Research at Humboldt State University. We initially accessed tall redwoods by shooting arrows trailing fishing filament over

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sturdy branches 80 m high in the crowns. Nylon cord was attached to the arrow

end of the filament, hauled over the branches, and then used to haul a 10 mm

static rope into position. One end of the rope was anchored at ground level,

leaving the other end of the rope free for ascent via single rope technique. A

pulley was installed near the top of the tree, and nylon cord was left in the tree

between research days to maintain the climbing path. Movement within the

crowns was facilitated by the use of an arborist-style, split-tail lanyard system

(Jepson 1997).

Sampling Regime

General approach. I established sampling locations at 10 m height

intervals in each tree in order to quantify the spatial distribution of certain

physiological parameters (i.e., xylem pressure potential and stomatal

conductance) as well as light level, path length, and foliar variation. These

sample locations were distributed between the lowest available foliage (i.e., 40 to

60 m) and the highest accessible foliage (i.e., 95 to 110 m). In each tree, I

included at least one pair of inner/outer crown sampling points at the same height

to evaluate potential effects of different path lengths on water relations and foliar

morphology.

Physiological parameters. I visited each tree twice: October 2000 (dry

season) and January 2001 (wet season). On each visit I collected triplicate

predawn and midday foliage samples from 10 m height intervals and measured

xylem pressure potential (ψ_x) using a Scholander-Hammil pressure chamber

(PMS Instrument Company, Model 600). At each sampling location, I also

measured horizontal path length. This measurement included lengths of branch

segments, their diameters, and numbers of branch junctions from the main trunk

to the sample. Wood volume for the measured path length was calculated from

these measurements using the formula for a frustum of a cone (i.e., volume $= \pi$ x

L x [$a^2 +$ ab $+ b^2$], where L = length, a = radius at distal end, b = radius at

proximal end). After measuring ψ_x, I sent leaf tissue from all samples to Northern

Arizona University for stable carbon isotope ($\delta^{13}C$) and nitrogen content analysis.

$\delta^{13}C$ serves as a measurement of stomatal conductance (Ehleringer & Osmond

1989) and, thus, is a long-term indicator of water stress.

Leaf morphology. In addition to xylem tensions and stable carbon isotope

ratios, I quantified leaf morphology and leaf anatomy of second-year foliage at

each sample location. The following variables were measured: surface area to

dry mass ratio (hereafter SA:M) of internodes, stomatal location (i.e., abaxial vs.

adaxial), and cross-sectional areas of vascular bundles. I measured surface

areas of 10 internodes from each sample using a digital surface-area meter

(Delta T instruments). Samples were then oven-dried at 68°C for one week and

weighed. The mean SA:M of internodes was calculated as cm^2/g dry mass.

For anatomical and morphological structures, I sampled three trees intensively, with ten needles from second-year internodes selected from each of the same sampling locations used for hydraulic measurements. The histochemical technique was adapted from a method used in mycorrhizal research (Peterson 1994). Sections were stained for lignin with 0.1% w/v berberine hemisulfate in distilled water for one hour, rinsed, and mounted in cytoseal. Sections were then photographed using a digital camera and UV fluorescent microscopy. Structures of interest were measured from digital photographs using the National Institute of Health software package, Image.

Light level. I evaluated light level at each sample location using digital hemispherical photography (Nikon® Coolpix 950 digital camera fitted with a fisheye lens and Scanopy® self-leveling "O" mount) and canopy analysis software (WinScanopy®). The software tracks the sun's path over an artificial "hemisphere" created from the photograph and calculates the amount of solar radiation reaching the point at which the photo was taken. Photosynthetically active photon flux density (PPFD, mol/m^2day) for both direct and diffuse light was used to calculate total site factors (i.e., amount of PPFD at sample point / total available PPFD above canopy) from photographs taken at every sample location. PPFD is expressed as a daily mean. To avoid speculation regarding redwood's growing season, PPFD was calculated over the course of an entire year.

Data analyses. I used total site factors, horizontal path-length, branch wood volume, number of branch junctions, and height above ground as independent variables in a stepwise multiple regression analysis with SA:M of second-year internodes as the dependent variable. I compared leaf SA:M and stable carbon isotope ratio between inner and outer crown positions using two-sided paired t-tests. Within-crown gradients of leaf anatomy were evaluated by regressing two dependent variables (i.e., cross sectional area of vascular bundles and proportion of total stomata on the adaxial leaf surface) against height above ground. The program JMP 4.0 (SAS Institute 1999) was used for these analyses.

Manipulative Experiments

Hydraulic supply vs. light. I evaluated water stress and light intensity as possible causes of redwood foliar variation by applying a set of manipulative treatments (i.e., increased sapwood/leaf area ratio, decreased sapwood to leaf area ratio, increased light, and decreased light) to foliage in each study tree. After one year's growth, I examined the foliar response by measuring SA:M of first- and third-year internodes. Potential hydraulic variation from branch to branch was controlled for by comparing first-year foliage, which formed under the treatment's influence, to third-year foliage from the same branch, which developed before the treatment was in place. I also evaluated the effect of these

treatments on the stable carbon isotope ratio in five of the trees. I accomplished the manipulations by the following methods.

1) Hydraulic supply. I attempted to decrease water available to foliage by partial girdling of branches. Stems were cut to a depth of approximately one third their diameter and splinted with a steel angle bar and hose clamps. Because stem conductivity is proportional to the cross sectional area of sapwood and to the square of tracheid cross-sectional area (i.e., porosity; Tyree & Ewers 1991), the sapwood/leaf area ratio and, consequently, the amount of water available to foliage can be decreased by partially cutting the sapwood of a branch.

I attempted to increase the amount of water available to foliage by removing half of the leaves from a branch. Removal of foliage increases the sapwood/leaf area ratio and, consequently; the hydraulic sufficiency of the stem to supply water to the remaining foliage (i.e., leaf specific conductivity). This approach is based on the assumption that increased capacity in the stem will result in increased water flowing to the leaves. It has never been tested.

2) Light. I decreased light levels by hanging 2 x 2 m pieces of 50% shade cloth above sample locations. I used selective pruning to increase light levels by removing small branches above south-facing foliage. The mean increase in light levels due to pruning, measured with before and after hemispherical photography, was 202%.

<u>Foliar absorption</u>. Foliage in five trees was subjected to experimental misting to test its ability to absorb moisture directly. Three treatment/control sets were installed between 100 and 105 meters in each tree. These consisted of unmisted foliage (control), covered foliage (sealed in aluminum foil to suppress transpiration), and treatment foliage, which received one hour of hand misting with water. After treatment, ψ_x was measured with a Scholander-Hammil pressure chamber.

<u>Data analyses</u>. In the sapwood/leaf area ratio vs. light experiment, rank-transformed SA:M was used as the response variable in a randomized complete block ANOVA. In order to control for tree-to-tree variation, tree was used as a blocking factor. The response variable was the difference in SA:M between first- and third-year foliage. Even under normal circumstances, redwood foliage exhibits a significant decrease in SA:M with age, so new growth could not be compared directly with older growth under the same treatment. I analyzed $\delta^{13}C$ data from five trees with a single factor ANOVA on rank scores.

In the misting experiment, xylem pressure potential data were analyzed with a Friedman rank sum test, which is analogous to a two-way ANOVA with tree as a blocking factor. Rank transformations were used in both experiments because residuals of untransformed data were not normally distributed.

RESULTS

Vertical Gradients

Xylem pressure potential. A strong relationship exists between xylem pressure potential (hereafter ψ_x) and height in redwood (Figures 1 & 2). During the 2000 dry season, both predawn and midday ψ_x were highly correlated with height ($R^2 = 0.76$ and $R^2 = 0.91$, respectively, for pooled data from ten trees). Rainy season measurements exhibited weaker correlations ($R^2 = 0.42$ and $R^2 = 0.74$, midday and predawn, respectively). In general, ψ_x was more negative during the dry season than the rainy season, and more negative at midday than before dawn (Table 1). At heights between 95 and 100 meters, midday ψ_x was 43% more negative than predawn ψ_x during the dry season, and midday ψ_x was 27% more negative than predawn ψ_x during the wet season. Comparisons across dry and wet seasons were consistent for both midday and predawn measurements with rainy season ψ_x being more relaxed (less negative) in both cases (i.e., 21% during dry season and 11% during wet season).

Dry season ψ_x was consistently more negative than wet season ψ_x, and predawn ψ_x never equilibrated with the gravitational potential gradient (GPG) during the dry season. During the wet season, however, several trees yielded ψ_x values that were less negative than the GPG. Two examples of this unusual pattern are the trees Demeter, in which predawn measurements were taken after

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a night of rainfall (Figure 3), and Paradox, in which predawn measurements were taken after a night of dense fog (Figure 4).

Stable carbon isotope ratios. Carbon isotope ratios ($\delta^{13}C$) were also strongly correlated with height. $\delta^{13}C$ became less negative (i.e., foliage became ^{13}C enriched) with increasing height in all ten trees (Figure 5). Leaf nitrogen content was uncorrelated with height ($R^2 = 0.004$).

Light. The light environment in all of the study trees exhibited a strong vertical gradient. Photosynthetically active photon flux density (PPFD), canopy openness, and the total site factor all increased with height (Figure 6). In general, redwood forest canopies are dark and redwood crowns are dense. Only 26% of the sample points in this study had total site factors greater than 0.2. Even at the height of 100 meters, one sampling location yielded a total site factor of 0.11.

Morphology and anatomy of leaves. Redwood foliage varied significantly with height in both its external morphology and internal anatomy. With increasing height, needles became shorter, rounder in cross section, and more tightly appressed to the shoot axis (Figure 7). Though not quantified, redwoods also altered their shoot orientations with height; lower-crown foliage was held horizontally while upper-crown foliage was held vertically. The SA:M of second-year internodes decreased strongly with height in all ten trees (Figures 8 & 9). The change in SA:M was at least partially due to a change in cross-sectional

shape. While mid-leaf cross-sectional areas remained roughly constant (Figure 10), the cross-sectional thickness to width ratio increased with height (Figure 11).

These external changes in foliar morphology were accompanied by internal changes in needle anatomy. The size of vascular bundles increased with height (Figure 12). Stomatal location also varied systematically with height. In the lower crown, most stomata were on the abaxial (lower) surface of needles, while in the upper crown, most stomata were on the adaxial (upper) surface of needles (Figure 13). Note that adaxial stomata in the upper crown are sheltered by the shoot because of the change in needle orientation relative to the shoot axis.

Multiple regression. Leaf SA:M was strongly correlated with both height above ground and path length. I used stepwise linear regression to select these two variables and eliminate branch volume, number of branch junctions, and light availability (total site factor) from the model (Table 2). Height alone explained 75% of the variation in SA:M with path length contributing another 4%. The addition of a "dummy" variable to account for tree-to-tree variation did not significantly alter the model.

Inner vs. outer crown. Comparisons of thirteen paired inner / outer crown samples show a clear crown-position effect on both leaf SA:M and $\delta^{13}C$. On average, horizontal path lengths for outer-crown positions were 4.6 times those of inner crown positions. Light level, as represented by direct site factors, was 33% greater in the outer than the inner crown. Second-year leaves from outer-

crown locations exhibited significantly reduced SA:M (P = 0.013, two-tailed paired t-test) and less-negative $\delta^{13}C$ (P = 0.0002, two-tailed, paired t-test) relative to those of the inner crown (Table 3).

Treatment Effects

Manipulations of light availability significantly affected SA:M and $\delta^{13}C$. Exposed foliage showed only 62% of the increase in SA:M between first- and third-year internodes exhibited by shaded foliage (Tables 4,5). The greater expansion of shaded foliage was accompanied by more negative $\delta^{13}C$ (Table 4,6).

Manipulations of sapwood/leaf area ratio also affected SA:M. Foliage from partially girdled stems had a 15% greater increase in SA:M between first- and third-year internodes than did foliage from partially pruned stems (Tables 4,7). Hydraulic manipulations had no effect on $\delta^{13}C$ (Tables 4,8).

Experimental misting of redwood foliage with water affected ψ_x of twigs (p = 0.006, Friedman rank sum test). There was a 20% increase in ψ_x relative to controls after one hour of misting, while ψ_x of twigs enclosed in aluminum foil to suppress transpiration did not differ significantly from controls (Figure 14).

DISCUSSION

There are pronounced variations of certain physiological and anatomical characteristics along the vertical gradient in a number of tall conifers. These patterns of variation may be far more distinct in some species than others. Changes in leaf morphology and anatomy with height, for example, appear to be inconsistent across taxa. While foliage of species such as grand fir (*Abies grandis*) changes in shape, size, and internal structure with height (O'Connell 1976), Douglas-fir (*Pseudotsuga menziesii*) exhibits no such patterns (Apple et al. 2002). Physiological characteristics, by contrast, have been studied far more intensively, and general patterns are consistent regardless of species. Parameters such as xylem pressure potentials (ψ_x) and stable carbon isotope ratios ($\delta^{13}C$) are strongly correlated with height and indicate that transporting water becomes increasingly difficult with height.

As with other conifers, ψ_x in redwood varies systematically with height. A clear decrease in ψ_x with height was observed in all ten trees regardless of weather conditions or time of day. Decreasing water potential with height is consistent with the cohesion/tension model of water transport and with empirical data collected from Douglas-fir (Baurele et al. 1999). This indicates that in the general sense, redwoods transport water by the same basic mechanism as other

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trees (i.e., bulk flow along a pressure gradient). As expected from this model of water transport, the characteristics of ψ_x gradients varied with climatic conditions.

The failure of predawn ψ_x to equilibrate with the GPG during the dry season was likely due to low soil water potential (ψ_s). While ψ_s clearly affects a plant's total water status (e.g., Royce, 2001), microclimatic variations also influence leaf and stem water potential. Nighttime transpiration, which has been observed in many plants (Donovan et al 2001), may also play a role in the low dry season, predawn ψ_x measured in redwood. Regardless of seasonal patterns in ψ_s, soil moisture most likely remains relatively constant from day to day. Daily weather patterns, on the other hand, are far less consistent, particularly during winter. Thus, the greater variation within the wet-season ψ_x data suggests that leaf-atmosphere interactions exert a much larger short-term influence on ψ_x than does soil moisture.

More interesting than the failure of dry season ψ_x to equilibrate to the gravitational potential gradient (GPG), however, is the "over-equilibration" observed during wet-season, predawn sampling. Two trees, Demeter and Paradox, exhibited predawn ψ_x significantly greater than the GPG when sampled during rainy and foggy weather. While close conformity of predawn ψ_x to the GPG has been observed in Douglas-fir (Baurele et al. 1999), ψ_x values greater than the GPG have never been measured in any tree. Because the GPG has

generally been discussed in the literature as a theoretical limit (Scholander et al. 1965, Zimmermann 1983), ψ_x levels should not exceed this limit if roots are the only organ providing water to the stem, even during extended periods of high humidity. The detection of ψ_x greater than the GPG suggests that water is entering the system from above ground level. This interpretation is strengthened by experimental evidence supporting direct foliar absorption of moisture in redwood.

While the ability to absorb atmospheric moisture in large quantities has been well documented in some vascular plant groups (e.g., bromeliads, see Benzing 1994), direct foliar absorption of water by conifers has been unknown until now. Given the degree to which one hour of experimental misting relaxed ψ_x (i.e., 20%) in redwood, it is likely that interception and foliar absorption of rain and fog play an important role its ecophysiology. This interpretation is supported by recent research conducted at the University of California Berkeley, where the development of sap-flux meters capable of detecting reverse sap flow have enabled a different approach to the question of foliar absorption. Exposure of young redwoods to fog during controlled, glasshouse experiments resulted in sap flow reversals, demonstrating the direct foliar absorption of fog (Burgess et al. 2000). Reverse sap flow has also been observed in the field in tall redwoods under wet climatic conditions (T.E. Dawson, pers. com.). These observations suggest that the conventional view of xylem function in trees as a uni-directional

system may, in fact, be overly simplistic. Indeed, it appears that redwoods possess the capacity for a more dynamic process of xylem sap flow involving reversals that are driven by changes in precipitation, humidity, and fog. The extent to which ψ_x might relax during an extended rainfall or fog event (indeed, whether it would ever relax sufficiently to enable embolism repair) is currently unknown. Regardless, foliar absorption may well be sufficient in quantity to reduce the degree of stomatal limitation experienced by upper-crown foliage. When considered in the context of the hydraulic limitation hypothesis (Ryan and Yoder 1997) and the climatic zone currently occupied by redwood, it seems likely that foliar absorption may contribute to redwood's ability to grow so remarkably tall.

As with ψ_x, a strong correlation exists between foliar $\delta^{13}C$ and height in redwood. As a measure of the degree of discrimination between ^{13}C and ^{12}C in leaf tissue, $\delta^{13}C$ can be affected by both early stomatal closure and photosynthetic rate. Often viewed as an indicator of long-term seasonal water status, the redwood $\delta^{13}C$ data strongly suggest that photosynthesis in upper-crown foliage is limited by stomatal conductance. The case for stomatal limitation is supported by the fact that leaf N, which is considered to be correlated with photosynthetic capacity (Evans 1989), remained constant with height in all ten trees. Even so, $\delta^{13}C$ patterns along the vertical gradient may be influenced by factors other than water stress. For example, leaf $\delta^{13}C$ in some conifers can be

more affected by light availability and nutrient content than by water stress

(Warren et al. 2001). Light availability, which increases dramatically with height in

redwood, clearly contributes to ^{13}C enrichment in redwood foliage. As with leaf

tissue along the height gradient, significant ^{13}C enrichment of outer relative to

inner crown foliage could be interpreted as either path length or light availability

effects. Experimental manipulations of light availability and sapwood - leaf area

ratio partially clarify this situation.

The significant reduction in $\delta^{13}C$ in shaded foliage confirms the light effect

on carbon isotope discrimination, but the lack of an experimental effect on $\delta^{13}C$

by sapwood/leaf area ratio manipulation may be due to flawed methodology.

First, partial girdling of the xylem resulted in severing of the phloem and may

have affected carbon translocation. Second, the foliage I chose for removal in the

sapwood/leaf area treatment (i.e., proximal to the trunk on small branches) was

older, and may have been lost through normal senescence anyway, thus

nullifying any effect. These problems might partially be mitigated in future studies

by manipulating the sapwood - leaf area ratio more aggressively. The removal of

most of the foliage from a branch, including most young foliage, would likely

avoid the problem of natural leaf loss due to senescence. It is doubtful, however,

that increased girdling would eliminate the resource allocation problem presented

by phloem disruption unless sapwood could be excavated from the stem carefully

without disturbing the majority of the phloem.

Like $\delta^{13}C$ and ψ_x, needle anatomy and morphology also vary

systematically with height in redwood. The observed reduction of foliar SA:M,

increase in vascular bundle size, and relocation of stomata from abaxial to

adaxial surfaces along the vertical gradient are changes that appear to be far

more dramatic in redwood than in other conifers. Leaf morphology of Douglas-fir,

for example, is influenced by tree age but not crown position (Apple et al. 2002).

The effect of age on Douglas-fir needles is similar to changes observed in

redwood along the height gradient. Mature Douglas-firs have shorter needles and

larger vascular bundles than saplings growing in similar environments. While

Douglas-fir attains increased foliar vasculature though a developmental change

that is independent of crown position, redwood needles appear to possess a high

degree of morphological plasticity. This plasticity is triggered, at least in part, by

light environment. Interestingly, patterns of foliar variation do not appear to be

consistent within higher-order taxa. Grand fir, though in the same family as

Douglas-fir, expresses increased thickness to width ratio and vascular bundle

size with increased height in its needles (O'Connell 1976). These anatomical

differences along the vertical gradient (reported from a 15 meter tall tree) are

similar to those seen in redwood, though less pronounced. The existence of

measurable differences in needle anatomy in such a small tree suggests that

taller grand firs might exhibit greater variation.

The reduction in SA:M with height in redwood is accompanied by a significant reduction in SA:M for outer-crown leaves relative to those of the inner crown at the same height. Like $\delta^{13}C$, the inner/outer crown difference could represent either path-length or light availability effects. The experimentally induced decrease in SA:M with increasing light availability supports the argument that light level is driving morphological variation. Surprisingly, light level was not a significant explanatory variable in the stepwise regression model, even with the addition of a "dummy" variable to control for tree to tree variation. This is likely because experimental manipulations were a more sensitive test of a light effect than the regression analysis, where any effect due to light would have been obscured by the height variable, which represents a combination of hydraulic factors and light level. It is still unclear whether or not hydraulic supply affects leaf SA:M. Sapwood/leaf area ratio manipulations did induced a significant change in foliar morphology, but not in the expected direction. The design flaws relevant to the $\delta^{13}C$ experiment discussed above make the significant increase in foliar SA:M induced by partial girdling impossible to interpret. While the question of hydraulic influence on leaf morphology is still unresolved, it seems clear that light availability is at least one of the causes.

Currently, redwood research is focusing attention on foliar absorption and sap flow. The use of ratio-method sap flow meters has allowed the measurement of extremely low flow rates (Burgess et al. 2001) and the demonstration of

reverse sap flow. Work is now underway to correlate flow rate and direction with microclimatic variables in individual tree crowns. Because of the unusual ability to absorb moisture though leaves, foliar anatomy and morphology may exert even more influence over hydraulic function in redwood than in other species. The consequences of leaf variation on redwood water dynamics, however, remain obscure. Traits exhibited by upper-crown foliage (i.e., reduced surface area and placement of stomata in sheltered positions) appear to be xeromorphic adaptations allowing foliage to persist in a hydraulically challenging environment. The devotion of proportionally more vascular tissue to individual needles also suggests that water transport at the tops of tall redwoods demands a specialized anatomy. These observations suggest the function of redwood foliar variation needs to be examined in terms of water transport. Two specific questions should be addressed in future research. First, what are the consequences of variations in foliar morphology on transpiration rates? For example, boundary layers around the sheltered stomata of upper needles may result in reduced transpiration. Second, what are the consequences, if any, of morphological variation on foliar absorption of moisture? It is possible that the relocation of stomata to the adaxial surface in the upper crown of the tree may facilitate absorption by positioning stomata where water will be held in the small capillary spaces between the adaxial surface and the shoot axis. This would allow more time for absorption and raise the possibility that foliar absorption may be more critical in the upper

crown. Addressing these items would not only lead to improvements in our

general understanding of conifer ecophysiology, but they would also help answer

the question of whether redwood foliar variation is an adaptive trait.

Figure 1. Dry season xylem pressure potentials (ψ_x) along the vertical gradient in tall redwoods. Values are derived from twig samples of 10 trees. The gravitational potential gradient (GPG) is indicated in red.
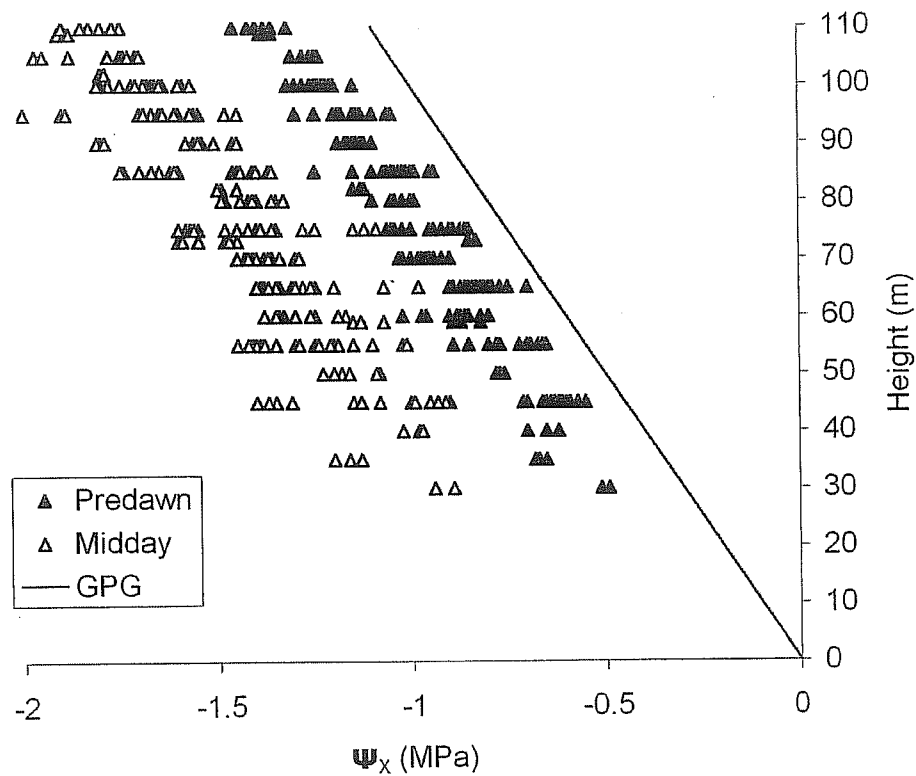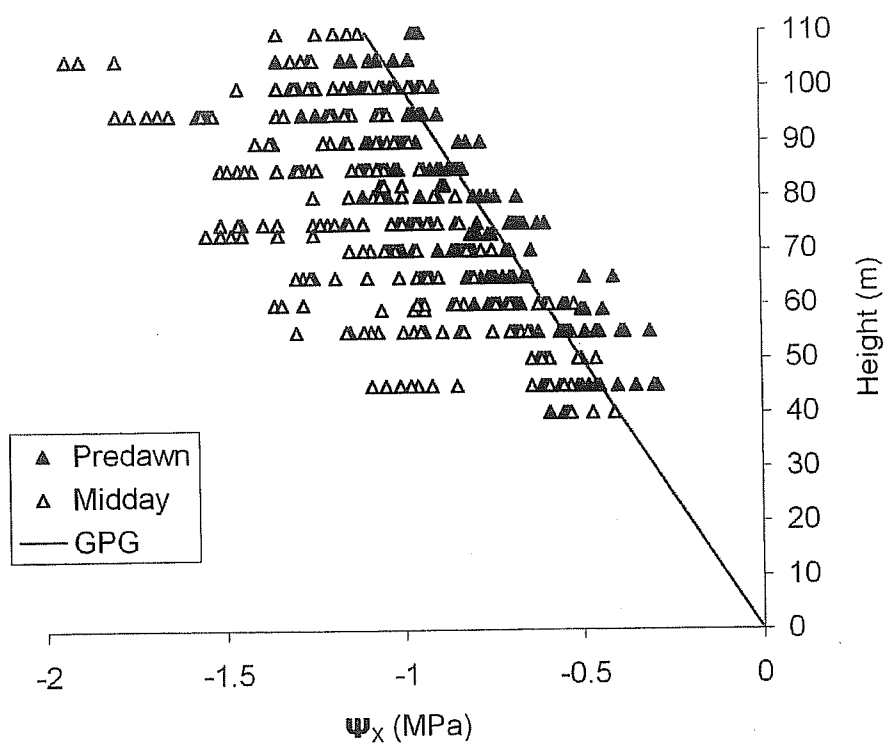
Figure 2. Wet season xylem pressure potentials (ψ_x) along the vertical gradient in tall redwoods. Values are derived from twig samples of 10 trees. The gravitational potential gradient (GPG) is indicated in red.
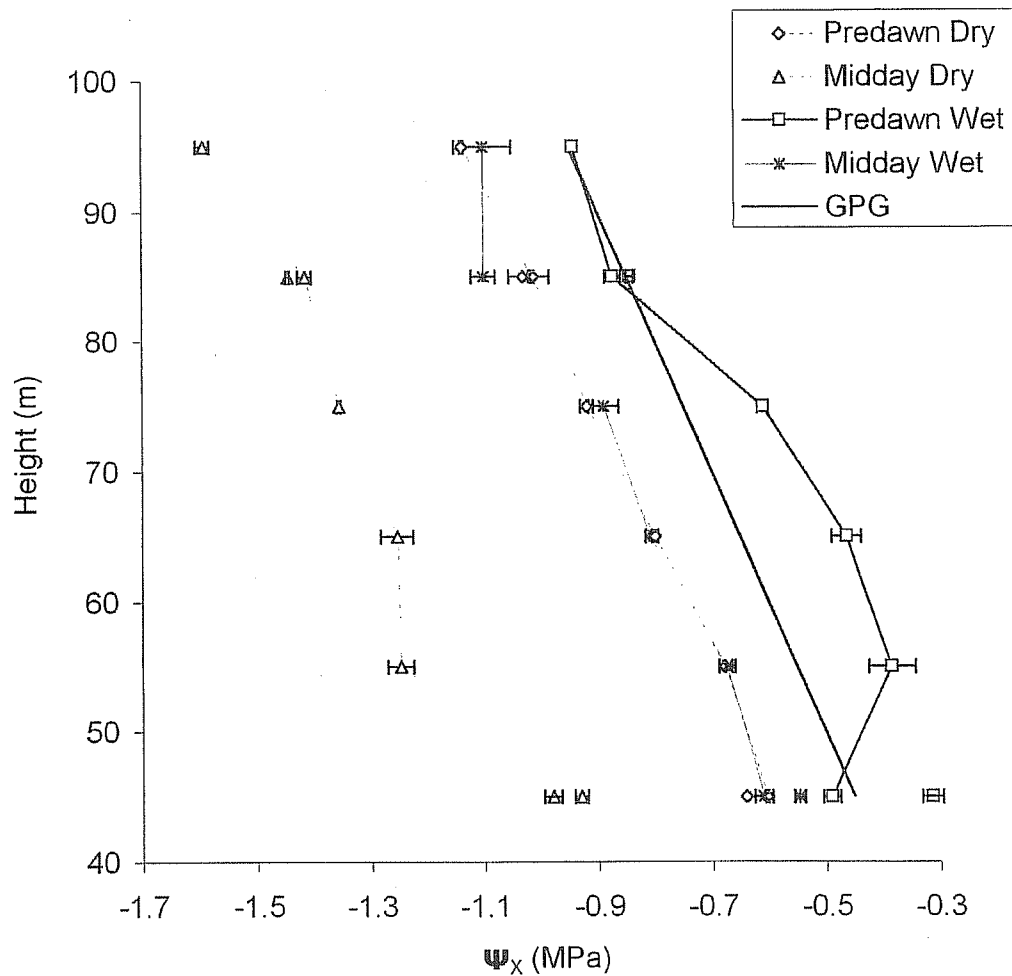
Figure 3. Midday and predawn xylem pressure potentials (ψ_x) in the inner crown of the redwood, Demeter, during the wet and dry seasons of 2000. Values are means ± one standard error (n = 3 twigs). Values not connected by lines are for outer-crown positions. The gravitational potential gradient (GPG) is also indicated.
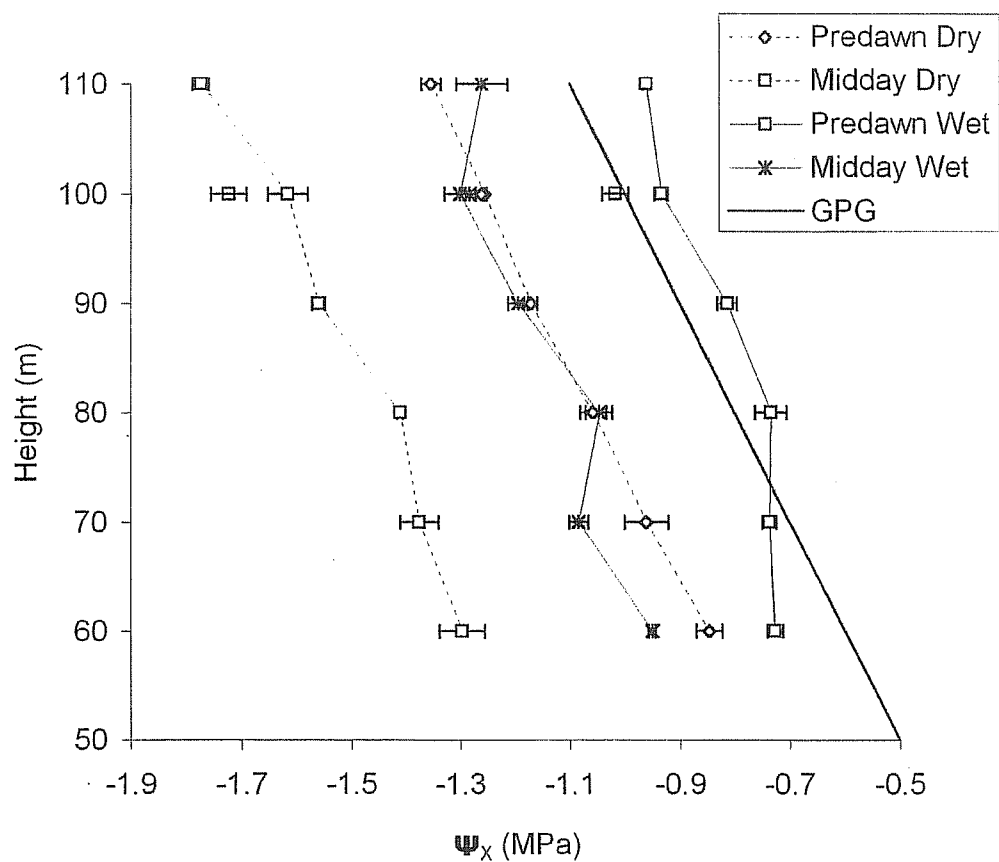
Figure 4. Midday and predawn xylem pressure potentials (ψ_x) in the inner crown of the redwood, Paradox, during the wet and dry seasons of 2000. Values are means ± one standard error (n = 3 twigs). Values not connected by lines are for outer crown positions. The gravitational potential gradient (GPG) is also indicated.
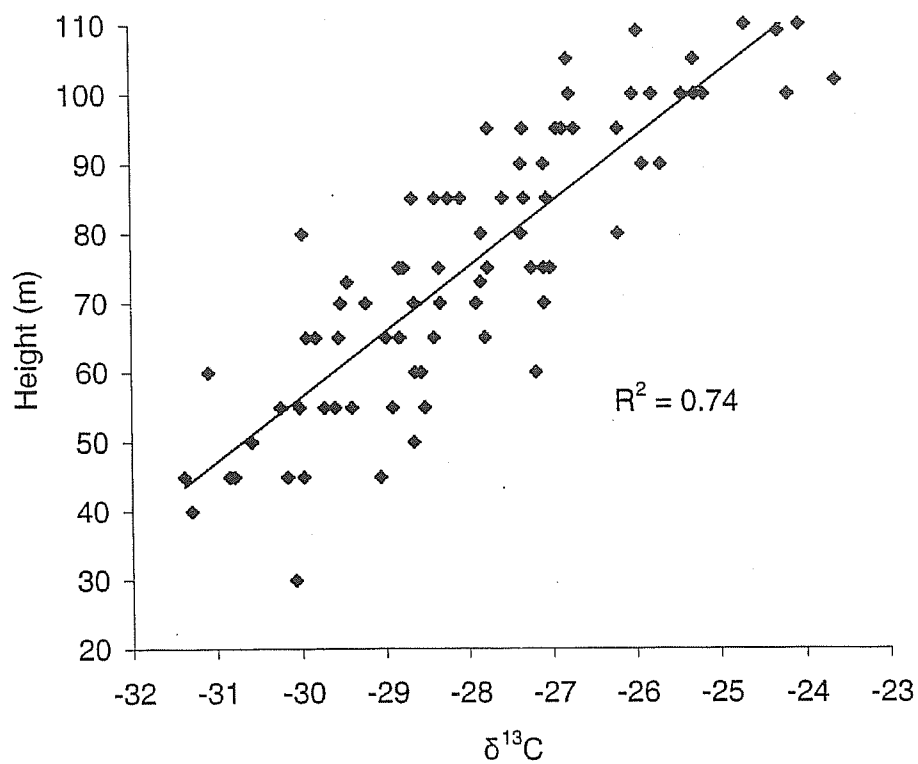
Figure 5. Enrichment of foliar ^{13}C content along the vertical gradient in tall
redwoods. Values are derived from three twig samples in each of 10
trees.
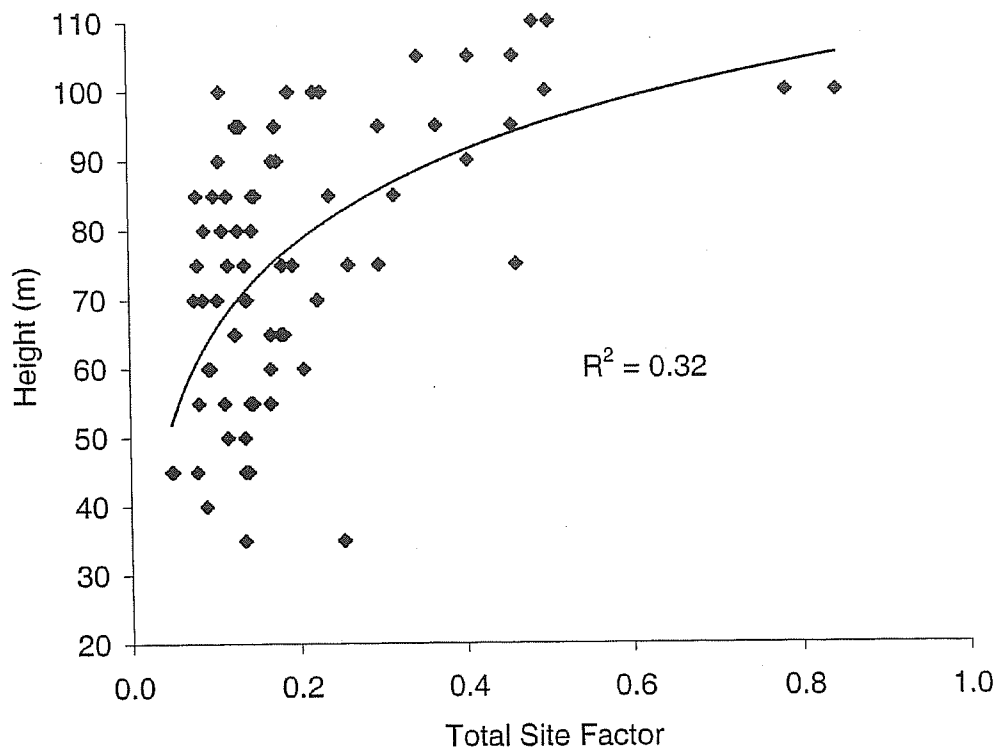
Figure 6. Light availability along the vertical gradient in the inner crowns of tall

redwoods expressed as total site factors (i.e., the proportion of above-

crown PPFD). Values are derived from hemispherical canopy

photographs taken in 10 trees.
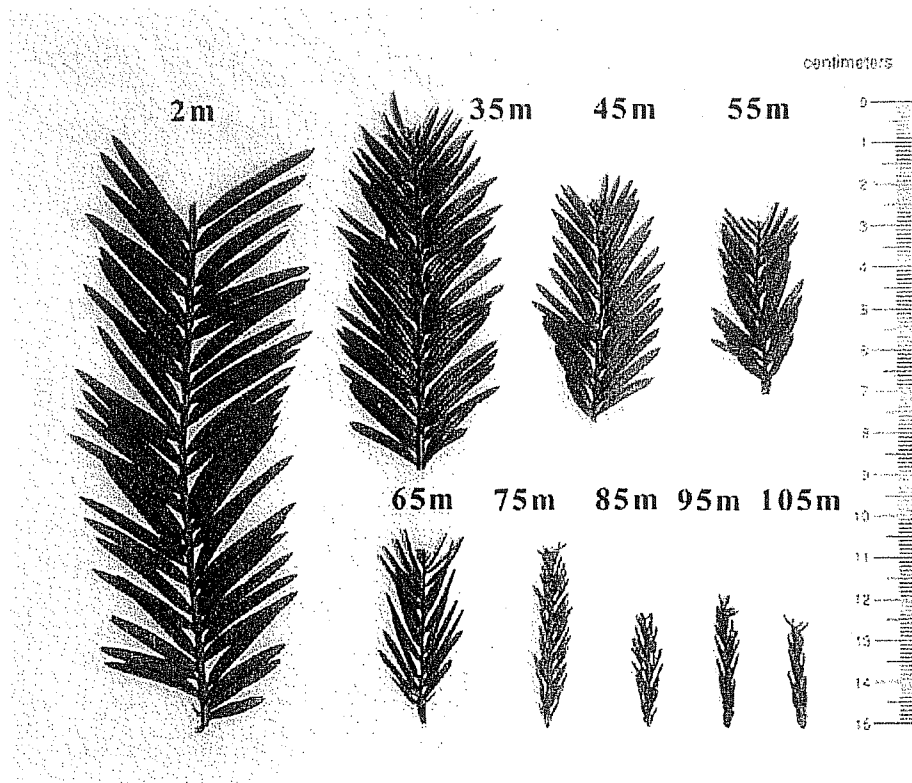
Figure 7. Variation in morphology of second-year internodes along the vertical

gradient in a single redwood tree at Humboldt Redwoods State Park, CA.
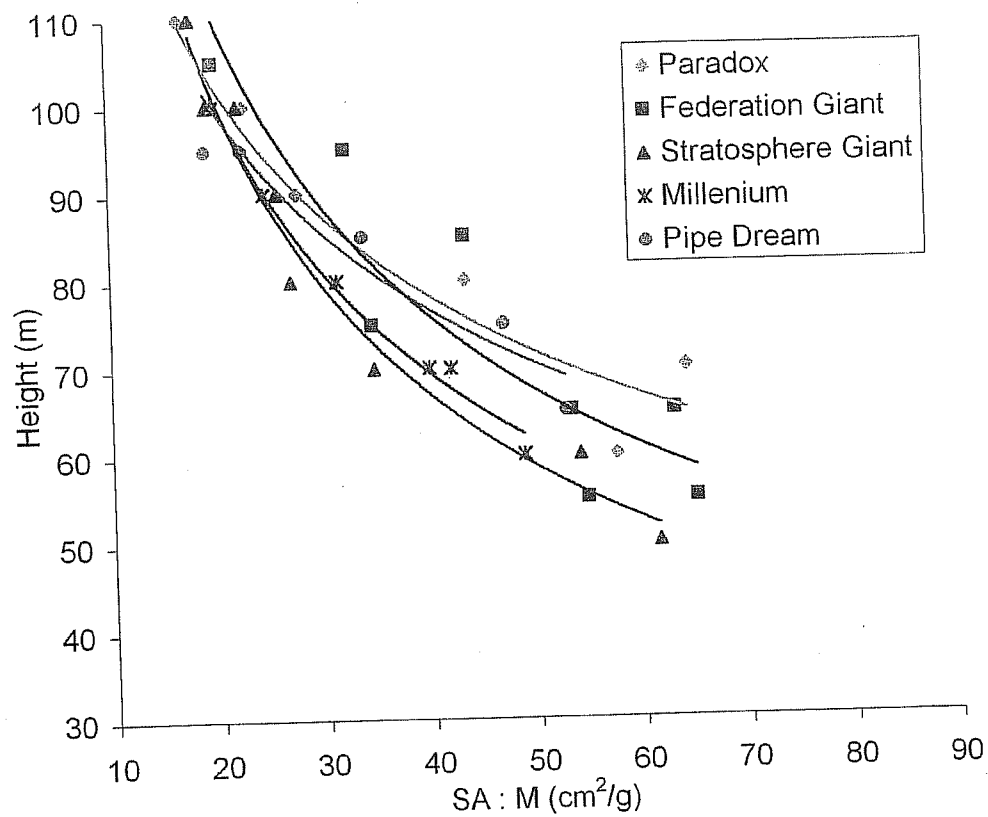
Figure 8. Surface area to mass ratio (SA:M) along the vertical gradient in the inner crowns of tall redwoods. Values are derived from second-year internodes on twigs of 5 trees in Humboldt Redwoods State Park, CA.
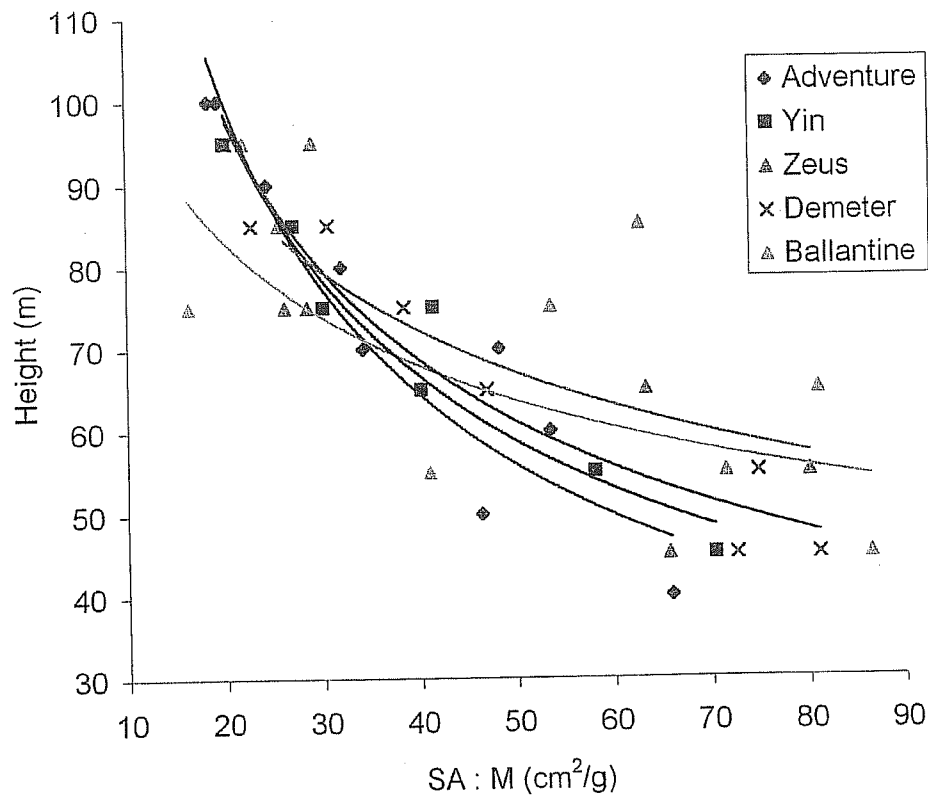
Figure 9. Surface area to mass ratio (SA:M) along the vertical gradient in the inner crowns of tall redwoods. Values are derived from second-year internodes on twigs of 5 trees in Prairie Creek Redwoods State Park, CA.
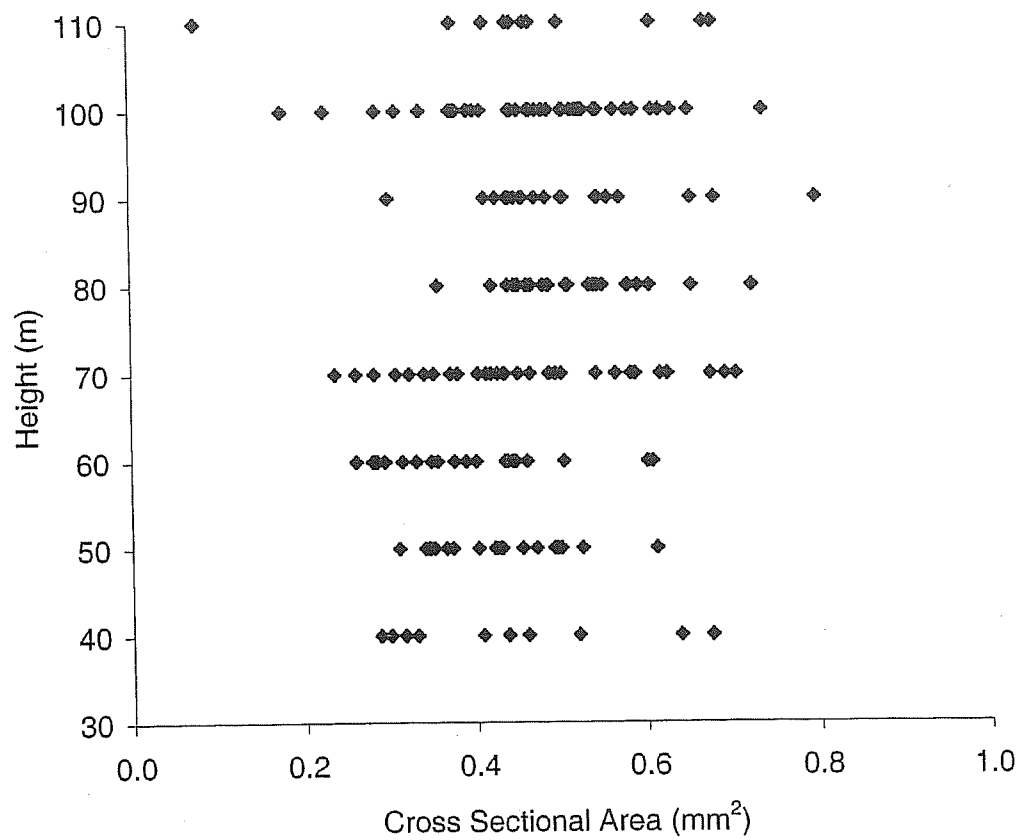
Figure 10. Cross sectional areas of needles along the vertical gradient in the inner crowns of tall redwoods. Values are derived from twigs of second-year internodes on 10 trees.
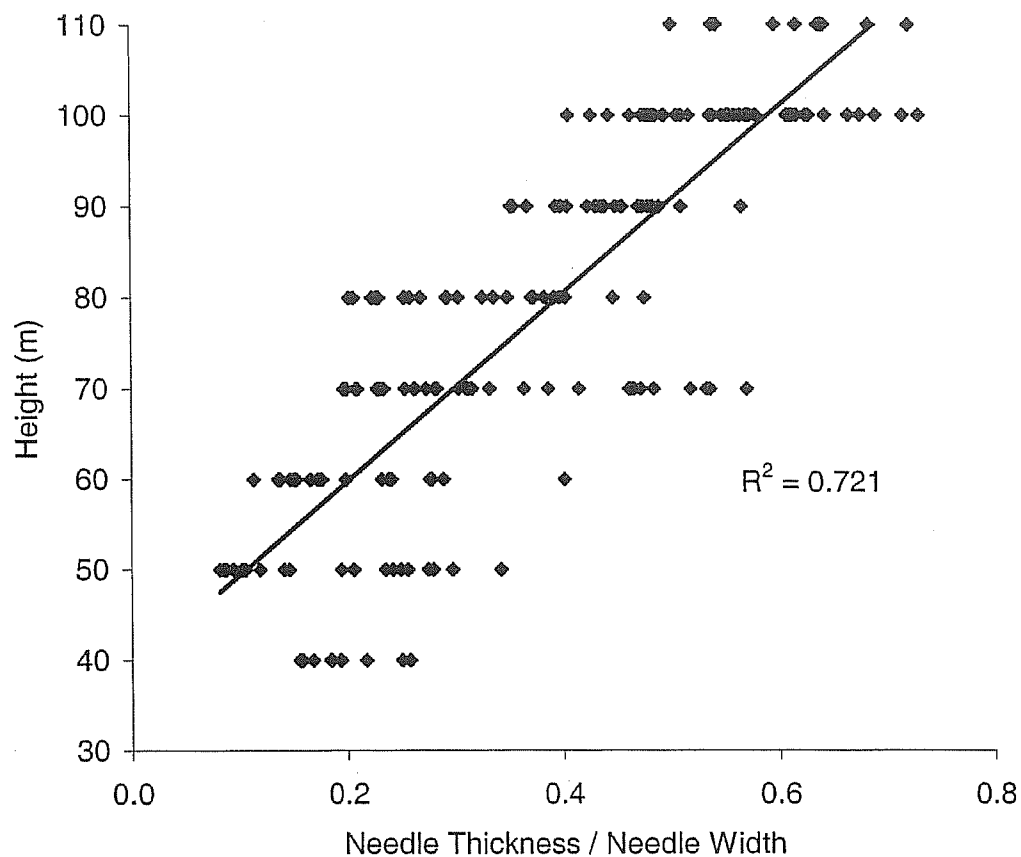
Figure 11. Cross-sectional thickness to width ratios of needles along the vertical

gradient in tall redwoods. Values are derived from second-year

internodes on twigs of 10 trees.
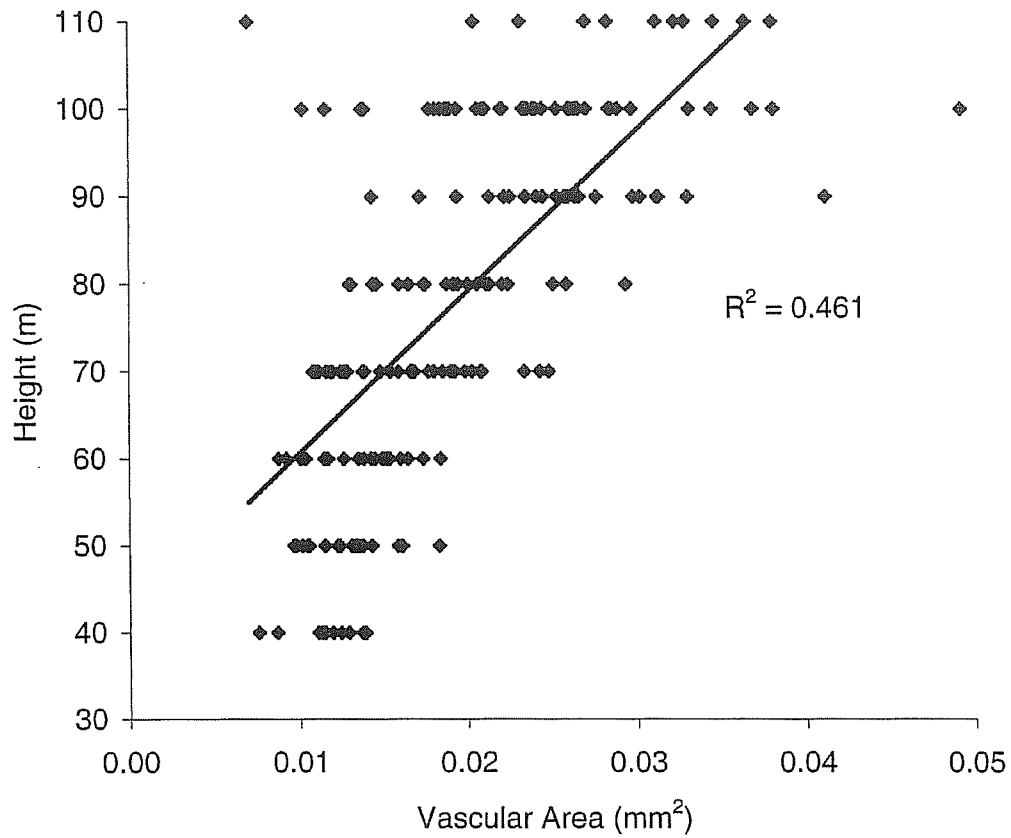
Figure 12. Mid-leaf cross-sectional areas of vascular bundles (including xylem, phloem, and transfusion tissue) along the vertical gradient in tall redwoods. Values are derived from second-year internodes on twigs of 10 trees.
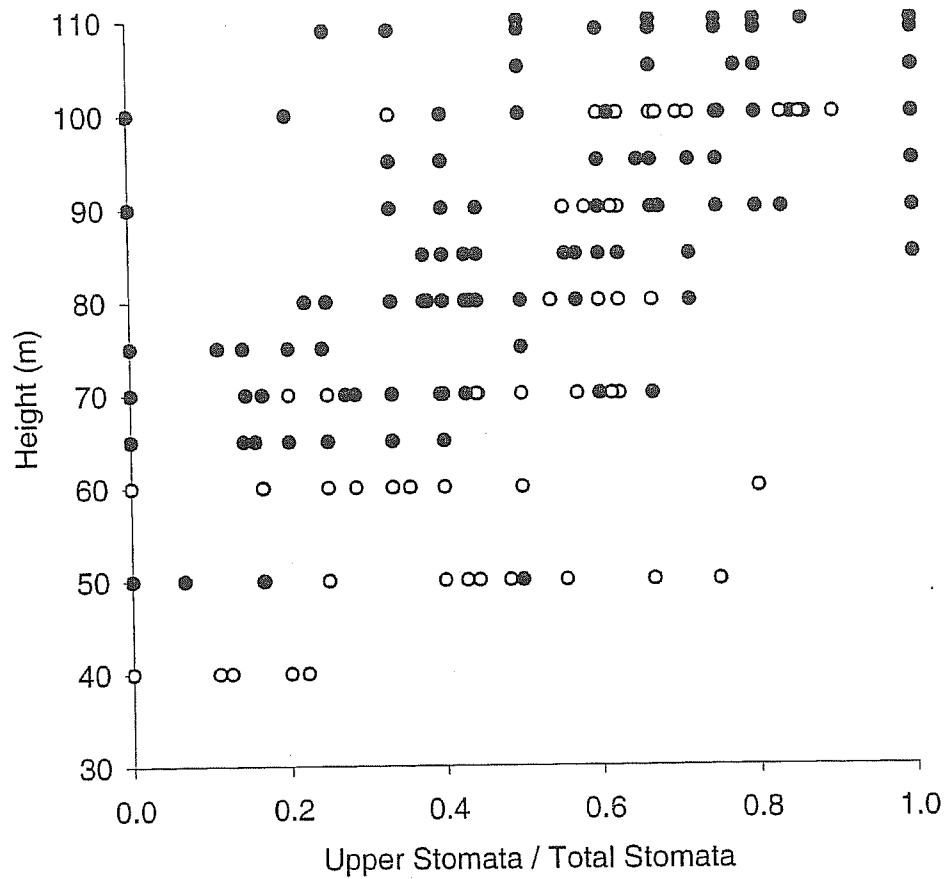
Figure 13. Proportions of stomata on upper surfaces of mid-leaf cross sections along the vertical gradient in tall redwoods. Values are derived from second-year internodes on twigs of two trees in Humboldt Redwoods State Park (filled circles) and one tree in Prairie Creek Redwoods State Park (unfilled circles), CA.
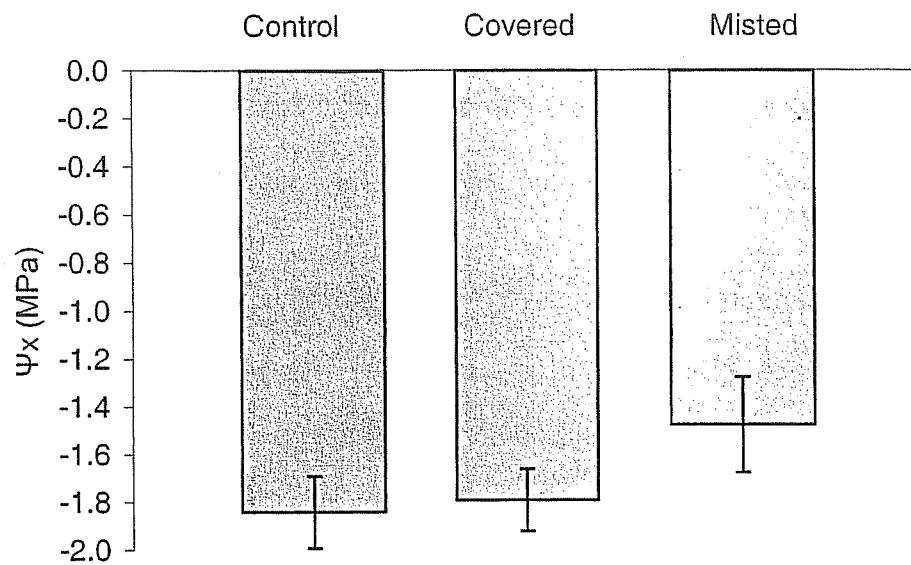
Figure 14. Relaxation of xylem tension (ψ_x) after one hour of misting upper crown

foliage of tall redwoods with liquid water. Foliage in the covered treatment

was enclosed in aluminum foil prior to misting. Values are means ± one

standard error (n = 5 trees in Humboldt Redwoods State Park, CA) for

twigs between 105 and 110 m above the ground.

Table 1. Predawn and midday xylem pressure potentials (ψ_x) observed between 95 and 100 m above the ground at both Prairie Creek and Humboldt Redwoods State Parks. Values are means ± one standard error (n = 10 trees).

	Predawn ψ_x	Midday ψ_x
Dry Season	-1.16 (0.012)	-1.66 (0.023)
Wet Season	-1.03 (0.016)	-1.31 (0.040)

Table 2. Multiple regression model of leaf surface area to mass ratio (SA:M) versus height above ground, path-length (horizontal distance from trunk), branch wood volume proximal to sample point, and number of branch junctions.

	Coefficients	P	Cumulative R^2
Height	-0.999	< 0.0001	0.75
Path length	-1.521	0.0014	0.79
Intercept	122.3		

Table 3. Surface area to mass ratio (SA:M) and stable carbon isotope ratio ($\delta^{13}C$) for inner/outer crown foliage in tall redwoods. Values are means ± one standard error (n = 10 trees). Differences between inner and outer crown positions were significant for both SA:M (P = 0.013) and $\delta^{13}C$ (P = 0.0002). Mean horizontal path lengths and light availability (total site factor) are indicated for each crown position.

Crown position	SA:M cm^2/g	$\delta^{13}C$	Path length (m)	Total site factor
Inner	43.4 ± 6.1	-28.23 ± 0.53	1.4 ± 0.33	0.21 ± 0.07
Outer	33.4 ± 4.8	-27.34 ± 0.56	6.2 ± 0.58	0.28 ± 0.07

Table 4. Separation of group means in manipulative experiments on upper crown foliage of tall redwoods. Values are mean differences between first- and third-year internodes ± one standard error for untransformed data. Means with different superscript letters for a given contrast differed significantly ($P < 0.05$) Control values are listed for reference and were included in analysis.

Source of variation	Experimental contrast	SA:M	$\delta^{13}C$
Light availability	High (exposed)	$5.72^a \pm 1.32$	$1.01^a \pm 0.23$
	Low (shaded)	$9.19^b \pm 1.32$	$-0.06^b \pm 0.23$
	Control	9.30 ± 0.98	0.25 ± 0.23
Sapwood - leaf area ratio	High (pruned)	$10.36^a \pm 0.42$	$0.56^a \pm 0.21$
	Low (girdled)	$11.87^b \pm 0.34$	$0.40^a \pm 0.24$
	Control	5.57 ± 0.93	0.31 ± 0.18

Table 5. ANOVA results for experimental manipulation of light availability in the upper crowns of tall redwoods (n = 10 trees). The dependent variable was difference in surface area to mass ratio (SA:M) between first- and third-year internodes on twigs.

Source of variation	Degrees of freedom	Sum of squares	F-ratio	P-value
Tree	9	383.0	2.186	0.1298
Treatment	1	105.8	5.435	0.0447
Error	9	175.2		

Table 6. ANOVA results for experimental manipulation of light availability in the upper crowns of tall redwoods in Humboldt Redwoods State Park (n = 5 trees). The dependent variable was the difference in stable carbon isotope ratios ($\delta^{13}C$) between first- and third-year internodes on twigs.

Source of variation	Degrees of freedom	Sum of squares	F-ratio	P-value
Treatment	1	57.6	18.89	0.0025
Error	8	24.4		

Table 7. ANOVA results for experimental manipulation of sapwood/leaf area ratio in the upper crowns of tall redwoods (n = 10 trees). The dependent variable was difference in surface area to mass ratio (SA:M) between first- and third-year internodes on twigs.

Source of Variation	Degrees of freedom	Sum of squares	F-ratio	P-value
Tree	9	466.9	36.55	0.0001
Treatment	1	10.56	7.440	0.0294
Error	7	9.938		

Table 8. ANOVA results for experimental manipulation of sapwood/leaf area ratio in the upper crowns of tall redwoods in Humboldt Redwoods State Park (n = 5 trees). The dependent variable was the difference in stable carbon isotope ratios ($\delta^{13}C$) between first- and third-year internodes on twigs.

Source of variation	Degrees of freedom	Sum of squares	F-ratio	P-value
Treatment	1	0.450	0.0529	0.825
Error	7	59.6		

BIBLIOGRAPHY

Apple, M., K. Tiekotter, M. Snow, J. Young, A. Soeldner, D. Phillips, D. Tingey, and B. J. Bond. 2002. Needle anatomy changes with increasing tree age in Douglas-fir. Tree Physiology 22: 129-136.

Bauerle, W. L., T. M. Hinckley, J. Cermak, J. Kucera, and K. Bible. 1999. The canopy water relations of old-growth Douglas-fir trees. Trees 13: 211-217.

Benzing, D.H. 1994. How much is known about Bromeliaceae in 1994? Selbyana 15:1-7.

Burgess, S. O., M. A. Adams, N. C. Turner, C. R. Beverly, C. K. Ong, A. A. H. Khan, and T. M. Bleby. 2001. An improved heat pulse method to measure low and reverse rates of sap flow in woody plants. Tree Physiology 21:589-598.

Burgess, S. O., E. Dubinsky, and T. E. Dawson. 2000. The role of fog in the ecology and water relations of coast redwood. Unpublished manuscript.

Dixon, H.H., and Joly, J. 1895. On the ascent of sap. Royal Society, (London) Philosophical Transactions B 186, 563-567.

Donovan, L. A., M. J. Linton, and J. H. Richards. 2001. Predawn plant water potential does not necessarily equilibrate with soil water potential under well-watered conditions. Oecologia 129: 328-335.

Ehleringer, J. R. and C. B. Osmond. 1989. Stable isotopes. Chapter 13 *in* R. W. Pearcy, J. R. Ehleringer, H. A. Moony, and P. W. Rundel, editors. Plant Physiological Ecology: Field Methods and Instrumentation. Chapman and Hall.

Evans, J. R. 1989. Photosynthesis and nitrogen relationships in leaves of C_3 plants. Oecologia 78: 9-19.

Ewers, F. W. and M. H. Zimmermann. 1984. The hydraulic architecture of balsam fir (*Abies balsamea*). Physiologia Plantarum 60: 453-458.

Gartner, B. L. 1995. Patterns of xylem variation within a tree and their hydraulic and mechanical consequences. Pp. 125-149 *in* B. L. Gartner, editor. Plant Stems: Physiology and functional morphology Academic Press, San Diego, CA.

Grassi, G. and U. Bagnaresi. 2001. Foliar morphological and physiological plasticity in *Picea abies* and *Abies alba* saplings along a natural light gradient. Tree Physiology 21: 959-967.

Gutschick, V. P. 1999. Biotic and abiotic consequences of differences in leaf structure. New Phytologist 143: 3-8.

Holbrook, N. M., A. L. Fredeen, G. W. Koch, and S. C. Sillett. 1996. Water potential gradients in a tall Douglas-fir tree. Tree Physiology

James, S.A., and Bell, D. T. 2000. Influence of light availability on leaf structure and growth of two *Eucalyptus globulus* ssp. *globulus* provenances. Tree physiology 20: 1007-1018.

Jepson, J. 1997. The tree climber's companion. A compact reference and training manual for tree climbers. Beaver Tree Publishing, Longville MN.

Korstian, C.F. 1925. Some ecological effects of shading coniferous nursery stock. Ecology 6: 48-51.

Le Roux, X., T. Bariac, H. Sinoquet, B. Genty, C. Piel, A. Mariotti, C. Girardin, and P. Richard. 2001. Spatial distribution of leaf water-use efficiency and carbon isotope discrimination within an isolated tree crown. Plant, Cell and Environment 24: 1021-1032.

Niinemets, U. 1997. Acclimation to low irradiance in *Picea abies:* influences of past and present light climate on foliage structure and function. Tree Physiology 17: 723-732.

Niinemets, U. 2002. Stomatal conductance alone does not explain the decline in foliar photosynthetic rates with increasing tree age and size in *Picea abies* and *Pinus sylvestris*. Tree Physiology 22: 515-535.

O'Connell, M. 1976. Variation of internal leaf structure as a function of crown position in *Abies grandis*. Master's thesis, Humboldt State University, Arcata CA.

Peterson, R. L. 1994. Histochemistry of Ectomycorrhizae. Pp. 107-120 *in* J. R. Norris, D. J. Read, and A. K. Varma, editors. Techniques for Mycorrhizal Research. Academic Press, London.

Royce, E. B., and M. G. Barbour. 2001. Mediterranean climate effects. I. Conifer water use across a Sierra Nevada ecotone. American Journal of Botany 88: 911-918.

Ryan, M. G., and B. J. Yoder. 1997. Hydraulic limits to tree height and tree growth. Bioscience 47: 235-242.

Scholander, P. F., H. T. Hammel, E. D. Bradstreet, and E. A. Hemmingsen. 1965. Sap pressure in vascular plants. Science 148: 339-346.

Sellin, A. 2001. Hydraulic and stomatal adjustment of Norway spruce trees to environmental stress. Tree Physiology 21: 879-888.

Sperry, J. S. and M. T. Tyree. 1990. Water stress induced xylem cavitation in three species of conifers. Plant Cell and Environment 13: 427-436.

Stenberg, P, S. Palmroth, B. J. Bond, D. G. Sprugel, and H. Smolander. 2001. Shoot structure and photosynthetic efficiency along the light gradient in a Scots pine canopy. Tree Physiology 21: 805-814.

Tyree, M. T., and F. W. Ewers. 1991. The hydraulic architecture of trees and other woody plants. New Phytologist 119: 345-360.

Tyree, M. T., M. E. D. Graham, K. E. Cooper, and L. J. Brazos. 1982. The hydraulic architecture of *Thuja occidentalis*. Canadian Journal of Botany 61: 2105-2111.

Vallardes, F. and R. W. Pearcy. 1998. The functional ecology of shoot architecture in sun and shade plants of *Heteromeles arbutifolia* M. Roem., a California chaparral shrub. Oecologia 114: 1-10.

Warren, C. R., J. F. McGrath, M. A. Adams. 2001. Water availability and carbon isotope discrimination in conifers. Oecologia 127: 476-486.

Zimmermann, M. H. 1971. Tree structure and function. Springer-Verlag, New York.

Zimmermann, M. H. 1983. Xylem structure and the ascent of sap. Springer-Verlag, New York.