

Integrated responses of hydraulic architecture, water and carbon relations of western hemlock to dwarf mistletoe infection

F. C. MEINZER,¹ D. R. WOODRUFF² & D. C. SHAW³

¹USDA Forest Service, Forestry Sciences Laboratory, 3200 SW Jefferson Way, Corvallis, OR 97331, USA, ²Department of Forest Science, Oregon State University, Corvallis, OR 97331, USA and ³Wind River Canopy Crane Research Facility, University of Washington, 1262 Hemlock Road, Carson, WA 98610, USA

ABSTRACT

Dwarf mistletoe (*Arceuthobium* spp.) is a hemiparasite that is said to be the single-most destructive pathogen of commercially valuable coniferous trees in many regions of the world. Although its destructive nature is well documented in many respects, its effects on the physiology of its host are poorly understood. In the present study, water and carbon relations were characterized over a range of scale from leaf to whole tree in large (40- to 50-m-tall) individuals of western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) that were either heavily infected, or uninfected with hemlock dwarf mistletoe (*Arceuthobium tsugense*). Specific hydraulic conductivity (k_s) of infected branches was approximately half that of uninfected branches, yet leaf-specific conductivity (k_L) was similar because leaf area:sapwood area ratios ($A_L:A_S$) of infected branches were lower. Pre-dawn and minimum leaf water potential and stomatal conductance (g_s) were similar among infected and uninfected trees because adjustments in hydraulic architecture of infected trees maintained k_L despite reduced k_s . Maximum whole-tree water use was substantially lower in infected trees (approximately 55 kg d⁻¹) than in uninfected trees (approximately 90 kg d⁻¹) because reduced numbers of live branches in infected trees reduced whole-tree $A_L:A_S$ in a manner consistent with that observed in infected branches. Maximum photosynthetic rates of heavily infected trees were approximately half those of uninfected trees. Correspondingly, leaf nitrogen content was 35% lower in infected trees. Foliar $\delta^{13}\text{C}$ values were 2.8‰ more negative in infected than in uninfected individuals, consistent with the absence of stomatal adjustment to diminished photosynthetic capacity. Adjustments in hydraulic architecture of infected trees thus contributed to homeostasis of water transport efficiency and transpiration on a leaf area basis, whereas both carbon accumulation and photosynthetic water use efficiency were sharply reduced at both the leaf and whole-tree scale.

Key-words: *Arceuthobium* spp.; *Tsuga heterophylla*; carbon isotope ratio; leaf-specific conductivity; photosynthesis; stomata; water use efficiency.

INTRODUCTION

Dwarf mistletoes (*Arceuthobium* spp. Viscaceae) are hemiparasitic vascular plants that infect conifers, primarily members of the Pinaceae. It has been estimated that dwarf mistletoes reduce wood production by 11.3 million m³ year⁻¹ in the forests of the western United States alone (Hawksworth & Wiens 1996). Dwarf mistletoes develop an endophytic system within the branches of host trees, creating infections that may initially appear as spindle-shaped swellings (Geils, Tovar & Moody 2002). As is the case with many species of dwarf mistletoe, western hemlock dwarf mistletoe (*Arceuthobium tsugense*) infections remain localized on branches rather than becoming systemic. Diminutive aerial shoots appear at the swellings within 2–7 years of infection. Eventually, the infection sites undergo further swelling, causing the host tree to form densely packed, fan-shaped branches at the swollen region. These so-called ‘witches’ brooms’ are characteristic of advanced infections that can ultimately result in the death of entire lateral branch systems. Despite their diminutive size, dwarf mistletoes thus have a profound impact on the allometry and crown architecture of trees with advanced infections.

Although much is known about the biology, physiology and ecology of mistletoes (Hawksworth & Wiens 1996; Geils *et al.* 2002), the nature of vascular connections with their hosts (Kuijt 1960; Calvin & Wilson 1996), and the extent to which particular species are water, carbon or nutrient parasites (Glatzel 1983; Ehleringer, Cook & Tieszen 1986; Marshall & Ehleringer 1990; Marshall, Dawson & Ehleringer 1994), relatively little is known about the nature of their impact on the long-term physiological performance of their hosts. In the case of dwarf mistletoes, their small biomass and surface area relative to their hosts make it seem unlikely that they constitute a major sink for water, carbon and nutrient resources. Their impact on tree allometry thus seems disproportionate to their ability to divert resources from their host. Certainly, branch swellings

Correspondence: Frederick C. Meinzer. Fax: +1 541 758 7760; e-mail: fmeinzer@fs.fed.us

at the sites of infection can be expected to have a pronounced impact on branch hydraulic architecture, which in turn should influence stomatal regulation of gas exchange (Meinzer 2002; Mencuccini 2003). However, assessing the impact of infection-induced changes in branch hydraulic architecture on tree water and carbon relations requires measurements over the entire range of scale from leaf to whole-tree.

The available literature suggests a range of host allometric and physiological responses to dwarf mistletoe infection. Although it has been suggested that the development of massive brooms increases the overall ratio of leaf area to sapwood cross-sectional area ($A_L : A_S$) of the host tree (Tinnin & Knutson 1980; Broshot, Larsen & Tinnin 1986; Sala, Carey & Callaway 2001), this may be offset by eventual increases in branch mortality (Tennakoon & Pate 1996). Heavily infected individuals of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) and western larch (*Larix occidentalis* Nutt.) had significantly greater $A_L : A_S$ than uninfected individuals, but no significant difference in $A_L : A_S$ was observed among lightly infected and uninfected trees (Sala *et al.* 2001). In Douglas-fir, increased $A_L : A_S$ in heavily infected trees resulted in greater sap flux density (sap flow per unit sapwood area) than in uninfected trees, but rates of whole-tree water use in the two groups was similar because reduced total sapwood area in infected trees fully compensated for increased sap flux density. In contrast, the significant increase in $A_L : A_S$ in heavily infected western larch trees was not sufficient to have a significant influence on sap flux density or whole-tree water use. At the leaf level, intrinsic water use efficiency (WUE) was significantly reduced in infected individuals of both species as reflected in their more negative foliar $\delta^{13}\text{C}$ values. The relative contributions of changes in stomatal conductance and photosynthetic capacity to reduced $\delta^{13}\text{C}$ values in needles of infected trees were not determined. Similar N content in needles of uninfected and heavily infected trees suggests that photosynthetic capacity may not have been reduced in infected trees. Consistent with this, Logan, Huhn & Tissue (2002) reported that neither N content nor photosynthetic capacity were reduced in needles from white spruce (*Picea glauca* (Moench) Voss) trees infected with the dwarf mistletoe *Arceuthobium pusillum*.

As dwarf mistletoe infections develop over many years with progressive effects on tree architecture, the wide range of reported allometric and physiological consequences of infection may reflect observation of transient adjustments rather than steady-state behaviour. Furthermore, it is not clear whether host tree responses are largely a direct consequence of diversion of resources by the parasite, or whether the mere presence of the infection requires marked shifts in allometry to maintain homeostasis of key physiological processes at the leaf level. In this article we describe integrated responses of hydraulic architecture, water and carbon relations of western hemlock to dwarf mistletoe infection. Our objectives were to characterize interactions between tree architecture and physiological performance at the leaf, branch and whole-tree scales, and

to determine the impact of infection on the availability of N, a key limiting nutrient in the study site. We hypothesized that large adjustments in tree allometry and architecture associated with infection contribute to homeostasis of critical physiological processes and that the impact of the parasite on the N nutrition of its host would be negligible. The old-growth trees studied were of similar size and age. As the development of the infection is known to be slow, the characteristics of the heavily infected individuals studied were likely to reflect long-term adjustments to infection.

METHODS

Field sites and plant material

Hemlock dwarf mistletoe (Hennon Beatty & Hildebrand 2001) occurs from northern California to south-east Alaska where it is known from three subspecies (Wass & Mathiasen 2003). The subspecies at our study site is western hemlock dwarf mistletoe (*Arceuthobium tsugense* (Rosendahl) G.N. Jones ssp. *tsugense*). Clusters of 3- to 13-cm-long aerial shoots with reduced, scale-like leaves, begin to appear about 2 years after infection (Geils *et al.* 2002). The aerial shoots tend to be more common in high light environments within any given forest (Shaw & Weiss 2000) and apparently are only needed for reproduction. Plants are dioecious and produce many small, nectar-bearing flowers during mid-late summer. After pollination, the fruits take 13–14 months to mature. Therefore, a single female plant may have the previous year's maturing fruit and open, receptive, flowers at the same time. Seeds are explosively discharged horizontally up to 15 m from a single-seeded berry, causing dwarf mistletoe populations to be clumped based on stand composition and structure.

The study took place at the Wind River Canopy Crane Research Facility (WRCCRF), Wind River Experimental Forest (WREF), in south-west Washington State (Shaw & Greene 2003) where the annual precipitation is 2223 mm, of which <10% falls during the period June to September, and mean annual temperature is 8.7 °C. The eight primary study trees (Table 1) were located in a 4 ha plot of old-growth (500-year-old) Douglas-fir, western hemlock and western red cedar (*Thuja plicata* Donn) forest under the canopy crane. The stand density was 427 trees ha⁻¹, and basal area 82.9 m² ha⁻¹. The Douglas-fir trees (35 ha⁻¹) were from the original population that occupied the site between 1500 and 1600 AD, whereas the western hemlock trees (224 ha⁻¹) were successfully reproducing in the understorey, with the largest individuals being <250 years old. All western hemlock trees on the WRCCRF plot have been surveyed for dwarf mistletoe infection severity using Hawksworth's six-class dwarf mistletoe rating system (DMR), wherein a DMR of zero represents an uninfected tree, and a DMR of 6 represents a severely infected tree (Hawksworth 1977). Three large, uninfected trees (DMR = 0) and five severely infected (DMR = 6) trees of approximately the same diameter, height and age were chosen for measurements of sap flow, stomatal conductance,

Tree	Diameter (m)	Sapwood area (m ²)	Number of branches	
			live	dead
Uninfected				
260*	0.94	0.254	151	26
383*	0.89	0.279	163	33
419*	0.79	0.207	156	42
Mean ± SE	0.87 ± 0.04 a	0.247 ± 0.02 a	157 ± 4 a	34 ± 5 a
Infected				
2002*	0.87	0.237	92	45
2040	0.87	0.262	89	74
2054	0.79	0.279	120	66
2119*	1.05	0.248	59	63
2131*	0.73	0.198	139	46
Mean ± SE	0.86 ± 0.05 a	0.245 ± 0.01 a	100 ± 14 b	59 ± 6 b

Values followed by different letters within each column differ significantly at $P \leq 0.05$. *Trees utilized for measurements of gas exchange, water potential and stomatal conductance.

Table 1. Stem diameter at 1.3 m, sapwood area and numbers of live and dead branches for the western hemlock trees studied

leaf water potential and photosynthetic gas exchange characteristics (see below). Trees infected with hemlock dwarf mistletoe are spatially aggregated into distinct infection centres, with the 4-ha canopy crane plot containing one large infection centre occupying approximately 1 ha (Shaw, Freeman & Mathiasen 2000) and estimated to be ≤ 200 years old. As extensive destructive sampling is not allowed in the forest plot under the canopy crane, samples required for characterizing the impact of dwarf mistletoe infection on branch hydraulic architecture (see below) were obtained from two infected and two uninfected trees located on Trout Creek Hill in the WREF, about 5 km NW of the WRCCRF. These trees were of similar diameter and height to the eight primary study trees.

Sap flow

Variable length heat dissipation sap flow probes with a heated and reference sensor measuring length of 10 mm at the probe tip (James *et al.* 2002) were used to determine sap flux density at radial depths of 1.5, 5.5, 9.5 and 15 cm near the base (approximately 2.5 m) of the north side of the trunk of each tree listed in Table 1. For probe installation, two 38-gauge (2.58-mm-diameter) holes, separated axially by 10 cm, were drilled into the sapwood. The sensors were coated with thermally conductive silicone heat sink compound prior to insertion. All probes were protected from potential sunflecks by reflective insulation. Signals from the sap flow probes were scanned every minute and 10-min means were recorded by a data logger (CR10X; Campbell Scientific Corp., Logan, UT, USA) equipped with a 32-channel multiplexer (AM416; Campbell Scientific). Concurrent differential voltage measurements across the copper thermocouple leads were converted to a temperature difference between the heated and reference sensor (ΔT), which was converted to sap flux density (v ; $\text{g m}^{-2} \text{s}^{-1}$) using the empirical calibration of Granier (1985):

$$v = 119 k^{1.231}$$

where $k = (\Delta T_m - \Delta T)/\Delta T$, and where ΔT_m is the temperature difference when sap flux density is assumed to be zero. The mass flow of sap corresponding to each probe (F ; g s^{-1}) was calculated as:

$$F = vA,$$

where A (m^2) is the cross-sectional area of the sapwood calculated as the ring area centred on the 10-mm-long sensor and extending to midway between two sensors of successive depth. The innermost sensor was considered to measure the sap flux density to the estimated depth of heartwood. Whole-tree water use (kg h^{-1} or kg d^{-1}), was calculated as the sum of the four values of F measured along the radial profile, and was assumed to be equal to total daily transpiration. Irradiance and vapour pressure deficit (VPD) above the canopy (70–80 m) were obtained from data collected by the WRCCRF.

Hydraulic architecture and water relations

Hydraulic conductivity was measured on branches collected from the upper crowns of two uninfected (DMR = 0) and two severely infected (DMR = 6) trees growing at the Trout Creek Hill site. The branches were accessed by climbing the trees using a double rope method. Five to seven branches were excised from each tree for a total of 22 branches. Upon excision, the branches were sealed in plastic bags and transported to the laboratory where 10- to 15-cm-long segments were excised and cleared of emboli by vacuum infiltration in water. Segments from infected branches were centred on the spindle-shaped swellings resulting from infection. Hydraulic conductivity (k_h ; $\text{kg m MPa}^{-1} \text{s}^{-1}$) was calculated as:

$$k_h = J_v/(dP/dx)$$

where J_v is flow (kg s^{-1}) and dP/dx is the pressure gradient (MPa m^{-1}) imposed across the segment with a high pressure flow meter similar to that described by Tyree *et al.* (1993). Specific conductivity (k_s) was calculated by dividing k_h by

the mean functional xylem area at the two ends of the segment, and leaf-specific conductivity (k_L) was calculated by dividing k_b by the leaf area distal to the segment. Branch leaf areas were estimated by multiplying the total dry mass of the foliage by the area to dry mass ratio of a subsample of needles. Leaf areas of the subsamples were measured with a digital scanner and IMAGEJ version 1.27 image analysis software (<http://rsb.info.nih.gov/ij/>).

The canopy crane and its suspended gondola provided access to the upper crowns of the primary study trees for measurements of leaf water potential (Ψ_l) with a pressure chamber (PMS Instrument Company, Corvallis, OR, USA), and stomatal conductance (g_s) with a steady-state porometer (LI-1600, Li-Cor Inc., Lincoln, NE, USA). Both Ψ_l and g_s were measured concurrently on 6 d during the summer of 2002 (1, 17 and 31 July; 8 and 28 August; 19 September). On each day, four to six rounds of measurements were conducted on two to four branches on each of three severely infected (DMR=6) and the three uninfected (DMR=0) trees. Initial morning measurements of Ψ_l (0545–0630 h) were used for values of pre-dawn water potential (Ψ_{pd}) and the lowest observed daily values were used for minimum leaf water potential (Ψ_{min}). Although measurements were not always commenced prior to dawn due to logistical constraints associated with the use of the canopy crane, the initial values of Ψ_l are referred to hereafter as 'pre-dawn' for purposes of convention.

Leaf gas exchange and carbon isotope ratios

Gas exchange measurements were conducted from the canopy crane with a portable photosynthesis system equipped with a red/blue LED source and CO_2 injector (LI-6400; Li-Cor Inc.) The instrument was zeroed and the chemicals replaced prior to use each day. For determination of the

dependence of assimilation rate on intercellular CO_2 concentration ($A-C_i$ curves), photosynthetic photon flux density was held at $1500 \mu\text{mol m}^{-2} \text{s}^{-1}$ and humidity and temperature were held near ambient values. The cuvette CO_2 concentration was initially set near ambient, progressively lowered to $50 \mu\text{mol mol}^{-1}$, increased directly to ambient, and then progressively increased until no further response of the assimilation rate was observed. Two $A-C_i$ curves were determined for each of three trees in DMR class 6 and DMR class 0 for a total of 12 curves. The measurements were completed on 5 d (29 August; 5 and 18 September; 16 and 17 October) during the late summer and early autumn of 2002. Maximum assimilation rates (A_{max}) of uninfected and severely infected trees were estimated from the asymptotes of curves fitted to pooled data from the six $A-C_i$ curves determined for each DMR class. Assimilation rates (A) at ambient CO_2 concentration ($350 \mu\text{mol mol}^{-1}$) were estimated from data used to generate $A-C_i$ curves. Foliage on which gas exchange was measured was collected, dried and ground, and analysed for $\delta^{13}\text{C}$ and nitrogen content at the Idaho Stable Isotope Laboratory (ISIL, Moscow, ID, USA) on a Finnigan Delta Plus isotope ratio mass spectrometer (Finnigan, Bremen, Germany) coupled to a Carlo Erba elemental analyser (NC 2500; CE Elantech, Inc., Lakewood, NJ, USA).

RESULTS

Whole-tree water use

Daily water use was consistently and significantly greater ($P < 10^{-6}$) in uninfected than infected trees throughout the entire 2002 growing season (Fig. 1). Whole-tree water use increased during the spring, attained maximum values during June and July and declined slowly during August and September. Seasonal courses of daily water use appeared to

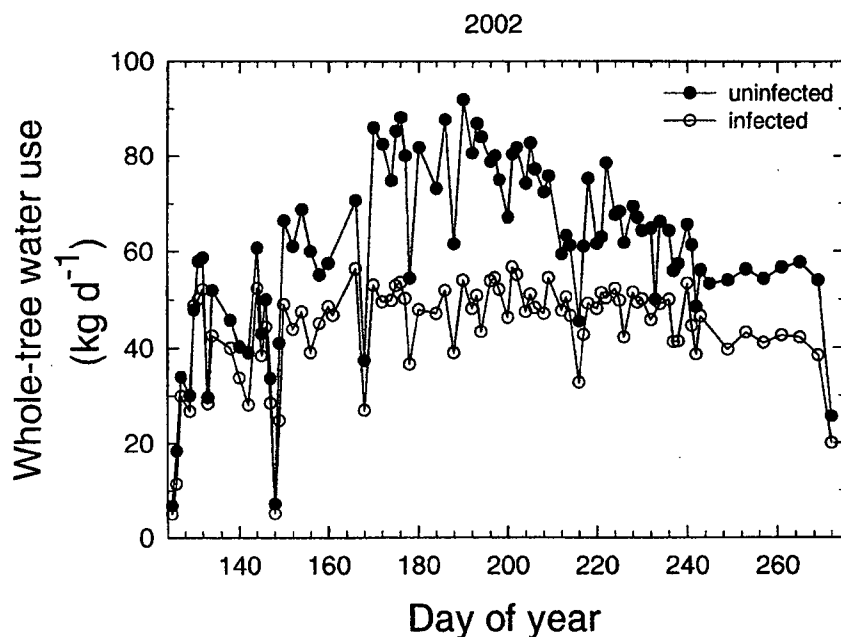


Figure 1. Seasonal courses of mean daily water use for infected and uninfected trees.

be determined largely by seasonal trends in temperature, vapour pressure deficit and total daily irradiance rather than seasonal drought because maximum values were sustained over a 2-month period between mid-June and mid-August during which less than 55 mm of precipitation fell (WRCCRF online database: <http://Departmentswashington.edu/wrccrf/database.html>). When values of mean daily water use for infected and uninfected trees were plotted against each other, it became apparent that on days when water use of uninfected trees was low, water use for the two groups was similar, approximating a 1:1 relationship (Fig. 2). However, increasing departure from a 1:1 relationship was observed as water use of uninfected trees began to exceed approximately 36 kg d^{-1} . The adequate fit of a single curvilinear relationship to the data suggested that relative responses of infected and uninfected trees to seasonal changes in environmental variables were similar. The ratio of daily water use by infected versus uninfected trees was approximately 0.62 when water use of infected trees was near maximal, which was similar to the ratio of live branches on infected versus uninfected trees (0.64, Table 1). Daily time courses of water use in infected and uninfected trees were consistent with seasonal time courses in that whole-tree sap flow for the two groups tended to be similar when irradiance and vapour pressure deficit were low (Fig. 3, right), but became increasingly different as irra-

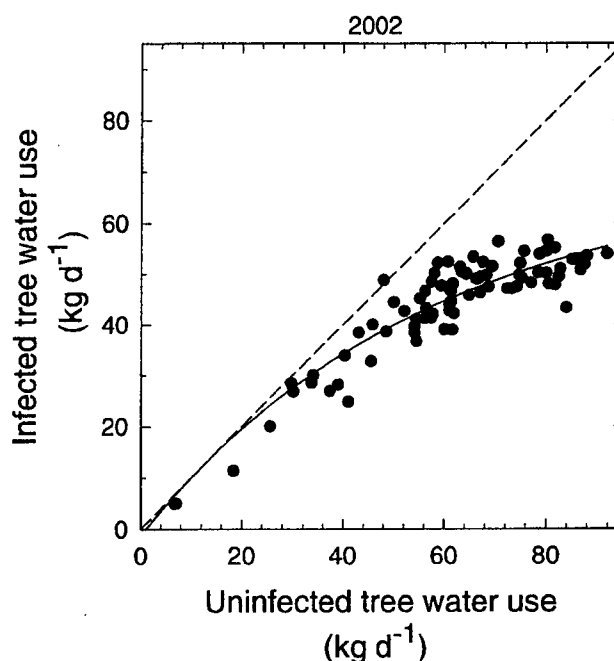


Figure 2. Mean water use of infected trees on a given day in relation to that of uninfected trees on the same day. The dashed line represents a 1:1 relationship.

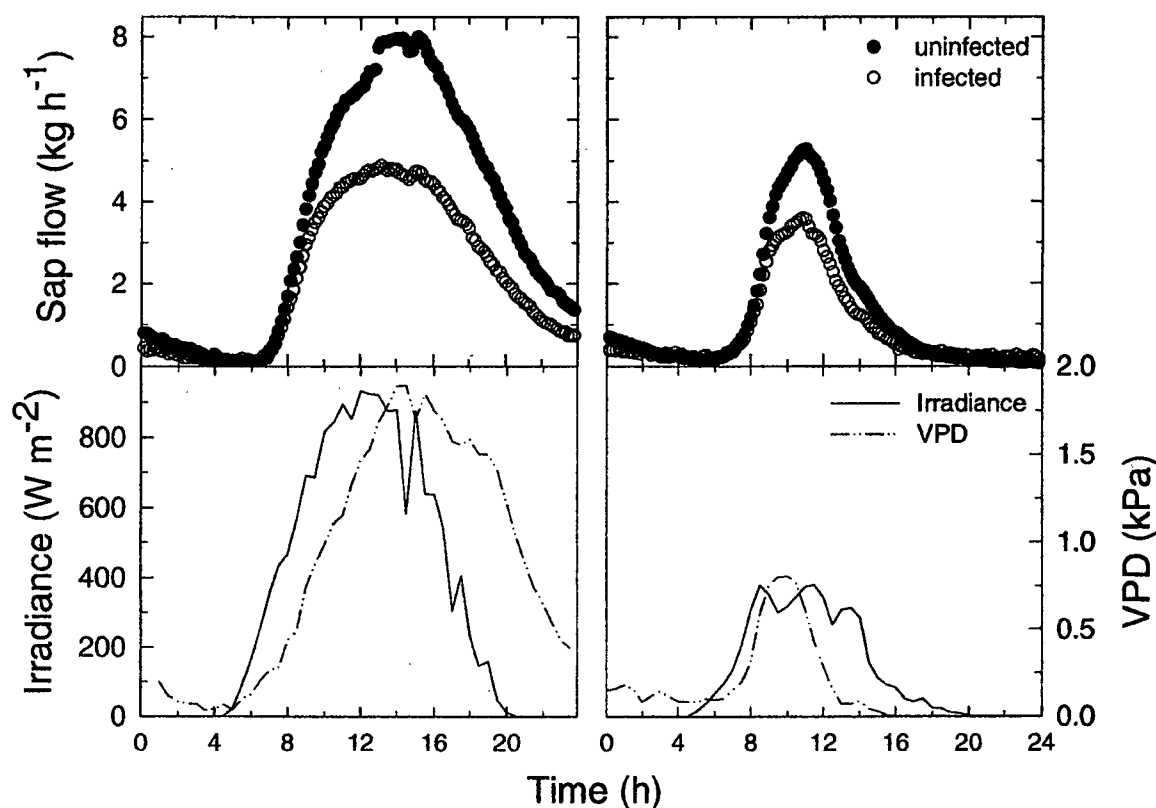


Figure 3. Time courses of irradiance, VPD and mean whole-tree sap flow for infected and uninfected trees on day 175 (left) and day 168 (right) 2002.

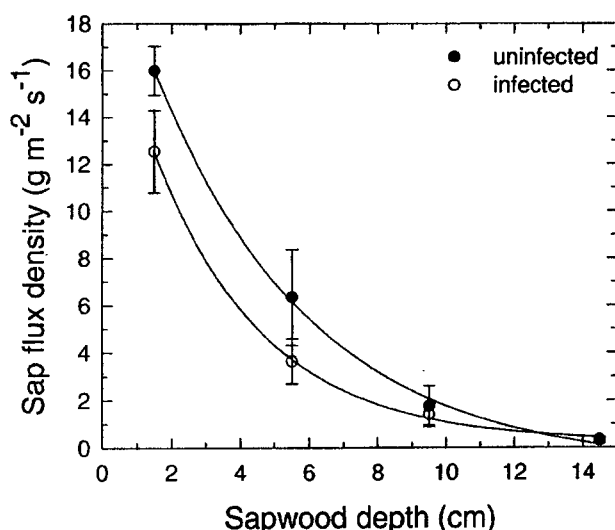


Figure 4. Mean ($\pm$ SE) radial profiles of sap flow for infected and uninfected trees measured between 1000 and 1400 h on day 175, 2002.

diance and VPD increased (Fig. 3, left). Sap flux density of both infected and uninfected trees declined exponentially with increasing depth in the sapwood, reaching zero between approximately 12 and 15 cm (Fig. 4). The relative rates of decline in sap flux density with increasing sapwood depth were nearly identical for the two groups, but mean sap flux density was consistently greater in the outermost sapwood of uninfected trees.

Hydraulic architecture and water relations

The mean diameters of branches used for hydraulic architecture measurements were nearly identical (Table 2). However, branches from infected trees had approximately half as much leaf area distal to the segments used for hydraulic conductivity measurements as branches from uninfected trees, causing $A_L : A_S$ to be about 43% lower in infected branches. Although infection of branch segments with dwarf mistletoe reduced k_b and k_s by 50%, k_L was not significantly different in infected and uninfected branches because reduced k_b in infected branches was offset by reductions in $A_L : A_S$. Both infected and uninfected branches appeared to share a common relationship between branch segment k_L and total leaf area distal to the segment (Fig. 5). Consistent with the similarity of k_L at the

branch level (Table 2), no significant differences in mean g_s , Ψ_{pd} , or Ψ_{min} in upper crown foliage of infected and uninfected trees were observed (Table 3).

Leaf gas exchange

Inspection of $A-C_i$ curves for foliage on infected and uninfected trees indicated that in uninfected trees, A increased more rapidly with C_i and reached maximum values at saturating levels of C_i that were about twice as high as those in infected trees (Fig. 6, Table 3). Differences in A_{max} at saturating C_i were associated with variation in leaf N content (Fig. 7a) and mean leaf N content was about 50% greater in uninfected trees (Table 3). Foliar $\delta^{13}C$ values, an integrated measure of intrinsic WUE, became less negative with increasing N content (Fig. 7b) and mean $\delta^{13}C$ values were significantly greater in foliage from uninfected trees (Table 3). The total range of foliar $\delta^{13}C$ was about 4‰ among the 12 branches sampled (Fig. 7b). The foliar $\delta^{13}C$ values of infected trees were consistent with the observed lack of stomatal adjustment to their diminished photosynthetic capacity, which allowed relative stomatal limitation of photosynthesis to decrease, leading to increased C_i/C_a and therefore increased discrimination against ^{13}C during CO_2 fixation. In addition to reducing foliar N content and photosynthetic capacity, dwarf mistletoe infection thus resulted in sharply reduced intrinsic WUE.

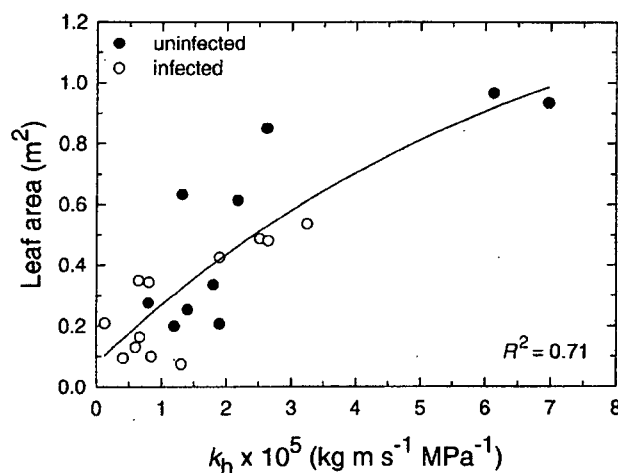


Figure 5. Relationship between hydraulic conductivity of branch segments and leaf area distal to the segments.

Table 2. Hydraulic architecture of western hemlock branches uninfected ($n = 10$) and infected ($n = 12$) with dwarf mistletoe

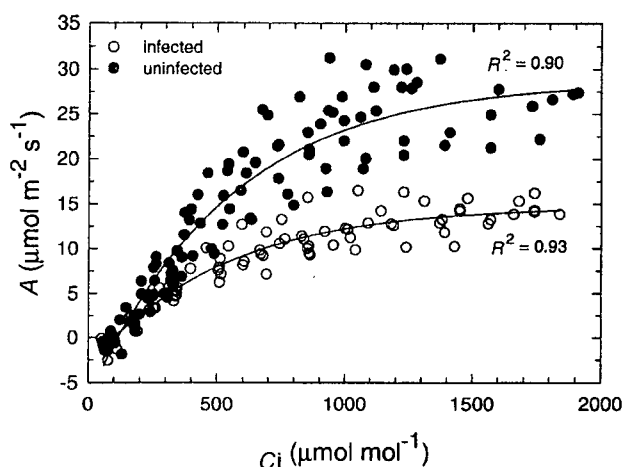
Condition	Diameter (cm)	A_L (m ²)	$A_L : A_S$ (m ² cm ⁻²)	$k_b \times 10^5$ (kg m s ⁻¹ MPa ⁻¹)	k_s (kg m ⁻¹ s ⁻¹ MPa ⁻¹)	$k_L \times 10^5$ (kg m ⁻¹ s ⁻¹ MPa ⁻¹)
Uninfected	0.86 $\pm$ 0.08 a	0.53 $\pm$ 0.10 a	0.82 $\pm$ 0.06 a	2.63 $\pm$ 0.68 a	0.40 $\pm$ 0.05 a	5.16 $\pm$ 0.71 a
Infected	0.88 $\pm$ 0.07 a	0.28 $\pm$ 0.05 b	0.47 $\pm$ 0.06 b	1.31 $\pm$ 0.29 a	0.19 $\pm$ 0.03 b	5.42 $\pm$ 1.24 a

Values are means ($\pm$ SE). Values followed by different letters within each column differ significantly at $P \leq 0.05$ for A_L , $P \leq 0.001$ for $A_L : A_S$, and $P \leq 0.002$ for k_s .

Table 3. Stomatal conductance, water status, nitrogen content, and gas exchange characteristics of western hemlock leaves on branches uninfected and infected with dwarf mistletoe

Condition	g_s ($\text{mmol m}^{-2} \text{s}^{-1}$)	Ψ_{pd} (MPa)	Ψ_{min} (MPa)	N (%)	$\delta^{13}\text{C}$ (‰)	A_{max} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	A ($\mu\text{mol m}^{-2} \text{s}^{-1}$)
Uninfected	68 ± 5 a	-0.84 ± 0.12 a	-1.78 ± 0.44 a	1.01 ± 0.03 a	-25.6 ± 0.2 a	23.6 ± 1.7 a	5.6 ± 0.5 a
Infected	70 ± 9 a	-0.80 ± 0.16 a	-1.89 ± 0.48 a	0.66 ± 0.03 b	-28.4 ± 0.3 b	12.0 ± 0.9 b	4.4 ± 0.1 b

Values of g_s , Ψ_{pd} and Ψ_{min} are means ($\pm$ SE) of measurements made on two to four branches on each of three trees on the six dates given in the text. Values of N content, $\delta^{13}\text{C}$, A_{max} and A are means ($\pm$ SE) of six branches. Values followed by different letters within each column differ significantly at $P \leq 0.05$.

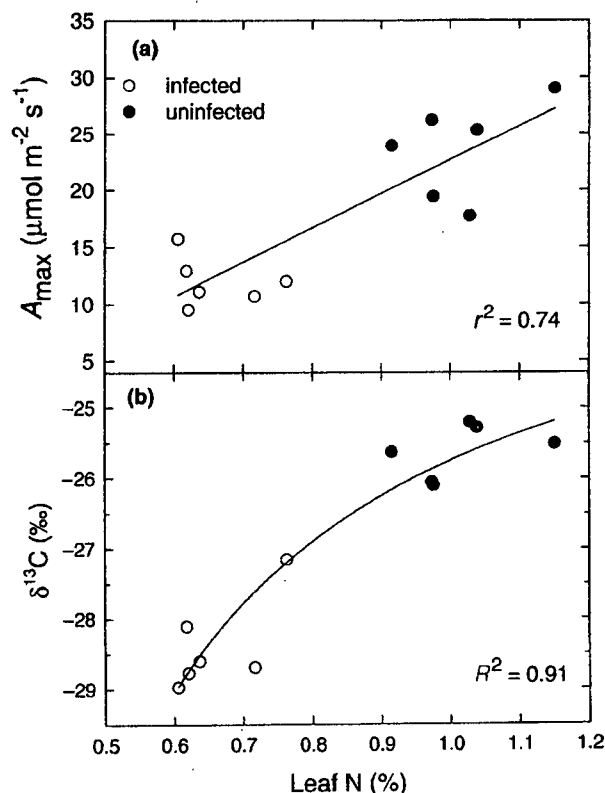
**Figure 6.** $A-C_i$ curves for foliage on intact branches of infected and uninfected trees. Each curve was fitted to data collected from six branches.

DISCUSSION

Dwarf mistletoe infection induced pronounced adjustments in hydraulic architecture of western hemlock at the branch and whole-tree scales that conserved water transport efficiency on a leaf area basis. Maintenance of nearly constant k_L through reductions in leaf area achieved homeostasis of g_s and leaf water status despite dramatic reductions in the water transport efficiency of infected branch xylem. At the whole-tree scale, however, total water use by infected trees was sharply reduced, especially under conditions of high irradiance and evaporative demand that favoured high rates of water use by uninfected trees. Previous studies have shown a tight co-ordination of g_s and transpiration per unit leaf area with k_L that allows minimum values of Ψ_L to remain nearly constant (Meinzer & Grantz 1990; Hubbard *et al.* 2001; Meinzer 2002). Clearly, gradual processes such as changes in allometry that conserve k_L by altering features such as $A_L : A_s$ contribute to stability of g_s , leaf transpiration and therefore Ψ_L (Andrade *et al.* 1998; Bucci *et al.* 2004). Stomata can also react rapidly to stabilize leaf water status following changes in k_L , resulting from treatments such as partial defoliation (Pataki, Oren & Phillips 1998), shading of a fraction of total leaf area (Whitehead *et al.* 1996) and root pruning (Briggs & Wiebe 1982). More

recent evidence suggests that stomata respond to rapid changes in leaf k_L associated with daily cycles of embolism and its reversal within the leaf (Brodrick & Holbrook 2003; Nardini & Salleo 2003; Trifiló *et al.* 2003). However, the exact determinants of species-specific operating ranges of Ψ_L , k_L , and g_s are not known.

In contrast with the homeostasis of leaf level water relations, the photosynthetic capacity of foliage on infected branches was sharply reduced, presumably as a result of sequestration of N by the mistletoe. Even though leaf area of infected branches was reduced by 50% relative to uninfected branches (Table 2), their foliar N content was 35% lower (Table 3). These results imply that despite its small biomass relative to that of its host, the N requirement of

**Figure 7.** (a) Maximum assimilation rates determined from $A-C_i$ curves and (b) leaf $\delta^{13}\text{C}$ values in relation to leaf N content.

the mistletoe for activities such as reproduction (Pate, Truac & Kuo 1991) was sufficient to adversely affect the N budget of the host and photosynthetic capacity in this relatively N-limited ecosystem (Klopatek 2002). Given that maximum water use of infected trees was about 40% lower than uninfected trees (Fig. 2), despite nearly identical transpiration rates per unit leaf area, it can be estimated that total carbon accumulation of infected trees was probably reduced by more than 40% because photosynthesis on a leaf area basis declined. Mean photosynthetic rates estimated from A–C_i curves at ambient CO₂ concentration and saturating irradiance were 5.6 and 4.4 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for uninfected and infected trees, respectively. When this 21% reduction in photosynthesis is added to the 40% reduction associated with loss of leaf area, carbon accumulation is estimated to be about 60% lower in heavily infected trees. The impact of reduced leaf photosynthetic capacity on carbon balance was thus compounded at the whole-tree scale because total leaf area was sharply reduced at both the branch level and at the whole-tree level due to increased mortality of entire branches in the heavily infected individuals studied.

Stomatal conductance and A are known to co-vary in a consistent manner in numerous species (Schulze & Hall 1982). Linear relationships between A and g_s that pass through the origin will cause C_i/C_a, and therefore intrinsic WUE, to remain constant as A and g_s vary (Goldstein *et al.* 1996). However, a curvilinear dependence of A on g_s is often observed, which results in decreasing relative stomatal limitation of photosynthesis, and therefore declining intrinsic WUE, as A and g_s increase (Schulze & Hall 1982). In the present study, $\delta^{13}\text{C}$ values of foliage from infected trees indicated that their intrinsic WUE was reduced, presumably because g_s did not change proportionally to N-induced changes in A_{max}. These results are consistent with those of several previous reports of the effects of N supply on leaf gas exchange (Liu & Dickmann 1996; Harvey & van den Driessche 1997, 1999; Clearwater & Meinzer 2001).

The integrated responses of hydraulic architecture, water and carbon relations of western hemlock to dwarf mistletoe infection suggest that the selective advantage of maintaining homeostasis of long-distance water transport and leaf water relations characteristics dominates over any advantage associated with adjustments that contribute to homeostasis of the efficiency of water use during carbon accumulation. Because total water use by infected trees was sharply reduced, the impact of their diminished photosynthetic WUE on dry season carbon gain should have been negligible unless neighbouring trees depleted unutilized soil water. However, similar values of Ψ_{pd} for infected and uninfected trees suggest that their access to soil water was similar. In sites where water is more limiting, pre-emptive use of soil water by neighbouring healthy trees may place infected trees at a competitive disadvantage that is not a direct physiological consequence of dwarf mistletoe infection.

To our knowledge, only one prior study has attempted to assess integrated allometric and physiological responses of conifers to dwarf mistletoe infection (Sala *et al.* 2001). As

so few published studies are available, it is difficult to know whether most conifers exhibit a similar set of integrated responses. Consistent with our observation of diminished intrinsic WUE in western hemlock as indicated by a decline in foliar $\delta^{13}\text{C}$ values, Sala *et al.* (2001) reported significant decreases in foliar $\delta^{13}\text{C}$ values of heavily infected Douglas-fir and western larch trees. These observations imply that reduced photosynthetic capacity associated with reduced leaf N content may be a general response of conifers to dwarf mistletoe infection. However, no reduction in photosynthetic capacity or N content of needles was detected in individuals of white spruce infected with *A. pusillum* (Logan *et al.* 2002). In contrast with sharply reduced A_L : A_S in heavily infected western hemlock trees, Sala *et al.* (2001) observed significant increases in A_L : A_S of heavily infected Douglas-fir and western larch trees. Nevertheless, there was no significant difference in whole-tree water use between heavily infected and uninfected Douglas-fir trees in their study, whereas in our study water use of heavily infected western hemlock trees was substantially reduced as a result of their lower A_L : A_S. Regardless of different responses of whole-tree water use and allometry of western hemlock, Douglas-fir, and western larch to dwarf mistletoe infection, co-ordinated adjustments in transpiration and hydraulic architecture appeared to allow their leaf water status to be unaffected or marginally affected. It is possible that apparent disparities in allometric and physiological responses to dwarf mistletoe infection may arise from transient responses to different stages of infection rather than species-specific behaviour. Development of infections over many years may produce transient changes in allometry resulting from local hormonal imbalances induced by the parasite. Such responses might cause A_L : A_S to transiently increase before ultimately declining. The responses observed in the present study are likely to reflect quasi steady-state responses to late stages of infection because preliminary growth ring analyses of infected and uninfected trees suggested that growth of infected trees began to decline at least 80 years prior to the initiation of the study (D. Woodruff, unpublished observation).

The results of the present study have a number of implications for scaling between tree and stand level processes related to water and carbon fluxes. In forests containing an appreciable fraction of infected western hemlock trees, attempts to scale from whole-tree to stand-level transpiration based on sap flow of individual trees must include measurements in both healthy and infected trees and reliable estimates of the fraction of stand sapwood area represented by each. Conversely, stand-level estimates of both transpiration and photosynthesis obtained from techniques such as eddy covariance cannot be generalized unless relationships between levels of dwarf mistletoe infection and water vapour and CO₂ fluxes are characterized. The typically low site replication of eddy covariance measurements makes extrapolation of results to forest stands with different levels of infection particularly challenging. If carbon accumulation of heavily infected trees is reduced by as much as 60% as estimated above, then a relatively small

fraction of infected trees could significantly affect inferences about carbon balance of healthy stands. The clumped distribution of infected trees into infection centres (Shaw & Weiss 2000) compounds these scaling challenges.

ACKNOWLEDGMENTS

This research was supported by the USDA Forest Service Ecosystem Processes Program, and the Wind River Canopy Crane Research Facility located within the Wind River Experimental Forest, T.T. Munger Research Natural Area in Washington State, USA. The facility is a co-operative scientific venture among the University of Washington, the USDA Forest Service Pacific North-west Research Station and Gifford Pinchot National Forest. We are grateful to Amy Burke for her able assistance in the field and to Renée Brooks for valuable comments.

REFERENCES

- Andrade J.L., Meinzer F.C., Goldstein G., Holbrook N.M., Cavellier J., Jackson P. & Silvera K. (1998) Regulation of water flux through trunks, branches and leaves in trees of a lowland tropical forest. *Oecologia* **115**, 463–471.
- Briggs G.M. & Wiebe H.H. (1982) The effects of root pruning on the water relations of *Helianthus annuus* L. *Journal of Experimental Botany* **33**, 966–976.
- Brodribb T.J. & Holbrook N.M. (2003) Stomatal closure during leaf dehydration, correlation with other leaf physiological traits. *Plant Physiology* **132**, 2166–2173.
- Broshot N., Larsen L. & Tinnin R.O. (1986) *Effects of Arceuthobium americanum on Twig Growth of Pinus contorta*. Research Note PNW-RN-453. USDA Forest Service, Washington, DC, USA.
- Bucci S.J., Goldstein G., Meinzer F.C., Franco A.C., Campanello P. & Scholz F.G. (2004) Mechanisms contributing to seasonal homeostasis of minimum leaf water potential and predawn disequilibrium between soil and plant water potential in neotropical savanna trees. *Trees* in press.
- Calvin C.L. & Wilson C.A. (1996) Endophytic system. In *Dwarf Mistletoe: Biology, Pathology, and Systematics* (eds F.G. Hawksworth & D. Wiens) Agriculture Handbook 450, pp. 113–122. USDA Forest Service Washington, DC, USA.
- Clearwater M.J. & Meinzer F.C. (2001) Relationships between hydraulic architecture and leaf photosynthetic capacity in nitrogen-fertilized *Eucalyptus grandis* trees. *Tree Physiology* **21**, 683–690.
- Ehleringer J.R., Cook C.S. & Tieszen L.L. (1986) Comparative water use and nitrogen relationships in a mistletoe and its host. *Oecologia* **68**, 279–284.
- Geils B.W., Tovar J.C. & Moody B.H. (2002) *Mistletoes of North America*. USDA Forest Service, Rocky Mountain Research Station, Ogden, UT, USA.
- Glatzel G. (1983) Mineral nutrition and water relations of hemiparasitic mistletoes: a question of partitioning. Experiments with *Loranthus europaeus* on *Quercus petraea* and *Quercus robur*. *Oecologia* **56**, 193–201.
- Goldstein G., Meinzer F.C., Sternberg L., Jackson P., Cavellier J. & Holbrook N.M. (1996) Evaluating aspects of water economy and photosynthetic performance with stable isotopes from water and organic matter. In *Tropical Forest Plant Ecophysiology* (eds S.S. Mulkey, R. Chazdon & A.P. Smith), pp. 244–267. Chapman & Hall, New York, USA.
- Granier A. (1985) Une nouvelle méthode pour la mesure du flux de sève brute dans le tronc des arbres. *Annales Des Sciences Forestières* **42**, 193–200.
- Harvey H.P. & van den Driessche R. (1997) Nutrition, xylem cavitation and drought resistance in hybrid poplar. *Tree Physiology* **17**, 647–654.
- Harvey H.P. & van den Driessche R. (1999) Nitrogen and potassium effects on xylem cavitation and water-use efficiency in poplars. *Tree Physiology* **19**, 943–950.
- Hawksworth F.G. (1977) *The 6-class Dwarf Mistletoe Rating System*. Research Note RM-48. Rocky Mountain Forest and Range Experiment Station, USDA Forest Service, Fort Collins, CO, USA.
- Hawksworth F.G. & Wiens D. (1996) *Dwarf Mistletoes: Biology Pathology and Systematics*. Agricultural Handbook 709. USDA Forest Service, Washington, DC, USA.
- Hennon P.E., Beatty J.S. & Hildebrand D. (2001) *Hemlock Dwarf Mistletoe*. Forest Insect and Disease Leaflet 135. USDA Forest Service, Forest Health Protection, Pacific Northwest Region, Portland, OR, USA.
- Hubbard R.M., Ryan M.G., Stiller V. & Sperry J.S. (2001) Stomatal conductance and photosynthesis vary linearly with plant hydraulic conductance in ponderosa pine. *Plant, Cell and Environment* **24**, 113–121.
- James S.A., Clearwater M.J., Meinzer F.C. & Goldstein G. (2002) Heat dissipation sensors of variable length for the measurement of sap flow in trees with deep sapwood. *Tree Physiology* **22**, 277–283.
- Klopatek J. (2002) Belowground carbon pools and processes in different age stands of Douglas-fir. *Tree Physiology* **22**, 197–204.
- Kuijt J. (1960) Morphological aspects of parasitism in the dwarf mistletoes (*Arceuthobium*). *University of California Publications in Botany* **30**, 337–436.
- Liu Z.J. & Dickmann D.I. (1996) Effects of water and nitrogen interaction on net photosynthesis, stomatal conductance, and water-use efficiency in two hybrid poplar clones. *Physiologia Plantarum* **97**, 507–512.
- Logan B.A., Huhn E.R. & Tissue D.T. (2002) Photosynthetic characteristics of eastern dwarf mistletoe (*Arceuthobium pusillum* Peck) and its effects on needles of host white spruce (*Picea glauca* [Moench] Voss). *Plant Biology* **4**, 740–745.
- Marshall J.D. & Ehleringer J.R. (1990) Are xylem-tapping mistletoes partially heterotrophic? *Oecologia* **84**, 244–248.
- Marshall J.D., Dawson T.E. & Ehleringer J.R. (1994) Integrated nitrogen, carbon and water relations of a xylem-tapping mistletoe following nitrogen fertilization of the host. *Oecologia* **100**, 430–438.
- Meinzer F.C. (2002) Co-ordination of vapour and liquid phase water transport properties in plants. *Plant, Cell and Environment* **25**, 265–274.
- Meinzer F.C. & Grantz D.A. (1990) Stomatal and hydraulic conductance in growing sugarcane: stomatal adjustment to water transport capacity. *Plant, Cell and Environment* **13**, 383–388.
- Mencuccini M. (2003) The ecological significance of long-distance water transport: short-term regulation, long-term acclimation and the hydraulic costs of stature across life forms. *Plant Cell and Environment* **26**, 163–181.
- Nardini A. & Salleo S. (2003) Effects of the experimental blockage of the major veins on hydraulics and gas exchange of *Prunus laurocerasus* L. leaves. *Journal of Experimental Botany* **54**, 1213–1219.
- Pataki D.E., Oren R. & Phillips N. (1998) Response of sap flux and stomatal conductance of *Pinus taeda* L. trees to stepwise reductions in leaf area. *Journal of Experimental Botany* **49**, 871–878.
- Pate J.S., True K.C. & Kuo J. (1991) Partitioning of dry matter and

- mineral nutrients during a reproductive cycle of the mistletoe *Anyema linophyllum* (Fenzl.) tieghem parasitizing *Casuarina obesa* Miq. *Journal of Experimental Botany* **42**, 427–439.
- Sala A., Carey E.V. & Callaway R.M. (2001) Dwarf mistletoe affects whole-tree water relations of Douglas fir and western larch primarily through changes in leaf to sapwood ratios. *Oecologia* **126**, 42–52.
- Schulze E.-D. & Hall A.E. (1982) Stomatal responses, water loss and CO₂ assimilation rates of plants in contrasting environments. In *Encyclopedia of Plant Physiology*, Vol. 12B *Physiological Plant Ecology II Water Relations and Carbon Assimilation* (eds O.L. Lange, P.S. Nobel, C.B. Osmond, & H. Ziegler), pp. 181–230. Springer-Verlag, New York, USA.
- Shaw D. & Greene S. (2003) Wind River Canopy Crane Research Facility and Wind River Experimental Forest. *Bulletin of the Ecological Society of America* **83**, 115–121.
- Shaw D.C. & Weiss S.B. (2000) Canopy light and the distribution of hemlock dwarf mistletoe (*Arceuthobium tsugense* (Rosendahl) G.N. Jones ssp. *tsugense*) aerial shoots in an old-growth Douglas-fir/western hemlock forest. *Northwest Science* **74**, 306–315.
- Shaw D.C., Freeman E. & Mathiasen R.L. (2000) Evaluating the accuracy of ground based dwarf mistletoe rating: a test case using the Wind River Canopy Crane. *Western Journal of Applied Forestry* **15**, 8–14.
- Tennakoon K.U. & Pate J.S. (1996) Effects of parasitism by a mistletoe on the structure and functioning of branches of its host. *Plant Cell and Environment* **19**, 517–528.
- Tinnin R.O. & Knutson D.M. (1980) Growth characteristics of the brooms on Douglas-fir caused by *Arceuthobium douglasii*. *Forest Science* **26**, 149–158.
- Trifiló P., Gascó A., Raimondo F., Nardini A. & Salleo S. (2003) Kinetics of recovery of leaf hydraulic conductance and vein functionality from cavitation-induced embolism in sunflower. *Journal of Experimental Botany* **54**, 2323–2330.
- Tyree M.T., Sinclair B., Liu P. & Granier A. (1993) Whole shoot hydraulic resistance in *Quercus* species measured with a new high-pressure flow meter. *Annals of Forest Science* **50**, 417–423.
- Wass E.F. & Mathiasen R.L. (2003) A new subspecies of *Arceuthobium tsugense* (Viscaceae) from British Columbia and Washington. *Novon* **13**, 268–276.
- Whitehead D., Livingston N.J., Kelliher F.M., Hogan K.P., Pepin S., McSeveny T.M. & Byers J.N. (1996) Response of transpiration and photosynthesis to transient change in illuminated foliage area for a *Pinus radiata* D Don tree. *Plant, Cell and Environment* **19**, 949–9567.

Received 13 January 2004; received in revised form 12 March 2004; accepted for publication 12 March 2004