

ALTERNATIVE WATER SOURCES IN WESTERN CONIFERS VIA  
FOLIAR UPTAKE AND HYDRAULIC REDISTRIBUTION

By

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## ABSTRACT

### ALTERNATIVE WATER SOURCES IN WESTERN CONIFERS VIA FOLIAR UPTAKE AND HYDRAULIC REDISTRIBUTION

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Water is often the most limiting resource in a plant's environment. Plants that can maximize their ability to acquire water improve their chances of success. Outside of the traditional soil-plant-atmosphere continuum, plants can alternatively acquire water via foliar uptake of water and hydraulic redistribution (HR) of deep water. This study used greenhouse-based experiments to investigate water use and physiology in four conifer species native to the western U.S.: *Picea sitchensis* (Bong.) Carrière (PISI), *Pseudotsuga menziesii* (Mirb.) Franco (PSME), *Sequoia sempervirens* (D. Don) Endl. (SESE), and *Thuja plicata* Donn ex. D. Don (THPL). First, this work investigated the capacity for foliar water uptake using two different measurement methods (submersion in water versus exposure to water vapor in a fog chamber). Second, mesocosms were used to evaluate the capacity for HR of water and the possible effects of HR water on tree physiology. Analyses found that foliar uptake rates measured using a fog chamber were roughly three times greater than uptake rates measured using the submersion method. All species were capable of foliarly absorbing water; PSME generally had the greatest foliar uptake values while THPL had the lowest uptake capacity. Findings also suggested that PISI and SESE are capable of hydraulically lifting water and that this HR water can

sustain plant water potential and stomatal conductance during drought, even for neighboring trees. Collectively, this work provides a methodological comparison of two commonly-used methods to measure foliar water uptake capacity and also demonstrates that two important western conifer species (PISI and SESE) are capable of hydraulically redistributing water to enhance physiology (water potential and stomatal conductance) in the trees lifting water as well as in neighboring trees. The use of waters that have been foliarly absorbed and/or hydraulically lifted and redistributed may become increasingly important for trees in the western U.S., as climate models project that this region will likely continue to warm and dry in the coming decades.

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## INTRODUCTION

Plants live in the narrow space between the atmosphere and lithosphere where aboveground foliage reacts to minute changes in vapor pressure and humidity, and underground roots exchange water and nutrients with the rhizosphere. Plants are acutely sensitive to the moisture in their environment, because water is one of three most limiting resources to productivity and seedling survival (Cable 1969; Bloom et al. 1985; Craine and Dybzinski 2013). Regardless of nutrient and light availability, without sufficient water supply, plants are unable to uptake nutrients and unable to photosynthesize in daylight. Plants that can find and acquire water can better ensure their success. Many plants maximize water acquisition through foliar uptake (e.g. Limm et al. 2009; Goldsmith et al. 2013; Gotsch et al. 2014), hydraulic redistribution of deep water (e.g. Richards and Caldwell 1987; Dawson 1993), and/or increases in root surface area via fungal associations (e.g. Sánchez-Díaz and Honrubia 1994; Lehto and Zwiazek 2011).

### Foliar Uptake

California's summer fog bank is a low stratus layer formed by the convergence of warm and cool ocean currents off the coast. The bank moves inland towards low pressure areas created by the warming continental land mass (Byers 1953; Azevedo and Morgan 1974). Fog can be a source of water for plants through both fog drip to the soil (Byers 1953) and direct foliar uptake (Dawson 1998; Breshears et al. 2008; Limm et al. 2009). On average, across all ecoregions of the world, leaf wetting occurs greater than 100 days

annually, but can range from 29 to 174 days depending on the climatic region (Dawson and Goldsmith 2018). Summer marine fog along the Pacific coast of California has the highest frequency in northern and central California and declines toward Oregon and southern California (Oberlander 1956; Johnstone and Dawson 2010). Fog often penetrates coastal forests in the late evening and early morning hours (Azevedo and Morgan 1974) and can improve forest water status (the water status of an entire forest rather than an individual tree) by decreasing the vapor pressure deficit and therefore evapotranspirational water losses throughout an entire stand or forest, dripping water deposits down to the soil where they can be absorbed by plant roots, and depositing water on leaves where it can be absorbed directly into plants (Dawson 1998). This additional source of water in summer months is especially important because California's coast experiences a Mediterranean climate in which May to October are considered the dry season and solar radiation reaches its peak.

Leaves often spend much of their life wet, on average about  $9 \pm 3$  hours per day in both temperate and tropical forests, either partially or fully covered by a film of water (Dawson and Goldsmith 2018). Leaf wetness can result in measurable costs such as the promotion of pathogen development, reduction of photosynthetic gas exchange, leaching, and the uptake of toxic solutes (Dawson and Goldsmith 2018). Alternatively, leaf wetting events can promote foliar water uptake and improve leaf water status, especially in tall trees (Dawson and Goldsmith 2018; Kerhoulas et al. 2020b, a). This leaf-level benefit can scale up to serve whole plants, plant communities, and ecosystems (Dawson and Goldsmith 2018). The most obvious benefit of leaf wetting is that to water relations by

increasing plant water status and reducing water tension in foliage (Dawson and Goldsmith 2018; Guzmán-Delgado et al. 2018). Water tension determines the direction of water flow; traditionally, transpired water moves from soil to plant to atmosphere along a continuum of increasingly negative water potential (Jackson et al. 2000). Foliar uptake can reduce the differential between midday ( $\Psi_{md}$ ) and predawn ( $\Psi_{pd}$ ) water potentials, improve plant hydraulic functions, and may even provide a mechanism to reverse embolisms formed in the xylem of leaf veins and small branches (Dawson and Goldsmith 2018). For example, *Pseudotsuga menziesii* (Mirb.) Franco (PSME) needles can experience daily cycles of embolism and refilling, suggesting that this species relies fully or partially on foliar uptake to reverse daily embolisms (Woodruff et al. 2007). Improved plant water status can also enhance photosynthesis via increased stomatal conductance and promote cell expansion and growth via increased turgor pressure (Dawson and Goldsmith 2018). Additionally, increased turgor pressure due to increased  $\Psi$  (Koch et al. 2004) can also support greater mesoporosity, which enhances photosynthetic capacity via increased internal CO<sub>2</sub> diffusion rates (Mullin et al. 2009). Lastly, foliar wetting can reduce evapotranspiration (Byers 1953) and leaf and canopy temperature, which over the long-term, can improve carbon balance (Dawson and Goldsmith 2018). All precipitation events are at least partially intercepted by foliage, with only larger events substantially increasing soil water content. Thus, foliar uptake provides additional moisture enabling plants to supplement soil water uptake with precipitation inputs that wet foliage but do not increase soil moisture substantially (Dawson 1998; Breshears et al. 2008).

For foliar uptake to occur, leaf  $\Psi$  must be more negative than the atmosphere immediately surrounding it (Rundel 1982). Since foliar uptake is a function of plant  $\Psi$  (Breshears et al. 2008), under conditions of higher water stress, wetting events can provide a substantial water source for leaves. The positive correlation between foliar water uptake capacity and water stress (Burgess and Dawson 2004) suggests that this alternative water source can mitigate drought stress to improve plant survival during drought (Breshears et al. 2008). Water can enter the leaf in two forms, either as liquid or as vapor, and is driven by a gradient in either water potential or vapor concentration, respectively, between the leaf interior and the leaf boundary layer (the area immediately surrounding the leaf surface) (Berry et al. 2019). Foliar uptake is often measured as a change in mass that is then standardized by total leaf mass or leaf area, but can also be inferred from changes in physiology such as leaf water potential, gas exchange, and photosynthetic rates (Guzmán-Delgado et al. 2018; Kerhoulas et al. 2020b). There are still many unknowns regarding the pathway(s) for foliar water uptake. While there is considerable debate within the literature (Vaadia and Waisel 1963; Berry et al. 2019), it seems that foliar uptake predominantly occurs through stomatal pores (Burgess and Dawson 2004; Eichert and Goldbach 2008; Burkhardt et al. 2012; Guzmán-Delgado et al. 2018) and, in a smaller proportion, via a non-polar path through the lipophilic cuticle (Yates and Hutley 1995; Schreiber et al. 2001; Riederer 2006; Limm et al. 2009; Goldsmith et al. 2013). Similarly, there is debate within the literature between two common methods to expose foliage to water deposits: submerging foliage in liquid water versus exposing foliage to water vapor in a fog chamber (Limm et al. 2009; Dawson and

Goldsmith 2018; Kerhoulas et al. 2020b). It is unclear if/how complete submergence as opposed to a thin film of water resulting from exposure to a vapor cloud differ in their effects on the driving gradient of uptake, stomatal closure, and other traits that affect uptake.

Many plant species have the capacity for foliar uptake (Limm et al. 2009; Goldsmith et al. 2013; Gotsch et al. 2014); greater than 90% of the 124 different plant species yet tested demonstrate this ability (Dawson and Goldsmith 2018). Numerous plant species in the coastal redwood (*Sequoia sempervirens* (D. Don) Endl., SESE) ecosystem rely on fog water for some portion of their total water use (Burkhardt et al. 2012); some understory plants such as the western sword fern (*Polystichum munitum* (Kaulf.) C. Presl) are at times completely dependent on fog water (Dawson 1998). Coast redwoods are no exception, so much so that the latitudinal limits of the coast redwood distribution correspond with a specific fog threshold at both the northern and southern ecotones (Johnstone and Dawson 2010). Over the last century, fog frequency and coastal inversion strength has decreased (Dawson 1998), impacting coastally restricted species and increasing their drought sensitivity (Johnstone and Dawson 2010). Decreasing fog frequency will likely further restrict and threaten established redwood forests. Together this suggests that the species-specific capacity for foliar uptake may impact productivity of western conifers under future scenarios of warming and drying.

## Drought and Hydraulic Redistribution

Given the shifting climate and increases in drought frequency and severity (Griffin and Anchukaitis 2014), understanding how western conifers respond to drought is imperative to accurately model and predict their responses on individual and landscape scales under future climate conditions (Ambrose et al. 2015). As soil moisture declines, plant  $\Psi$  decreases under continued transpiration, which elevates the risk of cavitation, leading to a loss of hydraulic conductance and a further decline in xylem  $\Psi$  (Zimmermann 1983; Ambrose et al. 2015). Plants can close their stomata to reduce stomatal conductance ( $g_s$ ) of water vapor and maintain the integrity of the root-to-leaf pathway (Sperry et al. 1998). There are two contrasting approaches that plants often use to deal with potential cavitation: isohydry and anisohydry (Tardieu and Simonneau 1998). Isohydric plants typically exhibit strong stomatal control of transpiration in response to drought, maintain relatively high minimum leaf  $\Psi$ , and avoid severe xylem cavitation and hydraulic failure (Tardieu and Simonneau 1998; Ambrose et al. 2015). Due to this somewhat cautious stomatal regulation, isohydric species can be prone to carbon starvation during extended droughts (McDowell et al. 2008). Further, under excessively arid conditions, stomatal closure alone is not always sufficient to prevent cavitation, hydraulic failure, crown dieback, and even death (Brodribb and Cochard 2009; Skelton et al. 2017). Anisohydric plants, on the other hand, typically exhibit lower stomatal sensitivity during drought, maintaining high transpiration rates and allowing minimum leaf  $\Psi$  to decline to more negative values compared to isohydric plants,

consequently increasing the possibility for cavitation and hydraulic failure (Tardieu and Simonneau 1998; Ambrose et al. 2015). To accommodate these very negative  $\Psi$  values, anisohydric species often have wood anatomies that are highly resistant to cavitation (McDowell et al. 2008). While these two strategies can be helpful when thinking about plant water regulation, recent work suggests that stomatal regulation is likely more an expression of a trait-environment interaction rather than a fixed trait (Garcia-Forner et al. 2016; Hochberg et al. 2018). Thus, isohydry and anisohydry are opposite sides of a spectrum and species can occupy regions along the continuum rather than binarily falling directly into one camp or the other (Tardieu and Simonneau 1998; Ambrose et al. 2015; Voelker et al. 2018). Regardless of stomatal regulation strategy, drought can lower tree  $\Psi$  and  $g_s$  and therefore photosynthetic rate and net primary productivity (Ambrose et al. 2015). Given the variability of tree physiological responses to drought, rich conifer diversity in the western U.S., widespread drought-related forest mortality (Young et al. 2017), and model projections for a hotter and drier future climate in this region (Cook et al. 2015; Diffenbaugh et al. 2015), it is important to understand how alternative water sources such as hydraulically lifted and redistributed water from deep in the soil might buffer drought effects on trees.

Hydraulic redistribution (HR) is a process in which taproots transport deep groundwater to upper soil layers where it is later reabsorbed by more shallow roots. This movement of groundwater via plant roots into shallower soil horizons is driven by passive diffusion along  $\Psi$  gradients, as water will leave a host tree's hydrated root system

to permeate the surrounding soil with a lower  $\Psi$  (Richards and Caldwell 1987; Schwenke and Wagner 1992; Dawson 1993). In addition to improving the lifting tree's water supply by "banking" HR water in upper soil layers (Dawson 1993), more shallowly-rooted neighbors may rely on this supply of HR water during periods of drought. Hydraulic redistribution has been studied extensively in brush, grasses, and hardwoods (Richards and Caldwell 1987; Dawson 1993; Nadezhdina et al. 2008; Armas et al. 2011), but coniferous hydraulic redistribution (Simeone et al. 2019), especially in temperate or more mesic environments (Hafner et al. 2020), has been studied less thoroughly.

#### Common Mycorrhizal Networks

Common mycorrhizal networks (CMNs) are networks of hyphal pathways that can link and transfer resources among plants and can act as sinks themselves (Querejeta et al. 2003) while redistributing HR water to surrounding neighbors (Egerton-Warburton et al. 2007). Highlighting the potential importance of CMNs in interspecific resource sharing, research shows that water transferred between tree species via CMNs (Plamboeck et al. 2007) is not due to passive leakage from roots or hyphae into the soil and back into neighboring roots, suggesting that hyphae are responsible for the transfer between plants (Querejeta et al. 2003).

Mycorrhizal fungi play an important role in assisting host plants to obtain nutrients and water, their hyphae acting as extensions of the plant root system (Allen 1991). In mesic and arid regions, during times of drought, mycorrhizal hyphae aid plants by helping them access water in otherwise inaccessible soil pores, allowing plants to



continue photosynthesizing and avoid cavitation during the growing season when soil moisture can be at its lowest (Allen 2009). There are two types of mycorrhizal fungi associations, ecto- and endomycorrhizal, distinguished by the morphological difference of whether the fungus penetrates the root cell walls (Frank 1885, 1891; Allen 1991). One major group of endomycorrhizae are the vesicular-arbuscular mycorrhizae (VAM), describing the mycelial-fan that penetrates and spreads within root cortical cells, while ectomycorrhizae are identified by the presence of a mantle around the root exterior and a Hartig net formed between the root cortical cells of the host plant. The fungi that form VAM exhibit little specificity, while ectomycorrhizae vary in specificity (Allen 1991) from the highly specific *Alpova diplophloeus* (Zeller & C.W. Dodge) Trappe & A.H. Sm. with *Alnus* to *Amanita muscaria* (L.) Lam capable of partnering with a wide range of conifer hosts. *Pisolithus tinctorius* (Pers.) Coker & Couch, is another example of an ectomycorrhizal fungus capable of partnering with a wide range of host trees; as such, it has been spread worldwide through its use in forestry (Marx et al. 1982). Some mycorrhizal fungi (e.g., *Suillus lakei* (Murrill) Smith & Thiers) are capable of forming both arbutoid mycorrhizae with ericaceous shrubs and ectomycorrhizae with coniferous trees (Allen 1991; Smith and Read 1997). Likewise, some host plants, such as *Pseudotsuga*, are capable of forming both VAM and ectomycorrhizae (Harley and Smith 1983; Smith and Read 1997). In most cases, Pinaceae, which includes both *Picea* and *Pseudotsuga*, are primarily ectomycorrhizal, whereas all other conifer families, including Cupressaceae which possesses both *Sequoia* and *Thuja*, are dominantly vesicular-arbuscular mycorrhizal (Smith and Read 1997).

## Study Questions and Hypotheses

Given the importance of water for plant survival, the projections for a warmer and drier climate in the future, and the diversity of water sources that plants can use to supplement the traditional soil-plant-atmosphere continuum, this study investigated the capacity of four western conifer species to a) foliarly uptake water, b) hydraulically lift and redistribute water during drought, c) share HR water with neighboring trees during drought via CMNs, and d) use HR water to buffer physiological responses to drought. The investigation of foliar water uptake capacity also included a comparison between the submersion and fog chamber methods.

Study species included *Picea sitchensis* (Bong.) Carrière (PISI, Sitka spruce), *Pseudotsuga menziesii* (Mirb.) Franco (PSME, Douglas-fir), *Sequoia sempervirens* (D. Don) Endl. (SESE, coast redwood), and *Thuja plicata* Donn ex. D. Don (THPL, western redcedar). In the redwood forest ecosystem, SESE, PSME, and PISI are tall, dominant overstory trees, while THPL comprises a smaller component of this ecosystem, predominantly occupying the mid-canopy. Coast redwood and PSME are both especially important timber producing species of the Northwest. Sitka spruce and THPL are both highly susceptible to fire, and THPL will only grow where soil moisture is high. Coast redwood and THPL are both in Cupressaceae and form VAM associations, whereas PSME and PISI are both in Pinaceae and host ectomycorrhizal fungi, though Douglas-fir can form both VAM and ectomycorrhizal associations.

Specifically, this study investigated the following questions and corresponding hypotheses:

- 1) How does foliar water uptake capacity vary among species? Hypothesis: PSME will have the greatest capacity based on the results of Limm et al. (2009), and SESE will also have a high capacity for foliar uptake due to its coastally restricted range that correlates with a specific fog threshold.
- 2) How do the submersion and fog chamber methods to measure foliar uptake capacity compare? Hypothesis: uptake measurements will be greater with the submersion method than the fog method.
- 3) Do SESE and/or PISI hydraulically lift and redistribute water? Hypothesis: both species will hydraulically lift and redistribute water.
- 4) Do CMNs intra- and interspecifically transfer hydraulic redistribution water between neighboring trees? Hypothesis: CMNs transfer hydraulic redistribution water from source trees to intra- and interspecific neighboring trees.
- 5) Can hydraulic redistribution water help buffer tree physiological responses to drought in both the tree lifting water (donors) and in neighboring trees (recipients)?  
Hypothesis: hydraulic redistribution water will help maintain water potential and stomatal conductance during drought in both donor and recipient trees.

## METHODS

### Foliar Uptake

To quantify foliar water uptake capacity based on gravimetric changes in excised shoots (included both leaves and stems), the laboratory methods described in Limm et al. (2009) were followed. For each of the four study species (PISI, PSME, SESE, and THPL), foliar uptake capacity was evaluated using both complete submergence in water and immersion in water vapor via a fog chamber to evaluate uptake under more “natural” conditions than the submergence method (described below). The fog chamber (Figure 1) was constructed out of a metal frame (69 x 48 x 157 cm) covered in clear, polyvinyl sheeting (Blissun 4 Tier Mini Greenhouse) and included a waterproof fan (120 mm cooling case computer fan) to circulate air and fog, a reservoir containing a three-disk ultrasonic fog-generating device (Model HG-HJWJL-1315, BoTaiDaHong, ChengDu, China) in 5 L of deionized (DI) water below the fan, and a HOBO data logger (Model MX2300, Onset Computer Corporation, Bourne, MA) to record relative humidity and temperature within the chamber to confirm foggy conditions. For each method, there were six seedlings (~2-yrs old) per species; seedlings were from the local seed zone (092) and provided by The Jonsteen Company nursery (McKinleyville, CA). Prior to foliar uptake measurements, seedlings were planted in 1-gallon pots with a mix of soil and fertilizer conducive to mixed conifer forests of the coastal redwood ecosystem, watered

as needed (approximately once a week), and allowed to establish for six months in the Humboldt State University (HSU) Forestry Greenhouse.



Figure 1. A picture of the Blissun 4 Tier Mini Greenhouse used as a fog chamber in the foliar uptake experiment.

For both water uptake methods, three terminal shoots (~5 cm) were excised from each seedling (six seedlings per species), cut ends were wrapped with laboratory film (Parafilm; Pechiney Plastic Packaging, Chicago, IL) to prevent evaporation from the exposed stem surface, and each shoot was labeled with labeling tape. The starting mass of each shoot (including Parafilm and labeling tape) was measured ( $Mass_1$ ). For the submergence method, shoots were then submerged in DI water for 180 minutes to allow for foliar water uptake. For the fog chamber method, shoots were then hung from their

labeling tape in the fog chamber for 180 minutes to allow for foliar water uptake. We used this 180 minute submersion time to be comparable with previous studies (e.g., Limm et al. 2009). Following submergence or fog exposure, shoots were thoroughly patted dry with paper towels and the mass measured ( $Mass_2$ ). To account for potential residual water on shoots that was not blotted dry with the paper towel, the residual surface water was estimated by air-drying shoots briefly, re-weighing ( $Mass_3$ ), submerging shoots in water for 1 second, towel drying, and reweighing ( $Mass_4$ ). These masses were used to calculate foliar water uptake capacity using the following equation:

$$Uptake = (Mass_2 - Mass_1) - (Mass_4 - Mass_3) \quad (1)$$

where  $Mass_1$  is the mass before any wetting,  $Mass_2$  is the mass after 180 min of submergence or exposure to fog and towel-drying,  $Mass_3$  is the mass after brief air-drying, and  $Mass_4$  is the mass after brief resubmergence and towel-drying.

To standardize uptake, uptake was calculated on an area-basis, a mass-basis, and as a percent change in shoot water content (SWC). For each metric, uptake estimates from the three replicates per seedling were averaged into a single value. For the area-based calculations, leaves were removed from shoots and leaves and stems were scanned at 600 dpi (Epson America, Inc., Long Beach, CA). Projected shoot area (stem plus leaves) was digitally determined using Image J software (US National Institutes of Health, Bethesda, Md.) and used to standardize the uptake quantity per shoot area ( $\text{mg H}_2\text{O cm}^{-2}$ ). For the mass-based uptake calculations, stems and leaves (without Parafilm and labeling tape) were then dried at 60 °C for 72 hours and weighed to standardize

uptake quantity per shoot mass ( $\text{mg H}_2\text{O g}^{-1}$ ). For the SWC calculations, the following equation was used:

$$\% \text{ Change in SWC} = \left( \frac{(Mass_2 - (Mass_4 - Mass_3) - Mass_{dry})}{Mass_1 - Mass_{dry}} - 1 \right) * 100 \quad (2)$$

where  $Mass_{dry}$  is the total weight of the dried shoot and its leaves.

### Hydraulic Redistribution, Common Mycorrhizal Networks, and Drought:

#### Mesocosm Experiment

To investigate whether hydraulic redistribution and common mycorrhizal networks affect seedling water use, three-parted mesocosm experimental chambers were used: one central donor chamber flanked by two recipient chambers (Figure 2).

Mesocosms were constructed out of plastic aquariums (My Fun Fish Tank, As Seen On TV, 12 x 15 x 25 cm) painted matte grey to prevent light penetration and filled with soil and fertilizer conducive to mixed conifer forests of the coastal redwood ecosystem.

Fertilizer was a low N fertilizer and irrigated into the soil two weeks after establishment.

A hole (76 mm diameter) was drilled through one side of adjacent recipient chambers and opposite sides of the central donor chamber and the three mesocosms were held together

by a specialized adhesive (Weld-On 4 Acrylic Adhesive). Filter paper (20  $\mu\text{m}$ , GVS

Magna nylon membrane mesh, GE Osmonics) was placed in the holes between

mesocosms and allowed fungal hyphae ( $\sim 10 \mu\text{m}$ ) to move between mesocosms while

inhibiting the movement of water (Egerton-Warburton et al. 2007; Plamboeck et al.

2007). A hole (25 mm) was drilled in the bottom of the central mesocosm and the seedling taproot was passed through an air gap (~2 cm) into a container filled with perlite and tap water.

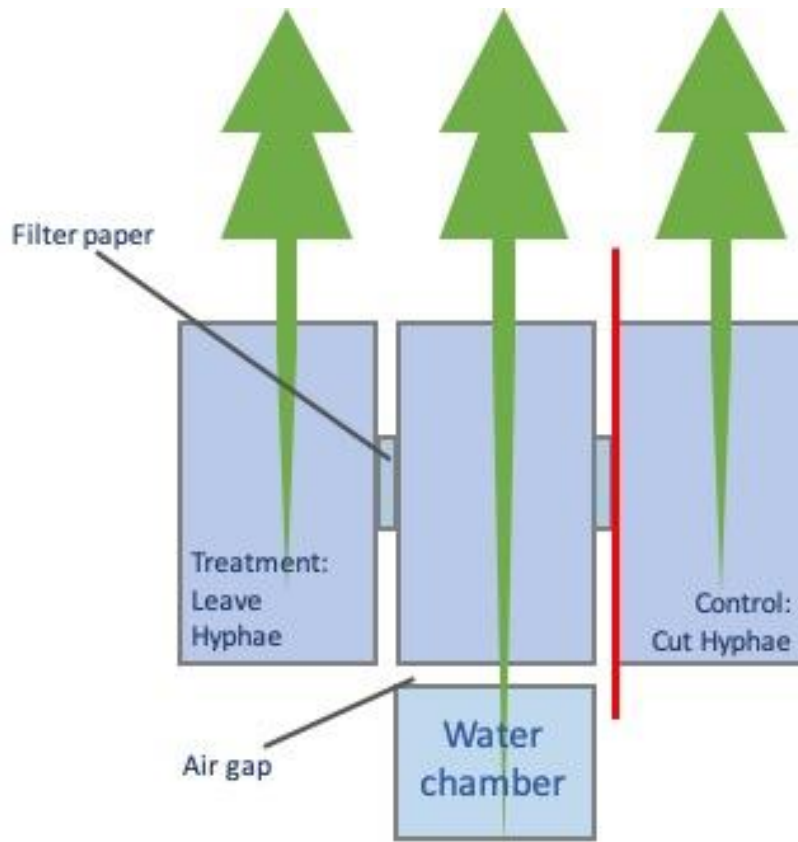


Figure 2. Diagram of a mesocosm showing the three-part chamber separated by filter paper. The central taproot passed through an exit hole and an air gap and into a container with water and perlite. In each chamber, one seedling was planted in a fertilized soil mixture inoculated with 19 mycorrhizal species. The experiment included one central donor seedling and two adjacent recipient seedlings. The filter paper was permeable to fungal hyphae but not to water.

Using the above three-part mesocosms, the following pairings were investigated, each with three replicates: 1) SESE donor with SESE recipients, 2) PISI donor with PISI recipients, 3) SESE donor with THPL recipients, and 4) PISI donor with PSME



recipients. All seedlings (~2-yrs old) were obtained from the local (092) seed zone and were provided by The Jonsteen Company nursery (McKinleyville, CA). Each mesocosm chamber was inoculated with mycorrhizae via watering with Soluble Maxx (approximately 28 g per 6 L of water) once at establishment and once after four weeks of establishment; this product contains a mixture of 10 ectomycorrhizal species and 9 endomycorrhizal species (Table 1). In addition to the central donor seedling's taproot accessing water, mesocosms were watered from above as needed to ensure adequate water to all seedlings. Mesocosms were grown in the HSU Forestry Greenhouse for 24 weeks to encourage establishment of both seedlings and mycorrhizae.

Table 1. List of endomycorrhizae and ectomycorrhizae species used to inoculate seedlings.

Endomycorrhizae	Ectomycorrhizae
<i>Glomus intraradices</i>	<i>Rhizopogon villosulus</i>
<i>Funneliformis mosseae</i>	<i>Rhizopogon luteolus</i>
<i>Glomus aggregatum</i>	<i>Rhizopogon amylopogon</i>
<i>Glomus etunicatum</i>	<i>Rhizopogon fulvigleba</i>
<i>Glomus deserticola</i>	<i>Pisolithus tinctorius</i>
<i>Rhizophagus clarus</i>	<i>Scleroderma cepa</i>
<i>Funneliformis monosporus</i>	<i>Scleroderma citrinum</i>
<i>Paraglomus brasilianum</i>	<i>Suillus granulatus</i>
<i>Gigaspora margarita</i>	<i>Laccaria bicolor</i>
	<i>Laccaria laccata</i>

At the end of this establishment period, baseline physiological measurements were collected from all seedlings. Unfortunately, there was low establishment success among seedlings, such that after 24 weeks the only functional mesocosms included: PSME-PISI-PSME ( $n = 3$ ) and THPL-SESE-THPL ( $n = 2$ ). To evaluate pre-drought seedling water status, midday leaf water potential ( $\Psi_{md}$ ) was measured on small

branchlets using a pressure chamber (Model 600, PMS Instruments, Corvallis, OR). To evaluate pre-drought seedling gas exchange capacity, a leaf porometer (Model SC-1, Decagon Devices Inc., Pullman, WA) was used to measure stomatal conductance ( $g_s$ ). To minimize destructive loss of plant material, only one  $\Psi_{md}$  measurement was taken per seedling; for  $g_s$ , three measurements were taken and then averaged into a single value for that seedling.

After 24 weeks of establishment and the measurement of baseline  $\Psi_{md}$  and  $g_s$  in seedlings, a drought was simulated to evaluate the potential for hydraulic redistribution and water exchange via CMNs. In each mesocosm, mycorrhizae were severed between one recipient chamber and the donor chamber to serve as a control. On the other side of each mesocosm, mycorrhizae were left intact between the remaining recipient chamber and the donor chamber. Watering from above was then terminated to simulate drought, with the lower reservoir, accessible only by the central chamber seedling's taproot, remaining as the sole water supply. Isotopically labeled (D-enriched,  $\delta D = 486\text{‰}$ ) deionized (DI) water was then used to fill the lower reservoir. In theory, water would move from the lower reservoir into the central chamber via taproot hydraulic redistribution. If water leakage from hyphae and/or roots into the soil occurred, labeled water should be detected in the soil of the central donor chamber. And, if water transfer occurred between chambers via CMNs, labeled water should be detected in the seedling and/or soil of the adjacent chamber with intact mycorrhizal connections but not in the adjacent chamber with severed mycorrhizal connections. To confirm that soil water could not pass through the filter paper outside of mycorrhizae, no labeled water in the soil or

seedling of the recipient chamber with severed mycorrhizae (the control) should be detected.

After one week of drought, seedling physiology as well as xylem water and soil water stable isotope compositions were measured. On each seedling,  $\Psi_{md}$  and  $g_s$  were again measured using the same methods as previously described, and one branch (with leaves removed) was harvested for xylem water extraction and stable isotope analysis. In each chamber, soil was sampled at 10 cm intervals (0, 10, and 20 cm depths) to visually confirm mycorrhizal colonization and extract soil water for isotopic analysis.

Unfortunately, during this destructive sampling phase, it was discovered that the filter paper had disintegrated and failed to create a barrier, and mycorrhizal colonization of the roots was minimal, so the study question related to CMNs could not be addressed.

Nevertheless, to investigate study questions related to hydraulic redistribution, all twig and soil samples were stored in a freezer until cryogenic vacuum extraction of water in the HSU Forest Physiology Lab. All water samples were sent to the University of New Mexico's Center for Stable Isotopes for isotopic analysis of  $\delta D$  and  $\delta^{18}O$  using a Picarro laser-based isotopic analyzer (L1102-I Water Isotopic Analyzer, Picarro, Santa Clara, CA) equipped with a micro-combustion module to remove organics that could potentially interfere with water isotope analysis. Organic molecules such as terpenes, alcohols, and green leaf volatiles have the potential to skew results, however, this module vaporizes samples before oxidizing them to convert organics into minute quantities of carbon dioxide and nascent water (West et al. 2010; Picarro 2012).

## Data Analysis

Statistical analysis was conducted using JMP 9 (SAS Institute, Cary, NC) and  $\alpha = 0.05$ . In general, outliers were removed if they were greater than two standard deviations from the mean. For ANOVA-based analyses, Shapiro-Wilk goodness of fit tests were used to test the assumption that data were normally distributed; if this assumption was violated, Kruskal-Wallis tests were used to detect significant differences among groups. Levine and Bartlett tests were used to test the assumption of equal variance among groups; if this assumption was violated, Welch tests were used to detect significant differences among groups. If significant differences among groups were found, Tukey's HSD multiple means comparisons were used to determine how groups differed. For the foliar uptake capacity investigation, one and two-way ANOVAs were used to test for significant effects of method (submergence versus fog exposure) and species on foliar uptake. Matched pair two-tailed  $t$ -tests were used to identify significant differences between the two treatments. Regression analyses were also used to determine the relationship between submersion-based estimates of uptake capacity and estimates based on the fog chamber method. For the hydraulic redistribution analysis, the fraction of HR water measured in soil and xylem water samples was calculated using a two-source mixing model (Dawson et al. 2002):

$$f_A = \frac{(\delta_t - \delta_B)}{(\delta_A - \delta_B)} \quad (3)$$

where  $f_A$  is the fraction of the total contributed by source A,  $\delta_t$  is the sample  $\delta D$ ,  $\delta_B$  is the  $\delta D$  of source B, and  $\delta_A$  is the  $\delta D$  of source A. To evaluate if HR water influenced

seedling physiology during drought, matched pair *t*-tests were used to compare pre- and post-drought differences in  $\Psi_{\text{md}}$  and  $g_s$ .

## RESULTS

## Foliar Uptake

Shoot water uptake on an area basis ( $\text{mg H}_2\text{O cm}^{-2}$  shoot area) was related to both treatment ( $p < 0.0001$ ) and species ( $p = 0.001$ ) with no interaction ( $p = 0.67$ ) (Table **2Error! Reference source not found.**). For shoot water uptake on a mass basis ( $\text{mg H}_2\text{O g}^{-1}$  foliage), treatment ( $p < 0.0001$ ), species ( $p < 0.0001$ ), and their interaction ( $p = 0.02$ ) were significant effects (Table 3). Similarly, when analyzing percent change in SWC, treatment ( $p < 0.0001$ ), species ( $p < 0.0001$ ), and their interaction ( $p = 0.001$ ) were significant effects (Table 4).

Table 2. Two-way ANOVA table for the effects of species, treatment, and their interaction (S x T) on foliar water uptake on an area basis.

	<i>df</i>	<i>F</i>	<i>p</i>
Species	3	6.8	0.001
Treatment	1	94.6	<0.0001
S x T	3	0.5	0.67

Table 3. Two-way ANOVA table for the effects of species, treatment, and their interaction (S x T) on foliar water uptake on a mass basis.

	<i>df</i>	<i>F</i>	<i>p</i>
Species	3	12.0	<0.0001
Treatment	1	153.5	<0.0001
S x T	3	03.7	0.02

Table 4. Two-way ANOVA table for the effects of species, treatment, and their interaction (S x T) on foliar water uptake as a percent change in shoot water content (SWC).

	<i>df</i>	<i>F</i>	<i>p</i>
Species	3	18.5	<0.0001
Treatment	1	111.6	<0.0001
S x T	3	06.9	0.001

Across the three metrics (area-based, mass-based, and percent change in SWC) and species, the submersion method resulted in significantly lower foliar uptake values than the fog method when assessed using a one-way ANOVA ( $p < 0.0001$ ), and paired *t*-tests confirmed this result ( $p < 0.0001$ ). On average, across species and metrics, the fog-based method estimated foliar water uptake capacity rates roughly three times higher than estimates calculated using the submersion method. In other words, when using the fog chamber, foliage absorbed 2.5 to 3 times as much water than when fully submerged in water for the same period of time.

Among species, there were differences in foliar water uptake capacity. When assessing foliar uptake standardized by projected leaf area (Figure 3A), the submersion method measured comparable uptake rates among PSME, SESE and THPL, with PISI having a significantly higher capacity than the other species ( $p = 0.02$ ). Area-based measurements from the fog method showed that PISI had higher uptake rates than THPL ( $p = 0.13$ ), with SESE and PSME rates falling moderately between PISI and THPL. On a mass basis (Figure 3B), submersion measurements indicated that PISI and PSME had higher uptake rates than THPL, with SESE rates falling moderately in the middle ( $p = 0.01$ ). Contrastingly, mass-based uptake measurements from the fog chamber ranked the species from highest to lowest in the following sequence: PSME, SESE, PISI, THPL ( $p = 0.001$ ). When assessing uptake as a percent change in SWC (Figure 3C), submersion-based measurements found that PISI and PSME had higher uptake rates than SESE and THPL ( $p = 0.0003$ ). Fog-based measurements of percent change in SWC ranked the species from highest to lowest capacity in the following sequence: PSME, SESE, PISI,

THPL ( $p = 0.0003$ ). Across all three metrics, the submersion method generally demonstrated that PISI had the highest uptake rate and THPL had the lowest uptake rate, while the fog method generally found that PSME had the highest uptake rate and THPL had the lowest uptake rate.



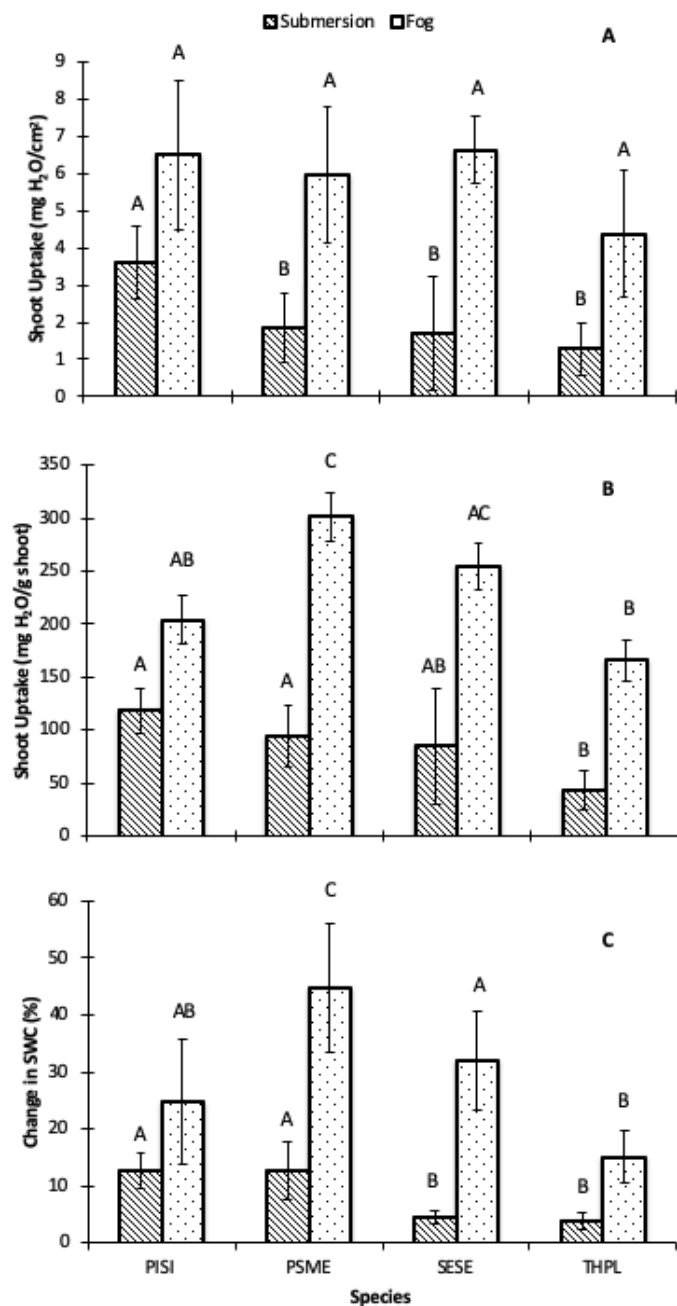


Figure 3. Mean ( $\pm$  SE) shoot (leaves and stem) water uptake capacity standardized by A) projected shoot area, B) shoot mass, and C) percent change in shoot water content (SWC) for each species (PISI = *Picea sitchensis*, PSME = *Pseudotsuga menziesii*, SESE = *Sequoia sempervirens*, and THPL = *Thuja plicata*) as measured via the submersion and fog chamber methods. Within a method, species not sharing the same letter are significantly different.

To relate the more easily acquired submersion-based uptake measurements to the more difficult to acquire fog-based uptake measurements, regression relationships between the two sets of values were explored. Although the fog chamber method consistently produced uptake measurements significantly higher than uptake rates measured using the submersion method for all species, in most cases it was not possible to develop a significant regression model for pooled species or for individual species using any of the three metrics ( $p > 0.05$ ) (Table 5), although for many of the non-significant relationships, the  $R^2$  values were quite high. However, a significant relationship between the two methods was found for THPL mass-based shoot water uptake ( $p = 0.01$ ,  $R^2 = 0.86$ ), which showed a declining exponential relationship between fog- and submersion-based measurements. A significant relationship between the two methods was also found for pooled species' change in percent SWC ( $p = 0.03$ ,  $R^2 = 0.24$ ), which showed a positive linear relationship between fog- and submersion-based measurements (Figure 4).

Table 5. Regression table for shoot (leaves and stem) water uptake on an area basis, mass basis, and percent change in shoot water content (SWC). Informative statistics are provided for models relating submersion-based measurements (x) to fog-based measurements (y). For each metric, relationships were explored for pooled species and individual species. Gray shading indicates significant relationships.

Metric	Taxa	Fit	$p$	$R^2$
Area-based uptake	All species	Linear	0.61	0.02
	<i>Picea sitchensis</i>	Linear	0.65	0.12
	<i>Pseudotsuga menziesii</i>	Exponential	0.24	0.33
	<i>Sequoia sempervirens</i>	Logarithmic	0.27	0.53
	<i>Thuja plicata</i>	Logarithmic	0.10	0.52
Mass-based uptake	All species	Logarithmic	0.52	0.02
	<i>Picea sitchensis</i>	Logarithmic	0.79	0.03
	<i>Pseudotsuga menziesii</i>	Logarithmic	0.08	0.71
	<i>Sequoia sempervirens</i>	Exponential	0.51	0.16
	<i>Thuja plicata</i>	Exponential	0.01	0.86
% Change in SWC	All species	Linear	0.03	0.24
	<i>Picea sitchensis</i>	Power	0.76	0.04
	<i>Pseudotsuga menziesii</i>	Power	0.38	0.26
	<i>Sequoia sempervirens</i>	Exponential	0.18	0.92
	<i>Thuja plicata</i>	Logarithmic	0.17	0.41

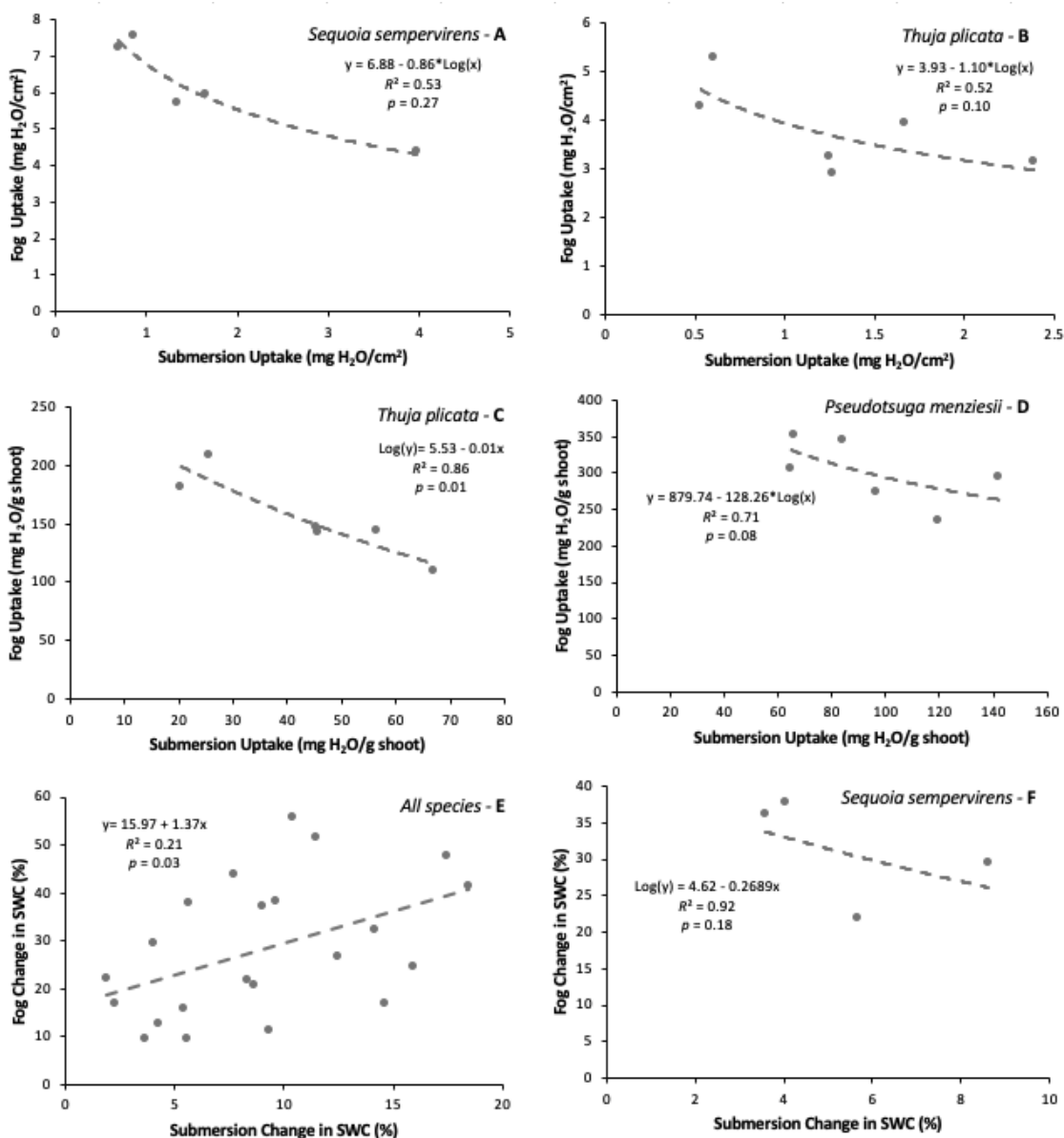


Figure 4. Regression relationships relating submersion-based estimates of foliar water uptake capacity to fog-based estimates for A) area-based uptake in *Sequoia sempervirens*, B) area-based uptake in *Thuja plicata*, C) mass-based uptake in *Thuja plicata*, D) mass-based uptake in *Pseudotsuga menziesii*, E) percent change in shoot water content (SWC) for all pooled species (*Picea sitchensis*, *Pseudotsuga menziesii*, *Sequoia sempervirens*, and *Thuja plicata*), and F) percent change in SWC in *Sequoia sempervirens*. These were the strongest six relationships found to relate these two methods.

## Hydraulic Redistribution and Drought

Due to limited sample sizes resulting from unforeseen seedling mortality in the mesocosms and destructive sampling techniques (for  $\Psi_{md}$  and xylem water  $\delta D$ ), it was not possible to statistically evaluate hydraulic redistribution capacity or physiological responses to drought and HR water in individual species. However, by pooling species as central chamber donors or adjacent chamber recipients, it was possible to evaluate HR capacity and physiology based on mesocosm position. Further, as explained in the methods section, due to filter paper failure and minimal evidence of mycorrhizal colonization, the mesocosms were not able to address the original research questions related to water exchange via CMNs. Thus, no distinction was made between recipient types (severed versus intact mycorrhizae); chambers and seedlings were simply considered recipients or donors.

Stable isotope signatures (Figure 5) and a two-source mixing model (Figure 6) showed that both PISI and SESE hydraulically lifted and redistributed water to recipient PSME and THPL, respectively. Within chambers, the proportion of HR water in soil water was generally comparable with seedling xylem water and varied minimally with depth (Figure 5). Seedlings in the Pinaceae mesocosms (PSME-PISI-PSME) generally had a higher proportion of HR water compared to seedlings in the Cupressaceae (THPL-SESE-THPL) mesocosms. In both mesocosm types, donor seedlings had a higher proportion of HR water compared to recipient seedlings. *Sequoia sempervirens* appeared to share greater proportions of HR water with its recipient seedlings (THPL), than PISI

did with PSME. Xylem water in donor PISI seedlings had a remarkably high proportion of HR water, essentially 100% ( $99 \pm 3\%$ ); xylem water in the corresponding PSME recipient seedlings had  $72 \pm 0\%$  HR water. Xylem water in SESE donor seedlings consisted of  $38 \pm 8\%$  HR water, and xylem water in THPL seedlings consisted of  $31 \pm 1\%$  HR water.

Confirming trends indicated via water stable isotopes, seedling physiology measurements also suggest that PISI and SESE were capable of hydraulically lifting water to share via diffusion through the soil with neighboring PSME and THPL seedlings, respectively, and that this HR water was able to sustain pre-drought physiologies during drought in donor and recipient seedlings. Pre-drought,  $\Psi_{\text{md}}$  was similar between donor and recipient seedlings, although it was slightly higher in donors compared to recipients, but this elevation was not significant ( $p = 0.35$ ) (Figure 7A,

). Post-drought,  $\Psi_{\text{md}}$  was again insignificantly higher in center donor seedlings compared to recipient seedlings ( $p = 0.20$ ). Comparing pre- and post-drought  $\Psi_{\text{md}}$ , no significant differences occurred in central donor seedlings ( $p = 0.16$ ) or in recipient seedlings ( $p = 0.43$ ). For  $g_s$ , there were no measurable differences between donors and recipients before the drought was initiated ( $p = 0.82$ ) (Figure 7B,

). Post-drought however, center donor seedlings had significantly elevated  $g_s$  values, about twice as high compared to the adjacent recipient seedlings ( $p = 0.02$ ). Comparing pre- and post-drought  $g_s$ , there were no significant differences in recipient seedlings ( $p = 0.10$ ), but in the center donor seedlings  $g_s$  was significantly higher post-drought compared to pre-drought ( $p = 0.0043$ ).



Table 6. One-way ANOVA table for the effect of mesocosm location (donor or recipient) on pre- and post-drought midday water potential ( $\Psi_{md}$ ) and stomatal conductance ( $g_s$ ) for all species pooled (*Picea sitchensis*, *Pseudotsuga menziesii*, *Sequoia sempervirens*, and *Thuja plicata*).

	Location	
Pre-drought $\Psi_{md}$	<i>df</i>	2
	<i>F</i>	1.96
	<i>p</i>	0.18
Post-drought $\Psi_{md}$	<i>df</i>	2
	<i>F</i>	3.62
	<i>p</i>	0.08
Pre-drought $g_s$	<i>df</i>	2
	<i>F</i>	0.062
	<i>p</i>	0.81
Post-drought $g_s$	<i>df</i>	2
	<i>F</i>	11.20
	<i>p</i>	0.0053

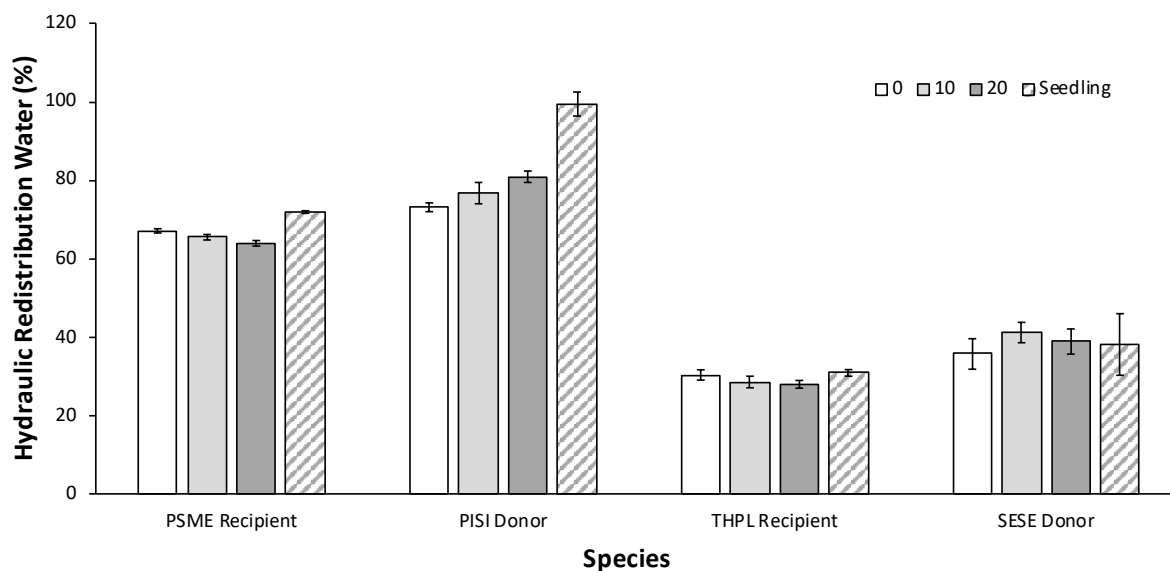


Figure 5. Mean ( $\pm$  SE) percent hydraulic redistribution water measured in soil water sampled from 0, 10, and 20 cm soil depths as well as in xylem water sampled from donor and recipient seedlings (PISI = *Picea sitchensis*, PSME = *Pseudotsuga menziesii*, SESE = *Sequoia sempervirens*, and THPL = *Thuja plicata*). Mesocosm types consisted of PISI donors paired with PSME recipients and SESE donors paired with THPL recipients.

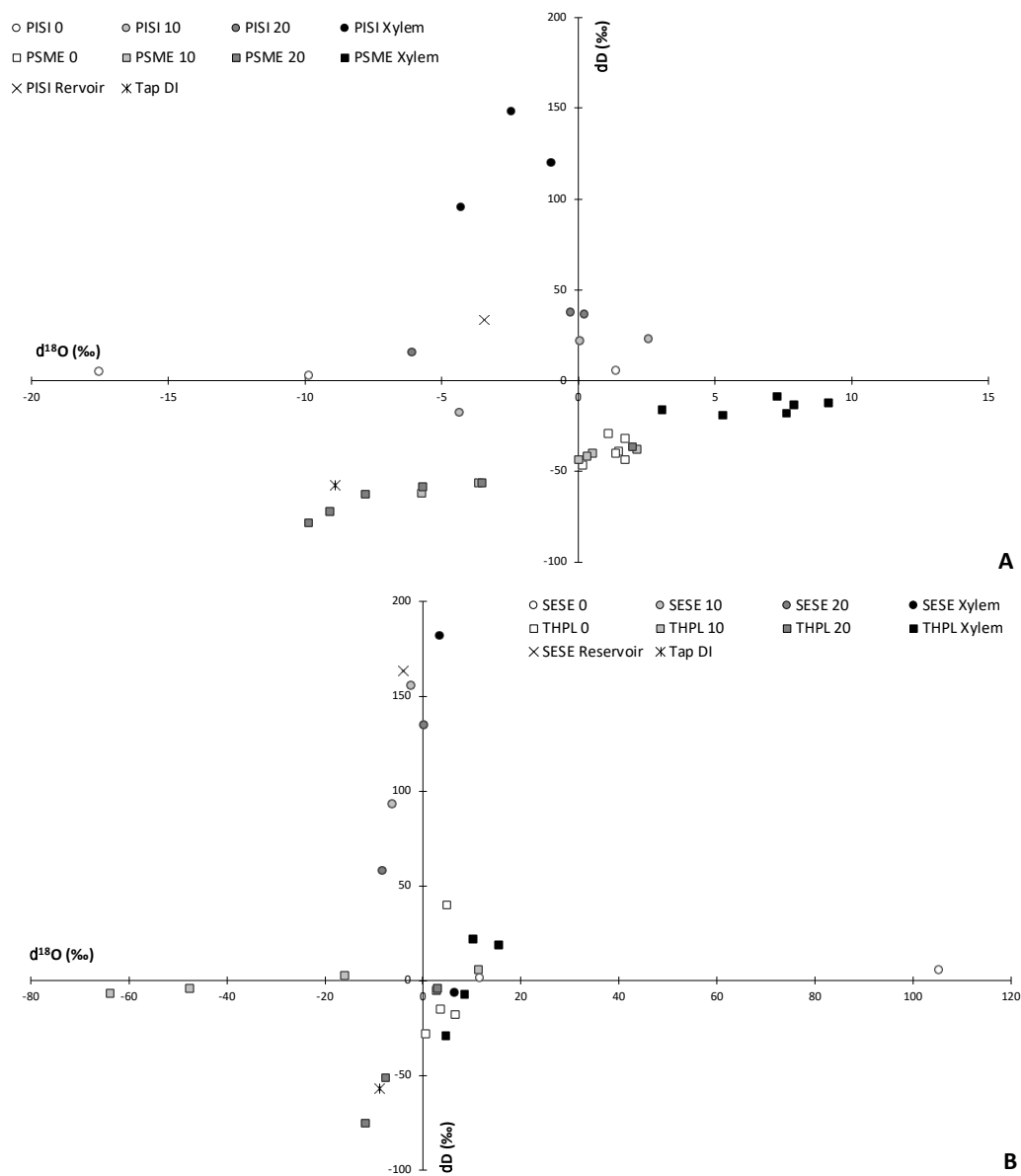


Figure 6. Stable isotope data for soil and seedling xylem water samples from mesocosms investigating hydraulic redistribution of water. A) Donor seedlings were PISI (*Picea sitchensis*) and recipient seedlings were PSME (*Pseudotsuga menziesii*). B) Donor seedlings were SESE (*Sequoia sempervirens*) and recipient seedlings were THPL (*Thuja plicata*). In each mesocosm chamber, soil water was sampled at 0, 10, and 20 cm depths. Isotopic signature of tap deionized (DI) water and water from donor reservoirs shown; tracer water added to reservoirs had  $\delta D$  485.93‰ and  $\delta^{18}O$  -8.90‰.

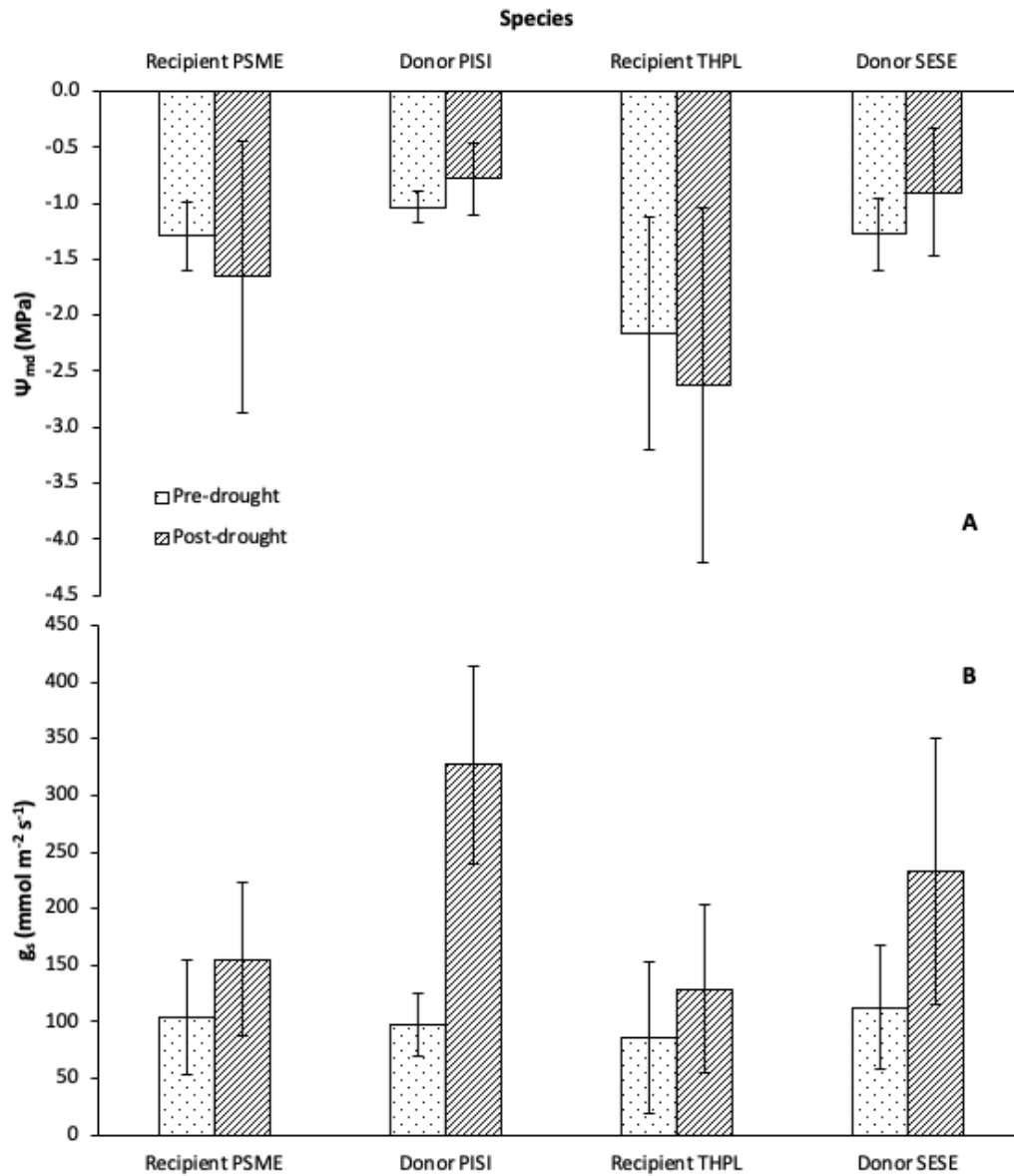


Figure 7. Mean ( $\pm$  SE) A) midday water potential ( $\Psi_{md}$ ) and B) stomatal conductance ( $g_s$ ) for donor and recipient seedlings during pre- and post-drought time periods. PISI = *Picea sitchensis*, PSME = *Pseudotsuga menziesii*, SESE = *Sequoia sempervirens*, and THPL = *Thuja plicata*). Post-drought  $g_s$  was significantly higher than pre-drought  $g_s$  in donor seedlings;  $\Psi_{md}$  did not differ significantly between positions or time periods and pre-drought  $g_s$  did not significantly differ between positions.

## DISCUSSION

Overall, this research was able to investigate study questions related to conifers' capacity for foliar water uptake, capacity for hydraulic redistribution, and capacity to use HR water to maintain physiology during drought. Due to unexpected seedling mortality in the mesocosm experiment, the investigation into if/how CMNs might aid the transfer of HR water between donor and recipient seedlings was not possible. Nevertheless, findings from this work provide novel information about foliar water uptake capacity in four western conifer species and how the evaluation of this capacity can vary among metrics and methods. Findings from this work also suggest that two western conifer species, PISI and SESE, are capable of hydraulically lifting water, sharing HR water with heterospecific recipient seedlings, and that all seedlings (donors and recipients) can physiologically benefit from HR water during drought.

### Foliar Uptake

This study's investigation comparing the submergence and fog chamber methods found that the submersion method yielded lower foliar water uptake values (for all measures of uptake) than the fog chamber method, suggesting that saturated vapor facilitates the greatest uptake. Despite foliage exposure times being the same for both methods, the lower uptake values for the submersion method suggest that complete submergence lowers uptake capacity and that certain uptake pathways (e.g., stomatal versus cuticular) may be more efficient than others. In contrast to this finding, another

study investigating Aleppo pine (*Pinus halepensis* P. Mill.) and sunflowers (*Helianthus annuus* L.) (Vaadia and Waisel 1963) found that water enters leaves more quickly when they are exposed to liquid compared to saturated vapor (i.e., fog). Nevertheless, findings from this study using four different conifer species consistently demonstrated that the submersion method can underestimate by about 3 times the actual foliar uptake capacities in realistic environments.

The submergence-based uptake rates measured in this study were consistently high compared to published rates using this same method. For comparison, the area- and SWC-based PSME and SESE submergence uptake rates measured in this study were approximately twice as high as rates measured by Limm et al. (2009). This difference suggests that uptake capacity may vary with geography, as the seedlings used in Limm et al. (2009) were sourced from the central portion of the SESE range while seedlings used in this study originated from a more northern portion of the SESE range. However, submergence area-based PISI uptake capacity measured in this study was again approximately twice as high as comparable rates measured by Kerhoulas et al. (2020) using seedlings sourced from the same seed zone. Speculatively, these high uptake measurements could relate to seedling water status, as dehydrated foliage has greater uptake rates than hydrated foliage (Burgess and Dawson 2004; Breshears et al. 2008), and it is possible that after 24 weeks in the greenhouse seedlings were somewhat water stressed despite attentive watering.

Among the four focal conifer species, this investigation found differences in foliar water uptake capacity. Consistently, THPL had the lowest foliar water uptake capacity,

suggesting that its unique leaf morphology with flattened sprays of scale-like leaves and conspicuous stomatal blooms may somehow limit water uptake (Figure 8). It is also possible that differences in the epidermal surface of THPL foliage somehow affect the boundary layer or decrease leaf water retention by increasing surface roughness (Berry et al. 2019). Additionally, cuticular differences among the four study species could help explain interspecific differences in uptake capacity, as cuticular pectins are associated with a range of uptake rates and capacities from fast but limited absorption to slow and abundant absorption, suggesting that these molecules can change cuticle porosity and hydrophobicity by affecting linkages and surfaces on the leaf (Boanares et al. 2018; Berry et al. 2019). Further, potentially the presence of an endodermis (a layer of thick-walled cells that surrounds transfusion tissue [xylem tracheids surrounding leaf veins] and separates it from the mesophyll) in Pinaceae (PISI and PSME) but not in Cupressaceae (SESE and THPL) (Chin and Sillett 2019) somehow supports greater uptake rates, although the mechanics of this relationship are unclear. Another possible explanation for the differences among species involves capacitance, as greater potential for foliar uptake is associated with increased capacitance (Berry et al. 2019), and increased capacitance is associated with greater wood density (McCulloh et al. 2014). PSME has the greatest wood density (Miles and Smith 2009) and capacitance (McCulloh et al. 2014) of the species studied in this experiment, while THPL has the lowest wood densities and capacitance values and PISI and SESE are mid-range. Thus, the uptake capacity among these four species ranks in the same sequence as their wood densities and capacitance potentials, suggesting that these three characteristics are related.



Figure 8. A close-up image of the stomatal bloom found on *Thuja plicata* foliage (Schlichter 2005).

Notably, the submersion method found that PISI had the highest foliar water uptake rates, particularly when evaluated on an area-basis. These high uptake rates could relate to the unique transfusion tissue present in PISI (Figure 9) that is not present in SESE or PSME (although the latter two species also have transfusion tissue). This unique transfusion tissue in PISI is made of distinct, parenchyma-free clusters of thin-walled tracheids that are substantially smaller than typical transfusion tracheids (Chin and Sillett 2017). Variability in transfusion tissue likely influences uptake capacity, as water travels more efficiently through tracheids than mesophyll cells (Oldham et al. 2010) and as cells in this tissue can collapse under water stress to protect leaves from damage and then later refill (Brodribb and Holbrook 2005; Ishii et al. 2014). Additionally, PISI leaves have

mesophyll plates that function like angiosperm bundle sheath extensions to divide the mesophyll into chambers (Chin and Sillett 2017). This compartmentalization likely provides a direct apoplastic route for water transport (Buckley et al. 2011), thereby enhancing outside-xylem water conductivity between vein and epidermis to enhance stomatal conductance (Chin and Sillett 2017) and foliar water uptake capacity.

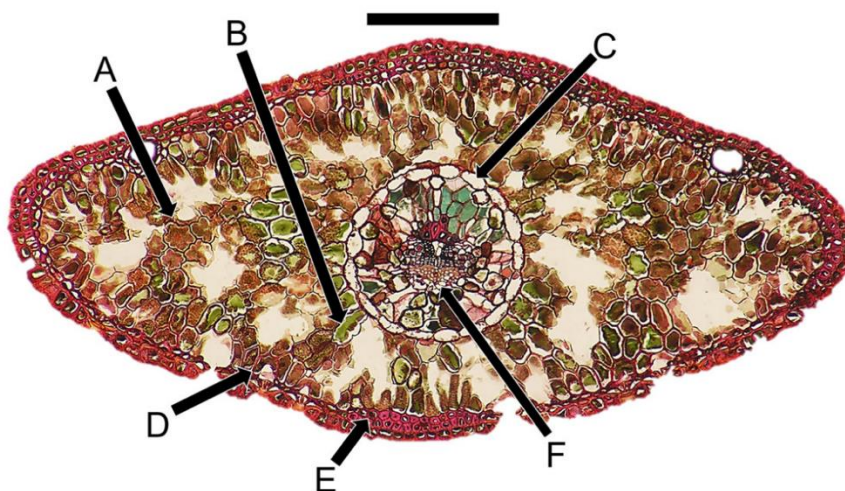


Figure 9. A fixed and stained leaf transverse section of a *Picea sitchensis* leaf from 95m above the ground, near the treetop shown with the adaxial-side down to reflect shoot orientation (Chin and Sillett 2017). The unique endodermis tissue is identified by label C.

Another notable trend was that the fog method measured the highest foliar water uptake capacity in PSME when evaluated on a mass-basis and as a change in SWC. This high capacity for foliar water uptake in PSME may result from abundant microscopic epiphyllic green algae enhancing water uptake (Chin and Sillett 2019) and/or a unique cuticular chemical composition such as more saccharide structures (Kerstiens 1996; Riederer 2006; Berry et al. 2019) that reduce resistance across this barrier. These high fog uptake rates in PSME suggest that this species may have an advantage over other species



due to a heightened ability to access and use fog water, which potentially increases water status, gas exchange, and productivity. *Sequoia sempervirens* may similarly experience increase in uptake capacity from the presence of beneficial microorganisms, such as endophytic fungi that live on the leaf surface and can directly penetrate into the leaf through stomata (Burgess and Dawson 2004). The interspecific differences in fog foliar uptake capacity suggest that PISI and THPL, with their comparatively low uptake rates, may be less affected by decreases in coastal fog frequency than SESE or PSME.

Since fog uptake capacities flux throughout the day and greatest uptake correlates with the timing and presence of fog within ecosystems (Berry et al. 2014), it is likely that foliar uptake capacities are greater than those measured in this experiment because measurements were conducted at midday and fog on the California coast typically occurs in the early morning hours, burning off by midday and early afternoon (Dawson 1998; Limm et al. 2009). The fact that across this study's four focal species, fog exposure increased SWC between approximately 15 and 45% indicates that this supplementary water is functionally important for these species. Thus, the 33% decline in fog frequency since the early 20th century along the Pacific coast of northern California (Johnstone and Dawson 2010) has the potential to adversely affect coniferous forests, particularly if coupled with a warming and drying climate (Cook et al. 2015; Diffenbaugh et al. 2015). Fog is a dominant climatic factor along the coast, and long-term reductions will likely continue to impact the water and carbon economy of redwoods and other coastal endemic species (Johnstone and Dawson 2010). With climate change, these important wetting events will likely continue to decrease, which will assuredly affect the coastal redwood

ecosystem (Limm et al. 2009) and possibly further restrict the native SESE distribution. Although, dendrochronological studies show a recent increase in SESE growth correlating with decreased fog frequency (Carroll et al. 2014), particularly in the northern portions of the SESE range, possibly indicating that if trees are not water stressed (as in the temperate rainforest of coastal northern California), fog can actually limit growth by reducing light availability. Thus, there are still many unknowns about the functional reliance of coastal conifers on fog water inputs and how climatic shifts in these inputs will influence temperate forests.

While these findings contribute to the understanding of foliar water uptake in these four species, there are still many unknowns concerning how plants supplement soil water with atmospheric water deposits. For example, it would be helpful to know more about the proportion of total water use that fog water contributes for a diverse collection of forest species and how foliar uptake capacities change across time (throughout the day, throughout a season, across a year, across leaf ages, and as a stand ages). The results of this study beg additional questions: Does fog water increase seedling survival? Does fog water uptake aid embolism reversal, especially in PSME (Woodruff et al. 2007)? And, does water uptake in tree crowns via bark and/or adventitious roots meaningfully influence tree water status and physiology?

### Hydraulic Redistribution and Drought

In addition to supplementing soil water with water absorbed directly via foliage, trees can also supplement plant-available soil water with hydraulically lifted and

redistributed water from deep in the soil profile. Soil and xylem water isotopic signatures from this study's mesocosm experiment confirmed that central donor seedlings of PISI and SESE were able to hydraulically lift deeper water and share this water with neighboring heterospecific recipient seedlings (PSME and THPL, respectively) through the soil. Unfortunately, due to the failure of the filter paper, no inferences can be made from this experiment about the role of CMNs in the sharing of water between donor and recipient seedlings. Nevertheless, this finding has important implications for the water cycle in mesic, conifer-dominated ecosystems where this phenomenon has received little attention. Hydraulically redistributed water has the potential to influence the rate of plant water use and might change the hydrological cycle of the entire forest (Jackson et al. 2000).

Physiologically, results indicate that HR water was able to buffer drought effects in PISI and SESE donor seedlings and PSME and THPL recipient seedlings. This buffering was evidenced by sustained  $\Psi_{\text{md}}$  and  $g_s$  in all seedlings between the pre- and post-drought time periods. This finding would be more powerful if there were control mesocosms without taproot access to water to confirm that without access to this water source, seedling  $\Psi_{\text{md}}$  and  $g_s$  decrease. However, because the original experiment was designed to investigate if/how HR water could influence drought physiology in donor and recipient seedlings, there were not mesocosms set up without access to a lower water reservoir. In any case,  $g_s$  curiously increased between the pre- and post-drought time periods, although this was only significant for the center donor seedlings. It is possible that the pre-drought conditions were less than ideal and drying out during the drought

period enhanced physiology. The lack of drought response in the adjacent recipient chambers coupled with the soil and xylem water isotopic signatures together suggest that the central seedling taproot drew water from the lower reservoir and that the water then left the donor seedling root system and travelled through the soil to the adjacent chambers and recipient seedlings.

Thus, isotopic and physiological evidence suggest that PISI and SESE are capable of hydraulically lifting water and sharing it with PSME and THPL neighbors, respectively. *Picea sitchensis* shared greater proportions of HR water with its recipient seedlings than *Sequoia sempervirens* did with its recipients. This may be due to differences in root structure between Pinaceae and Cupressaceae. It is possible that these potential differences may also play a role in Pinaceae's tendency to be primarily ectomycorrhizal and Cupressaceae's tendency to be primarily endomycorrhizal. However, there is a lack of comparative studies between the root anatomy of these two groups that might elucidate this difference. In other ecosystems, HR can provide weeks' worth of available water for neighboring plants during drought periods, effectively delaying the potential for mortality and increasing stand resistance to drought (Brooks et al. 2002). Results from this study suggest that similarly, HR water may provide a buffer for drought in mesic temperate forests for a diverse collection of species. This use of HR water could be particularly important for shallowly rooted understory plants (Dawson 1993) as well as establishing tree seedlings, and can therefore have ecosystem-wide effects by influencing plant survival, transpiration, and productivity. This confirmation that HR occurs in the temperate redwood forest highlights the need for further work in

this field to improve our understanding of hydrogeologic processes and nutrient cycling in this ecosystem.

Developing a greenhouse experiment exploring hydraulic redistribution via taproots and common mycorrhizal networks proved more challenging than expected. Several lessons could be learned that can improve future replications of this experimental design. An automatic watering system using drip irrigation is one method to ensure adequate and even moisture is delivered to each seedling. Ensuring temperatures and air movement in the greenhouse are maintained at an appropriate level for the entire duration of the experiment is also critical. Pests should be fully and completely eradicated as soon as they are discovered. “Blowout” is a condition indicating seedlings were shocked by their transplant and denotes imminent seedling death; this condition can be avoided by transplanting on cool, moist days when seedlings are fully hydrated. Certain amendments, such as a low N fertilizer and Superthrive, can be irrigated into the soil to help avoid this condition, though the use of fertilizer, specifically nitrogen, can impede the formation of mycorrhizal associations, so any fertilizer amendments should be used sparingly. Multiple inoculations help increase the chances of colonization as well. Lastly, roots have difficulty developing if they are completely submerged in water, so a medium that introduces aeration is necessary in the lower reservoir, such as a perlite and soil mixture.

## CONCLUSIONS

Western conifers have developed a variety of approaches to access water beyond just what is available in upper soil levels. Approaches include foliar uptake, hydraulic redistribution, and common mycorrhizal networks. *Picea sitchensis*, *Pseudotsuga menziesii*, *Sequoia sempervirens*, and *Thuja plicata* were assessed for their ability to absorb water via their foliage. Foliage had a greater capacity to absorb water when it was in fog vapor form versus when it was liquid and foliage was fully submerged. The submersion method underestimated uptake capacity by approximately three times. Although there were variations depending on the method and metric used, generally *Pseudotsuga menziesii* had the greatest capacity to uptake water via its foliage while *Thuja plicata* had the lowest. While further investigation is needed to more definitively tease out the mechanisms driving interspecific differences in uptake capacity, it is likely that variation in leaf morphology, epidermal surfaces, and anatomy could be responsible for observed differences between methods and among species. Additionally, wood density and capacitance could be driving the trends in uptake capacity measured in this study. In terms of accessing deeper water sources, *Picea sitchensis* and *Sequoia sempervirens* both appeared to hydraulically lift water and redistribute it to neighboring heterospecific (*Pseudotsuga menziesii* and *Thuja plicata*, respectively) trees, highlighting hydrologic complexities that could be further explored in the redwood forest ecosystem. Of particular interest, greater knowledge about the role of common mycorrhizal networks in redistributing water among species would greatly improve our understanding of water

movement through this forest type. These types of future investigations coupled with findings from this study will help scientists better understand and model water acquisition and usage in forests, allowing scientists and managers alike to be better prepared in the face of a changing climate.

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