NIGHTTIME WATER LOSS FROM C_3 PLANTS

by

AVA R. HOWARD

(Under the Direction of Lisa A. Donovan and Marc W. van Iersel)

ABSTRACT

Plants dominate many ecosystems and play a critical role in water cycling. Most plants have a C_3 photosynthetic pathway and water losses at night can include both transpiration (E_{night}) from the canopy and hydraulic redistribution (HR) from roots. The magnitude of water lost in these pathways is highly variable and a better understanding of the relationship between these processes and potential environmental drivers is needed. In greenhouse studies, we examined the effects of manipulating soil water and nitrogen availability on instantaneous measures of leaf conductance (g_{night}) and E_{night} in three focal species: *Populus angustifolia, P. balsamifera spp. trichocarpa* and wild *Helianthus annuus*. For all species g_{night} was at least five times greater than minimum leaf conductance, suggesting most g_{night} and E_{night} may be regulated. Based on known daytime responses to water limitation, we hypothesized that g_{night} and E_{night} would decrease when soil water availability was limited. Results from five studies and all three species supported this hypothesis. Based on the potential for transpiration to increase mass flow of mobile nutrients to roots, we hypothesized that g_{night} and E_{night} would increase under limiting soil nitrogen. Results from eight studies and all three species rejected this hypothesis. Nitrogen limitation sufficient to reduce biomass did not affect g_{night} or E_{night} when potentially confounding water stress was eliminated. Based on reductions of HR when transpiration is artificially increased by nighttime lighting, we hypothesized that naturally occurring E_{night} would reduce the magnitude of HR. We

tested this hypothesis in the greenhouse using *Artemisia tridentata*, *Helianthus anomalus* and *Quercus laevis*. Suppressing E_{night} with nighttime canopy bagging resulted in increased HR for *A. tridentata* and *H. anomalus* but not *Q. laevis*. Overall, our studies suggest that g_{night} and E_{night} vary in response to soil water but not nutrient availability and can affect the magnitude of HR. Variability in E_{night} should be incorporated into models of stand and ecosystem water flux. Increased understanding the variability in E_{night} should also be used to improve estimates of when and to what magnitude HR may play an important role in ecosystem hydrology.

INDEX WORDS: transpiration, conductance, C_3, nighttime, nocturnal, hydraulic redistribution, hydraulic lift, plant, soil water, soil nitrogen, *Helianthus*, *Populus*, *Artemisia*, *Quercus*

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AVA R. HOWARD

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by

AVA R. HOWARD

Major Professor: Lisa A. Donovan
Marc W. van Iersel

Committee: Chris Peterson
Ronald Hendrick
Kathrin Stanger-Hall

Electronic Version Approved

Maureen Grasso
Dean of the Graduate School
The University of Georgia
December 2008

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Plants play an essential role in cycling water though ecosystems (Chahine 1992). Leaves present an enormous surface area and water is constantly flowing across this surface from moist cells to the relatively dry air in a process known as transpiration. During the day the rate of transpiration (E_{day}) is high because stomata, pores in plant leaves, are normally maintained open (high daytime conductance; g_{day}) in order to allow CO_2 uptake for photosynthesis. Until recently it was widely accepted that at night most plants (C_3 and C_4) closed stomata in order to minimize water loss when photosynthesis is not occurring (Campbell and Reece 2005). However, over the last five years studies demonstrating substantial nighttime conductance (g_{night}) and transpiration (E_{night}) have accumulated (Musselman and Minnick 2000, Donovan et al. 2003, Bucci et al. 2005, Daley and Phillips 2006, Caird et al. 2007, Cavender-Bares et al. 2007, Dawson et al. 2007, Scholz et al. 2007, Seibt et al. 2007). Together these studies suggest that E_{night} is typically 5-15% of E_{day}. Continued water loss at night from plants has important implications for plant, ecosystem and catchment water balance studies (Dawson et al. 2007), plant water-use efficiency (Nemali unpublished) and uptake of pollutants into plants (Musselman and Minnick 2000, Seibt et al. 2007). Important methodological concerns also arise from E_{night} such as underestimation of soil water potential measured from plant predawn water potentials and underestimation of total transpiration by eddy covariance towers (Donovan et al. 2003, Fisher et al. 2007). The

commonality and implications of g_{night} and E_{night} underscores the importance of understanding the conditions that result in substantial nighttime water loss.

Most studies quantify nighttime water loss in natural populations and report correlations with environmental and other physiological variables (Benyon et al. 1999, Oren et al. 2001, Dawson et al. 2007, Kavanagh et al. 2007, Marks and Lechowicz 2007). These studies have demonstrated that g_{night} varies between species and within individuals across hours, nights, and seasons. Environmental factors leading to this variation have been suggested based mostly on correlations and a very few manipulative experiments. The research in this dissertation utilizes controlled studies to confirm field observations and test predictions while eliminating potentially confounding variables.

During the day, stomatal conductance is well known to be regulated with respect to changing soil water potential and atmospheric demand (Zeiger et al. 1987, Lambers et al. 1998, Nobel 2005). Declines in stomatal conductance occur in well-described patterns and allow plants to minimize use of available water for a given amount of CO_2 assimilated (Cowan 1977) and maintain soil-to-leaf hydraulic continuity (Sperry et al. 2002). However, little is known about how regulation may occur at night. Typical rates of E_{night} may not represent a substantial cost to plants when water is abundant in the environment, but could become a substantial cost when water becomes scarce. Research on natural populations suggests that g_{night} may be sensitive to seasonal changes in soil water availability (Donovan et. al. 2003, Grulke et al. 2004, Dawson et al. 2007). Greenhouse studies with wheat (Rawson and Clarke 1988) and live oaks (Cavender-Bares et al. 2007) also found g_{night} and E_{night} decreased in response to decreased soil water availability.

In chapter 2 and 3 we examine the effect of controlled water manipulations on g_{night} and E_{night} in *Helianthus annuus* and two *Populus* species. These species were chosen because they were amongst the first species to have substantial g_{night} and E_{night} documented under field conditions (Snyder et al. 2003). These species have a C_3 photosynthetic pathway and together allowed us to test our predictions in both an annual and trees. Chapter 2 and 3 include sustained and short-term soil water manipulations in order to determine whether changes in g_{night} and E_{night} under sustained limitations persist when plant leaves are produced under the same well-watered conditions. Our studies thereby teased apart changes in g_{night} from longer-term changes in leaf cuticle and stomatal size and density.

Variation in soil nutrient availability has also been suggested as a factor that may impact regulation of g_{night} and E_{night} (Snyder et al. 2003, Caird et al. 2007, Scholz et al. 2007). The Barber-Cushan model of root nutrient uptake predicts that increasing water flux to the rhizoplane by maintaining substantial E_{night} minimizes the formation of a nitrate depletion zone around plant roots (Barber and Cushman 1981, Barber 1995). If E_{night} increases nutrient acquisition then plants might benefit from the ability to regulate g_{night} in response to soil nutrient availability. However contradictory results have been reported regarding the effect of E_{night} on plant nitrogen uptake (McDonald et al. 2002, Snyder et al. 2008, but see Christman et al. *submitted*) and the response of g_{night} and E_{night} to soil nutrient availability (Ludwig et al. 2006, Christman et al. *submitted*, but see Scholz et al. 2007). One factor that may be contributing to the variable results is secondary water stress created by fertilization manipulations. The addition of fertilizer to soil adds ions that can lower the water potential of the soil solution. Also, sufficiently fertilized plants tend to grow larger than plants with limited nutrients. The larger plants transpire more water each day, which can result in reduced soil water availability. Chapter 2 and 3 determined

if nutrient availability directly affects g_{night} and E_{night} with carefully controlled experiments that eliminated potentially confounding secondary affects on plant water status.

To fully understand the impact environmental conditions may have on g_{night} and E_{night} it is necessary to understand what portion of g_{night} and E_{night} may be regulated. Observations of E_{night} have focused on total nighttime water loss, using methods of sap flux, leaf-level instantaneous gas exchange and weighing. These measures include water loss both through stomatal pores and through the remaining leaf surface or cuticle. Cuticular water loss is small compared to that through open stomata and is traditionally ignored in daytime measures. However, at night when stomata are partially closed and transpiration is typically only 5-15% of that during the day (Caird et al. 2007), a substantial portion g_{night} and E_{night} could be accounted for by cuticular losses or a physical limitation of stomata to close further. Water loss though the cuticle and through stomata after maximal possible closure is not subject to plant regulation and together can be considered minimal leaf conductance (g_{min}). Thus, to further understand the extent to which E_{night} is regulated, Chapter 2 and 3 quantify the proportion of naturally occurring g_{night} that is above g_{min}.

Before the commonality of g_{night} and E_{night} were appreciated it was well documented that at night plants can facilitate translocation of water in soil. Water can move into roots in wet soil, travel though the root system and leak back out into dryer soil. This passive process is called hydraulic redistribution (HR) and may occur when canopy water demand is low and a soil moisture gradient exists across the root system of a plant (Caldwell et al. 1998). HR provides several benefits to plants including reducing surface soil evaporative loss (when direction of redistribution is downward), increasing next-day transpiration, prolonging fine root lifespan, increasing microbial and mycorrhizal activity in the rhizosphere and overall improving carbon,

water and nutrient acquisition (Caldwell et al. 1998, Querejeta et al. 2003, Domec et al. 2004, Lee et al. 2005, Bauerle et al. 2008, Scott et al. 2008, Aanderud and Richards *submitted*).

Recently it has also been shown that HR and E_{night} can occur during the same night in the field (Donovan et al. 2003) and several authors have suggested that naturally occurring E_{night} may limit HR (Hultine et al. 2003, Caird et al. 2007, Dawson et al. 2007). Such a relationship between E_{night} and HR is supported by published studies that show that when "E_{night}" is experimentally increased by nighttime lighting, HR is diminished (Caldwell and Richards 1989, Caldwell 1990, Bauerle et al. 2008). However, nighttime lighting likely has other unintended impacts on plant and stomatal physiology. Chapter 4 provides the first controlled test of the impact of naturally occurring E_{night} on the magnitude of HR. Three species were used in Chapter 4: *Artemisia tridentata, Quercus laevis* and *Helianthus anomalus*. These species were chosen because substantial E_{night} and/or HR have been documented for these species in their native habitats (Caldwell et al. 1998, Espeleta et al. 2004, Caird et al. 2007). Additionally these species are native to habitats in which the necessary environmental conditions for E_{night} (dry nighttime air) and HR (substantial soil moisture gradient) often exist concurrently.

The chapters of this dissertation provide an essential base-line for understanding regulation of E_{night}, its biological significance and the environmental factors driving variation in its magnitude. The research presented here has important implications for improving estimates of ecosystem water flux and evapotranspiration inputs to weather models and accurately measuring plant water-use-efficiency and productivity.

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CHAPTER 2

HELIANTHUS NIGHTTIME CONDUCTANCE AND TRANSPIRATION RESPOND TO SOIL WATER BUT NOT NUTRIENT AVAILABILITY[1]

[1] Howard, A.R. and L.A. Donovan. 2007. *Plant Physiology*. 143:145-155.

ABSTRACT

We investigated the response of *Helianthus* species nighttime conductance (g_{night}) and transpiration (E_{night}) to soil nutrient and water limitations in nine greenhouse studies. The studies primarily used wild *Helianthus annuus,* but also included a commercial and early domesticate of *H. annuus,* and three additional wild species *(Helianthus petiolaris* Nutt.*, Helianthus deserticola* Heiser*,* and *Helianthus anomalus* Blake*).* Well-watered plants of all species showed substantial g_{night} (0.023-0.225 mol m^{-2} s^{-1}) and E_{night} (0.29-2.46 mmol m^{-2} s^{-1}) measured as instantaneous gas exchange. Based on the potential for transpiration to increase mass flow of mobile nutrients to roots, we hypothesized that g_{night} and E_{night} would increase under limiting soil nutrients, but found no evidence of responses in all six studies testing this. Based on known daytime responses to water limitation, we hypothesized that g_{night} and E_{night} would decrease when soil water availability was limited, and results from all four studies testing this supported our hypothesis. We also established that stomatal conductance at night was on average five times greater than cuticular conductance. Additionally, g_{night} and E_{night} varied nocturnally and across plant reproductive stages while remaining relatively constant as leaves aged. Our results further the ability to predict conditions under which nighttime water loss will be biologically significant and demonstrate that for *Helianthus*, g_{night} can be regulated.

INTRODUCTION

It is widely accepted that plants regulate stomatal aperture both to minimize water loss for a given amount of carbon assimilated and to minimize xylem cavitation (Cowan 1977, Sperry 2000). C_3 and C_4 plants fix carbon during the day and lose water from leaves as an unavoidable cost of getting CO_2 to the site of carboxylation. Although these plants are generally expected to close their stomata at night to conserve water when carbon gain is not occurring, significant

nighttime leaf conductance (g_{night}) and transpiration (E_{night}) have been observed in many C_3 species across a wide range of habitats (for review, see Musselman and Minnick 2000, Caird et al. 2007). Reported rates for g_{night} typically range from 0.01 to 0.25 mol m^{-2} s^{-1} and can represent greater than 50% of daytime conductance (g_{day}). E_{night} depends on both g_{night} and leaf-to-air vapor pressure deficit (VPD_l), but is usually 5% to 15% of daytime transpiration (E_{day}). To date, most studies document the magnitude of g_{night} and E_{night} and several have correlated these traits with environmental or physiological variables (Benyon 1999, Oren et al. 2001, Kavanagh et al. 2007). However, there have been few manipulative experiments that individually test the effect of environmental factors on the regulation of stomata at night.

Several researchers have speculated that nighttime water loss could enhance nutrient uptake by increasing mass flow of soluble nutrients to plant roots (Snyder et al. 2003, Daley and Phillips 2006, Caird et al. 2007). The Barber-Cushman model predicts that increasing water flux to the rhizoplane minimizes or eliminates the formation of a nitrate depletion zone around plant roots when conditions are appropriate for E_{night} (Barber and Cushman 1981, Barber 1995). Empirically, McDonald et al. (2002) demonstrated a benefit of increased transpiration on nitrate delivery and uptake by *Populus* plants. Although the Tanner and Beevers (2001) study is sometimes cited as contrary evidence, it dealt only with effects of transpiration on long-distance nitrogen transport within the xylem, not with mass flow delivery to roots. Thus, increased nutrient acquisition may represent a benefit that counters the cost of water loss at night. If nighttime water loss increases nutrient acquisition, then plants may benefit from the ability to regulate g_{night} in response to nutrient conditions. The effects of nitrate availability on g_{day} and E_{day} have been investigated and are variable (Chapin 1990, Fredeen et al. 1991, Ciompi et al. 1996, Cechin and Fumis 2004). Potential regulatory pathways are still being debated (Dodd et

al. 2003, Sakakibara et al. 2006). Two recent field studies with nutrient addition treatments found that g_{night} declined in response to nutrient additions (Ludwig et al. 2006, Scholz et al. 2007). However, the experimental designs of these studies did not permit direct effects due to reduced plant demand for nutrient acquisition regulating g_{night} to be separated from indirect effects of plant size or water status. More studies are needed that experimentally manipulate soil nutrient availability and test its effect on g_{night} and E_{night}, independent of confounding variation in soil and plant water potential.

During the day, stomatal conductance is regulated with respect to changing soil water potential and atmospheric demand to minimize use of available water during CO_2 uptake and maintain soil-to-leaf hydraulic continuity (Sperry et al. 2002). To further optimize use of limited soil water, regulation may also occur at night, reducing g_{night} and consequently E_{night}. This expectation held true for droughted wheat plants, where g_{night} decreased as compared to well-watered controls (Rawson and Clarke 1988). However, variable results have been obtained from studies that manipulated soil water potential with salt addition (Donovan et al. 1999) or through irrigation in the field (Donovan et al. 2003). At this time generalization about the effect of soil water availability on g_{night} and E_{night} is not possible, and further examination in controlled experiments is needed.

The magnitude of g_{night} and E_{night} may also vary temporally as leaves age or across plant reproductive stage (e.g. pre-reproductive, reproductive). Field studies have shown that small juvenile plants have higher g_{day} and E_{day} and lower water use efficiency than larger adults (Donovan and Ehleringer 1991, 1992). Leaf age has been shown to cause a decline in g_{day} in sunflowers (*Helianthus annuus;* Cechin and Fumis 2004). Similar to these daytime responses, Grulke et al. (2004) found higher g_{night} in large saplings than in mature trees, and Blom-Zandstra

et al. (1995) found g_{night} of rose leaves declined as leaves aged from three to six weeks. However, in both of these cases, direct effects of reproductive stage and leaf age cannot be differentiated from additional variables such as size and age. Controlled studies are needed to accurately assess the role of plant reproductive stage and leaf age on g_{night}.

Most measures of plant water loss include loss across both the cuticular and stomatal pathways operating in parallel. Because cuticular conductance ($g_{cuticular}$) is very small compared to daytime conductance through open stomata ($g_{stomata}$), its contribution to g_{day} has traditionally been ignored. However, when considering much lower magnitude g_{night} and E_{night}, cuticular losses may represent a substantial portion of the total measurement. Estimates of $g_{cuticular}$, ranging from 0.004 to 0.016 mol m^{-2} s^{-1}, have been derived from gas exchange measurements of intact leaves where stomatal closure has been induced by either leaf wilting (water stress) or exogenous abscisic acid (ABA) application (Rawson and Clarke 1988, Kerstiens 1995, Boyer et al. 1997, Burghardt and Riederer 2003, Nobel 2005). These estimates include water loss through the cuticle and maximally closed stomata, and thus represent a functional definition of $g_{cuticular}$. New techniques are available for estimating conductance and permeability of the cuticle separate from the stomatal pores, and they highlight the potential for variability in cuticular permeability (Schreiber et al. 2001, Santrueck et al. 2004, Kersteins 2006). However, it is still useful to measure water loss occurring though the cuticle plus stomata at maximal closure, because this represents a baseline that is not subject to short-term stomatal regulation.

We examined g_{night} and E_{night} in controlled greenhouse studies using wild *H. annuus*, *H. annuus* domesticates (commercial cultivar and Hopi domesticate), and a group of closely related wild species (*Helianthus anomalus* Blake, *Helianthus deserticola* Heiser and *Helianthus petiolaris* Nutt.). Substantial g_{night} (0.08-0.10 mol m^{-2} s^{-1}) has been reported for *H. annuus* and

H. anomalus in their native habitats (Snyder et al. 2003, Ludwig et al. 2006). The inclusion of several species allowed us to assess whether results for regulation of g_{night} and E_{night} can be generalized across closely related species. As large annuals, the *Helianthus* species were easily grown in the greenhouse, allowing experimental manipulation of soil treatments under controlled environmental conditions. This allowed for robust tests of environmentally stimulated regulation and nighttime water loss at different phases of maturity.

Our objective was to investigate issues of regulation and variation in g_{night} and E_{night}. Specifically, we addressed three questions: Are g_{night} and E_{night} regulated in response to soil nutrient and water availability? Under optimal soil conditions, do g_{night} and E_{night} vary nocturnally (within a night) and across leaf lifespan and plant reproductive stage? Finally, is g_{night} substantially larger than $g_{cuticular}$ when the latter is defined functionally as conductance though the cuticle and maximally closed stomata?

MATERIALS AND METHODS

The objectives were addressed in nine greenhouse studies carried out at the Biological Sciences Plant Growth Facility at the University of Georgia, Athens (Table 2.1). The studies included four wild annual *Helianthus* species (*Helianthus annuus, Helianthus anomalus* Blake, *Helianthus deserticola* Heiser and *Helianthus petiolaris* Nutt.), commercial *H. annuus* cv. Gray Stripe (referred to as *H. annuus* domesticate) and the Hopi domesticate of *H. annuus* (referred to as *H. annuus* Hopi). Achenes of the four wild *Helianthus* species were collected in Juab County, UT, except for the *H. annuus* from Keith Country, NE, used in the Fall 2003-2 and Fall 2004-2 studies, and the *H. petiolaris* collected in Washington County, UT. The achenes of *H. annuus* domesticate used in Fall 2003-2 and Spring 2006 studies were obtained from Carolina Biological. The achenes of *H. annuus* Hopi (PI 432504 NPGS Accession) used in Fall 2004-2

study were originally collected from Shungopovi Village, Hopi Indian Reservation, Navajo County, AZ.

The wild *Helianthus* species and the *H. annuus* Hopi achenes were germinated in Petri dishes and transferred to pots after the seedlings developed root hairs. The *H. annuus* domesticate achenes were sown directly into the study pots. The study pots (20-25 cm diameter) contained a mix of sand and Turface (fritted clay, Profile Products), except for the Fall 2003-1 and Fall 2003-2 studies that used all sand. All plants were grown in a greenhouse with natural daylight supplemented to 12 to 14 h with metal-halide lamps. Temperatures were generally set to be at or above 26ºC (day) and 16ºC (night). For the six studies that had greenhouse weather available for the growth interval (Fall 2004-1, Fall 2004-2, Spring 2005, Summer 2005, Fall 2005-1, and Spring 2006), the average night VPD_a and day VPD_a across studies (n=6) was 0.88 (SE=0.11) and 1.57 (SE=0.10) kPa, respectively.

Nutrient and water treatments

Nutrient treatments manipulated either total macro- and micronutrients (slow-release fertilizer, Osmocote Plus, Scotts-Sierra Horticultural Products) or manipulated just nitrogen (available only as nitrate). The latter was achieved with thrice weekly applications of a modified Hoagland solution containing 140 or 7 μg mL^{-1} nitrogen as nitrate. The sufficient and limited nitrate Hoagland solutions contained equal amounts potassium (176 μg mL^{-1} K) and phosphorus (31 μg mL^{-1} P). Additional macronutrients were calcium (50 μg mL^{-1} in high; 10 μg mL^{-1} in low), sulfur (8 μg mL^{-1} in high; 120 μg mL^{-1} in low) and magnesium (55 μg mL^{-1} in high; 6 μg mL^{-1} in low). Micronutrients included: Cl (0.443 μg mL^{-1}), B (0.068 μg mL^{-1}), Mn (0.027 μg mL^{-1}), Zn (0.033 μg mL^{-1}), Cu (0.008 μg mL^{-1}), Mo (0.012 μg mL^{-1}) and Fe (0.698 μg mL^{-1} as FeEDTA). In the three studies without a nutrient treatment, the plants either received the high

nitrate Hoagland solution or weekly application of 20:10:20 NPK soluble fertilizer (Peter's Peat-Lite Special, Scotts-Sierra Horticultural Products).

The soil water treatments consisted of supplying plants with ample water to maintain soils near field capacity (sufficient), and limiting the soil water availability (limited) either just prior to gas exchange measures or as a sustained treatment throughout the study. The limitation of soil water availability prior to gas exchange measures consisted of withholding water until visual wilting and depression of daytime gas exchange rates were achieved. The sustained water limitation in the Fall 2003-2 study consisted of watering every 4 to 5 d, beginning 2 weeks after germination. For the Fall 2005-2 study, leaf predawn xylem pressure potentials were sampled to accompany gas exchange measurements using a pressure chamber (Soil Moisture Equipment).

Gas exchange procedures

Leaf level measurements of daytime and nighttime gas exchange were made with a portable photosynthesis system (LI-6400, LI-COR). Measurements were made on a young fully expanded leaf of each plant, except when testing leaf age effects in the Spring 2005 study. The chamber light level was set to be 0 or 2000 μmol m^{-2} s^{-1} during the night and day, respectively. To view equipment and plants at night, we used green safety headlamps with intensity not detectable by an LI-190 sensor (0 μmol m^{-2} s^{-1} photosynthetic photon flux density; LI-COR) to avoid promoting stomatal opening. During the Fall 2004-1 study and part of the Spring 2005 study, leaves of some species were too small for the standard chamber, and an Arabidopsis (6400-15, LI-COR) chamber was used. This chamber lacks an internal light source, and daytime measurements were therefore only taken on sunny days when photosynthetically active radiation exceeded 1500 μmol m^{-2} s^{-1}.

For both chambers, air temperature was set to ambient, and CO_2 was supplied at 400 μmol mol^{-1}. Flow was set to 125 to 200 μmol s^{-1} at night and 700 μmol s^{-1} during the day. Chamber fan speed was set to high. To partially compensate for removal of the boundary layer due to the chamber mixing fan, chamber relative humidity was manually manipulated to a target 5% to 10% above ambient (assessed with open chamber). The standard chamber directly measures leaf temperature, and before every set of measurements, the leaf thermocouple was checked to ensure it was reading accurately to within 0.1 ^{o}C. Sample and reference infrared gas analysers were matched prior to each plant for nighttime measurements. Measurements were also made with an empty chamber or with dry paper in the chamber every four to six leaf measures to assess instrument error. Averaged by study, estimates of instrument error obtained with the standard or Arabidopsis chamber at night yielded values for g from 0.001 to 0.016 mol m^{-2} s^{-1}, which was always substantially lower than plant measures. Plant measures were logged when readings were stable and typically within 1-2 minutes of clamping onto the leaf.

Whenever possible, leaves were chosen that would fill the leaf chamber (6 cm^2 for standard chamber; 0.8 cm^2 for Arabidopsis chamber). When leaves that did not fill the chamber were used, all leaves in the measurement set (including those that filled the chamber) were marked before removal from the chamber to indicate placement of the chamber gaskets. The following day, gas exchange leaves were cut to remove all area that was not inside the chamber and scanned (Winfolia, Regent Instruments) to determine area. Leaves that did not fill the chamber were used in the Arabidopsis chamber in Fall 2004-1 (minimum area 0.45 cm^2) and in the standard chamber in Fall 2005-1 (minimum area 4.5 cm^2).

Daytime measurements were typically made between 9 am and 2 pm and nighttime measurements were typically made between 1 am and the beginning of astronomical twilight

(sun 12^0 below the horizon). Measures made at three times spaced though the night confirmed that this period captured maximum g_{night} but was well before a predawn stomatal opening would occur.

In the Fall 2005-2 study, nighttime water loss was measured both instantaneously using the LI-6400 as well as gravimetrically. Gravimetric measures of transpiration made over a 24-h time span were achieved by sealing the pot and root system in a bag, bagging all flower heads and weighing at the beginning and end of the day and night periods. To obtain water loss per area, all leaves were harvested the following day, and total leaf area was measured using a LI-3100 leaf area meter (LI-COR).

Assessment of cuticular water loss

$g_{cuticular}$ was defined functionally as conductance through the cuticle and stomata at maximum closure induced by either leaf wilting (water stress) or exogenous ABA application. As such, it includes both water loss through the cuticle and water loss through stomata at minimum aperture. The conductance provided by the LI-6400 (g_{night} or g_{day} in this study) is a total of both $g_{cuticular}$ and $g_{stomata}$ in parallel. Stomatal conductance at night was calculated as g_{night} minus $g_{cuticular}$ (Nobel 2005).

Cuticular water loss for excised, wilted leaves, was estimated both by weighing (Rawson and Clarke 1988) and by gas exchange measurements with the LI-6400. For weighing, excised leaves (cut end of petiole sealed with wax) were allowed to dry and wilt in the dark at ambient room temperature and VPD. Weights were taken approximately every 15 min, and, after initial rapid loss of water during which time stomata presumably closed, the linear relationship of water loss and time was used to estimate $E_{cuticular}$. During this period of linear water loss, $g_{cuticular}$ and $E_{cuticular}$ were also measured with the LI-6400 set to match ambient temperature and VPD. In the

Fall 2004-1 study, $E_{cuticular}$ from these methods, including all four species of wild *Helianthus*, were highly correlated (r^2= 0.939, P<0.0001, n=24). Thus, only the instantaneous LI-6400 measurements of $g_{cuticular}$ and $E_{cuticular}$ are reported.

$g_{cuticular}$ and $E_{cuticular}$ were also measured on leaves for which stomatal closure had been induced by exogenous ABA application. ABA was fed into the xylem sap of sufficiently-watered plants (Borel et al. 2001). Funnels were sealed around the stems of treatment plants and filled at predawn with degassed ABA solution (1.6 mol m^{-3} synthetic (±) ABA, 0.4 mol m^{-3} $Ca(NO_3)_2$ and 2.0 mol m^{-3} KH_2PO_4). Stems were drilled radially below the surface of the solution. Funnels were covered with silver foil and the solution was topped off as needed during the following day and night to ensure the drill hole was always below the surface of the solution. Plants were watered amply throughout the period of experimentation and gas exchange measures were made on the first leaf above the infusion point.

Leaf tissue analysis

In most of the nutrient treatment studies, leaves used for gas exchange were collected after measurement, dried, ground, and analyzed for nitrogen content (Carbo Era NA 1500 CN analyzer). When a factorial design of water and nutrient treatments was present, only the plants in the high water treatment were analyzed for leaf nitrogen. In the Fall 2004-1 study, gas exchange measurements were made on two dates per plant and these two leaves were combined for analysis of nitrogen content.

Biomass measures

Plants were generally harvested after reaching reproductive maturity and when plants began to show shoot senescence. Plants in the Fall 2003-2, Fall 2005-2 and younger age classes

in Fall 2005-1 studies were harvested before or shortly after the appearance of first flower. Plant shoots were divided into vegetative and reproductive components, dried at 60°C, and weighed.

Experimental design and statistical analysis

Experiments were either complete randomized block designs or completely randomized (Table 2.1). When gas exchange measurements were made across several days and nights, plants were grouped by block so that random effects due to night of measurement (e.g. VPD_a) were accounted for by the block effect. Measurements of different species or treatments made in one night and block were randomized to avoid confounding treatment results with effects of circadian rhythm or changing VPD though the night.

Most data were analyzed using a mixed-model ANOVA, with block treated as a random effect (PROC MIXED; SAS Institute, 2004, version 9.1) or with a general linear model ANOVA when blocking was not present (Fall 2005-2 study; PROC GLM; SAS Institute, 2004, version 9.1). In some cases, plant death, outliers, or difficulties with treatment application (e.g. ABA application, Spring 2006) resulted in an unbalanced design. When additional tests only involved two levels of a single variable, paired or independent *t* tests were used as appropriate. The Fall 2004-1 and Fall 2005-2 studies included repeated gas exchange measures during a 24-h period, and these data were analyzed in a repeated-measurement mixed model in PROC MIXED with an unstructured covariance matrix. In all analyses, variables were log transformed when necessary to approach model assumptions of normality of residuals and homogeneity of variance.

RESULTS

In all nine greenhouse studies (summarized in Table 2.1, 2.2), the four species of wild *Helianthus* plus domesticated *H. annuus* and *H. annuus* Hopi all showed substantial loss of water at night. For sufficiently watered plants, g_{night} averaged 0.098 mol m^{-2} s^{-1} (range, 0.023-0.225)

and E_{night} averaged 1.19 mmol m^{-2} s^{-1} (range, 0.29-2.46). Where available, g_{day} averaged 0.893 mol m^{-2} s^{-1} and E_{day} averaged 15.60 mmol m^{-2} s^{-1}. VPD_l for the gas exchange measurements averaged 1.30 kPa at night and 2.14 kPa during the day.

Response of g_{night} and E_{night} to soil nutrient and water manipulation

Six studies applied a soil nutrient treatment, four of which only manipulated soil nitrate (Table 2.1). There was no effect of nutrient limitation on g_{night} and E_{night} in any of these studies of *Helianthus* species (Figure 2.1, Table 2.2; $P>0.05$ for all). The nutrient limitation was substantial enough to significantly reduce vegetative shoot biomass in all six studies (Table 2.3) and reproductive biomass in the studies where plant growth continued into the reproductive stage (Fall 2003-1 micro- and macronutrient manipulation, $P<0.05$ for all species except *H. deserticola*; Fall 2004-1, Spring 2005, Summer 2005 nitrogen manipulation, $P<0.001$; data not shown). Leaf total nitrogen content was also measured in four of the six nutrient manipulation studies. The limited nitrate treatment imposed as a modified Hoagland solution resulted in lower leaf nitrogen content (Table 2.3). Leaf nitrogen was measured in only one study involving total macro- and micronutrient manipulation, and here the limited treatment resulted in significantly lower leaf nitrogen concentrations for *H. annuus* but not for *H. anomalus* or *H. petiolaris*.

In one of the nutrient limitation studies, Fall 2004-1, differences between wild *Helianthus* species were tested. A significant species effect was found (g_{night}, F-$statistic_{3,51}=3.08$, $P<0.05$; E_{night}, $F_{3,51}=3.03$, $P<0.05$), but a means separation test with Tukey's honestly significant difference showed differences to be minimal and only significant between *H. deserticola*, with the highest mean g_{night} and E_{night}, and *H. petiolaris* with the lowest ($P<0.05$).

Four studies applied soil water treatments (Table 2.1): sufficient (maintained near field capacity) and limited. Plants with limited water showed substantially reduced $g_{night,}$ E_{night}

($P<0.001$), g_{day}, E_{day}, and photosynthesis ($P<0.05$-0.001; Figure 2.2, Table 2.2). In the Fall 2004-2 study, g_{night} and E_{night} were assessed in both wild *H. annuus* and *H. annuus* Hopi, but there was no interaction between accession and response to soil water limitation for these traits ($P>0.05$). During Fall 2005-2, xylem pressure potentials were measured at three points though the night and were consistently and substantially lower in the water-limited *H. annuus* ($F_{1,14}=30.82$, $P<0.001$; Figure 2.3).

Variation in g_{night} and E_{night} nocturnally and across leaf lifespan and plant reproductive stages

A 24-h time course was measured for *H. annuus* in Fall 2005-2. g_{day}, E_{day} and photosynthesis showed typical patterns, increasing rapidly in the morning and declining during the afternoon. g_{night} and E_{night}, though low compared to daytime rates, increased through the night in the sufficiently-watered plants despite a small increase in atmospheric VPD (VPD_a) though the night (Figure 2.3; time effect for g_{night} and E_{night}, respectively, $F_{2,11}=31.2$, $P<0.001$; $F_{2,11}=32.37$, $P<0.001$). In addition to instantaneous gas exchange measures, gravimetric measures were used to estimate total E_{night} and total E_{day} during the same time period. E_{night} of sufficiently watered plants was 0.86 (SE=0.10) for instantaneous gas exchange and 0.22 (SE=0.01) mmol m^{-2} s^{-1} for gravimetric measures. These rates were 5.7% and 6.5%, respectively, of the daytime rates measured by the same methods. Measures of E_{night} and E_{day} made with instantaneous and gravimetric methods were correlated (E_{night} $r^2=0.78$, $P<0.001$, and E_{day} $r^2=0.87$, $P<0.001$; Spearman rank correlations). During this same night and day period, average VPD_a in the greenhouse was 0.6 kPa (SE=0.02) and 1.5 kPa (SE=0.12), respectively.

Repeated measures of g_{night} and E_{night} were also made on sufficiently-watered *H. annuus* in the Fall 2004-1 study and showed similar trends to those documented in 2005 (Figure 2.3).

g_{night} and E_{night} increased through the night (time effect, respectively: $F_{2,29}$=145.84, P<0.001; $F_{2,29}$=358.69, P<0.001) despite increasing VPD_a, and these trends were not affected by nitrate treatment (P>0.5).

The effect of leaf aging on g_{night} and E_{night} was initially assessed in the Spring 2005 study. Repeated measures of g_{night} and E_{night} were made on the same leaves of *H. annuus* across four weeks, starting when leaves were recently fully expanded. Start date for the four-week measurement sets were staggered across several weeks and used in the analysis to account for random environmental variation between nights. There was no decline in g_{night} or E_{night} due to leaf aging ($F_{1,321}$=0.83, P>0.3; $F_{1,321}$=0.57, P>0.4, respectively; Table 2.2). In the Fall 2005-1 study, leaf age effects were further assessed by comparing a young fully mature and older fully mature leaf of the same plant using sufficient nitrate treatment, 10-week-old plants. Here again, g_{night} and E_{night} did not differ with leaf age (t_{14}=1.21, P=0.2; t_{14}=1.22, P=0.2, respectively).

The effect of plant reproductive stage on g_{night} and E_{night} was assessed in the Fall 2005-1 study. For *H. annuus*, plant reproductive stage affected g_{night} and E_{night} under both sufficient and limited nitrate availability ($F_{2,46}$=17.45, P<0.001; $F_{2,46}$=15.96, P<0.001, respectively; Figure 2.4, Table 2.2). Pre-reproductive plants (5.5 weeks old) had higher g_{night} and E_{night} than did reproductive plants (10 or 15.5 weeks old.

The contribution of $g_{cuticular}$ to g_{night}

During the Fall 2004-1 and Spring 2005 studies, $g_{cuticular}$, functionally defined as water loss though the cuticle with stomata at maximal closure, was measured on excised, wilted leaves. In Fall 2004-1, g_{night} (stomatal and cuticular conductances combined) was higher than $g_{cuticular}$ for all four wild *Helianthus* species (Figure 2.5). In Spring 2005, g_{night} was again higher than $g_{cuticular}$ (Figure 2.5). In both studies, $g_{cuticular}$ measured on leaves was higher than instrument error

($P<0.001$), which averaged -7.5×10^{-6} mol m^{-2} s^{-1} during $g_{cuticular}$ measurements. During Spring 2006, $g_{cuticular}$ was measured on intact leaves of plants infused with exogenous ABA into the xylem. $g_{cuticular}$ was lower than g_{night} measured on intact leaves of control plants for both wild *H. annuus* and domesticated *H. annuus* (Figure 2.5).

Looking across all three studies, $g_{cuticular}$ for wild *H. annuus* ranged from 0.013 to 0.023 mol m^{-2} s^{-1} and there was good agreement between measures made with the two different techniques (Figure 2.5). Of the other three wild species, only the estimate of $g_{cuticular}$ for *H. deserticola* was substantially larger than the range for *H. annuus*. Not considering *H. deserticola*, calculated $g_{stomata}$ for wild *Helianthus* was on average five times greater than $g_{cuticular}$.

DISCUSSION

The *Helianthus* g_{night} reported here for greenhouse grown plants (0.023-0.225 mol m^{-2} s^{-1}) are within the range reported for two of these species in their native habitats (Snyder et al. 2003, Ludwig et al. 2006) and for C_3 and C_4 plants in general (Caird et al. 2007). The wild and domesticated *Helianthus* species in our studies had typical values for g_{day}, E_{day}, and photosynthesis (Table 2.2), and the g_{night} values were relatively large and greater than explained by $g_{cuticular}$.

In the Fall 2005-2 study, gravimetric measures were compared to instantaneous measures of transpiration. The gravimetric measures were approximately 4-fold lower, reflecting their integration over the entire night or day period, whereas instantaneous measures were timed to capture maximal E_{night} and E_{day} rates. However, there was a strong correlation between the two measurement techniques. Additionally, the percentage total E_{night} of total E_{day} measured gravimetrically over the 24-h gave an estimate of 6%, which agreed well with the 5% estimate

from instantaneous gas exchange measures during the same day/night period. This added validity to our estimates based on instantaneous measures.

Response of g_{night} and E_{night} to soil nutrient and water manipulation

We hypothesized that regulation might occur for increased g_{night} under limited nutrient conditions to increase bulk flow of soil solution to the roots and reduce the development of a nutrient depletion zone in the rhizosphere. Although the soil nutrient limitations were sufficient to limit shoot and reproductive biomass and generally to reduce leaf nitrogen concentration, they did not affect g_{night} and E_{night} in any of the wild *Helianthus* species or in domesticated *H. annuus*. Thus, for *Helianthus,* there is no evidence of nighttime stomatal regulation in response to soil nutrient limitations. Contrary to our *Helianthus* results, we have evidence that other species do respond to soil nutrient limitations imposed while controlling for plant water status, some with higher g_{night} (*Distichlis spicata, Populus balsamifera ssp. trichocarpa*), and others with lower g_{night} (*Arabidopsis thaliana*; M. Caird and A. Howard, *unpublished data*). A broader range of species needs to be tested to support any generalizations. The variable response of g_{night} to nutrient limitation may involve the same mechanisms that are currently being investigated for g_{day} responses, such as ABA, pH, and cytokinin signals (Dodd et al. 2003, Sakakibara et al. 2006).

Whether or not a species regulates g_{night} in response to soil nutrients, a plant that is transpiring at night may have increased uptake of nutrients such as nitrate. McDonald et al. (2002) demonstrated that *Populus* plants transpiring continuously (day and night), instead of only during the day, took up more nitrogen. Given that there is genetic variation for g_{night} and E_{night} (*Arabidopsis thaliana*; Christman et al. 2008), selection may favor high g_{night} and E_{night} in nutrient poor habitats if E_{night} provides a nutrient uptake benefit. The four wild *Helianthus*

species studied here are native to habitats differing in nutrient availability; *H. anomalus* and *H. deserticola* are endemic to nutrient poor desert dune habitats (Rosenthal et al. 2002, Brouillette et al. 2007). We found that *H. deserticola* did have higher g_{night} and E_{night} than *H. petiolaris*, consistent with the direction predicted by selection for higher g_{night} in lower nutrient habitats, but the magnitude of difference was relatively small and appeared largely driven by greater $g_{cuticular}$ in *H. deserticola*.

g_{night} and E_{night} did decline in response to water limitations that were generally sufficient to decrease leaf predawn xylem pressure potential, g_{day}, E_{day} and photosynthesis. Declines were such that g_{night} in the limited water treatments was generally within the range we recorded for functionally defined $g_{cuticular}$. For three of the four studies, the water limitation was short term and consisted of withholding water just prior to measurements on fully mature leaves, so that the effect on g_{night} could not be due to a long-term change in leaf structure, stomatal density or size, or cuticle. The decline in g_{night} and E_{nigh} due to water limitation demonstrates that guard cell regulation of nighttime water loss is possible, analogous to daytime regulation of water loss in response to soil drying. Our results agree with previous results showing lower g_{night} associated with decreased plant water status in *Hibiscus cannabinus* (Muchow et al. 1980), *Pseudostuga menziesii* (Running 1976, Blake and Ferrell 1977), and *H. anomalus* (Ludwig et al. 2006), and a water stress treatment resulting in decreased water loss at night in wheat plants (Rawson and Clarke 1988). The nighttime stomatal response to drought likely involves many of the same mechanisms that are currently being investigated for daytime responses, such as ABA and pH signals (Dodd 2003, Davies et al. 2005, Li et al. 2006), although this remains to be determined.

Variation in g_{night} and E_{night} nocturnally and across leaf lifespan and plant reproductive stages

Assessing temporal variation is necessary for interpereting the significance of instantaneous leaf-level measures of g_{night} and E_{night}. We complemented single instantaneous measures on most recently fully expanded leaves of mature plants with studies that assessed variation nocturnally, across leaf lifespan, and across plant reproductive stages. Beginning with nocturnal variation (across a single night), repeated measures during the night in two studies both showed a significant increase in g_{night} and E_{night}. A similar gradual increase in g_{night} has been observed in several other species including *Arabidopsis thaliana*, desert shrubs, and trees (Lasceve et al. 1997, Leymarie et al. 1998, 1999, Donovan et al. 2003, Bucci et al. 2004, Dodd et al. 2004, 2005), and potential regulatory mechanisms are being investigated (Lasceve et al. 1997, Gardner et al. 2006).

When g_{night} was measured across three nocturnal time-points for sufficiently watered *H. annuus* in Fall 2005-2, the increase in g_{night} was associated with an increase in VPD_a, although of small magnitude (from approximately 0.5 to 0.7 kPa; Figure 2.3). Thus, over this range of VPD_a, the correlation between g_{night} and VPD was not the negative relationship expected from daytime VPD_a responses (Franks and Farquhar 1999). However, a larger range of VPD_a is needed to test nighttime VPD responses. Correlative data from other studies suggest that g_{night} does decline in response to increased VPD_a, similar to g_{day}, and responses may be species specific (Oren et al. 2001, Bucci et al. 2004, but see Barbour et al. 2005 for contrast). Bakker (1991) found a decline in g_{night} in response to experimentally manipulated VPD. However, more studies are needed that experimentally manipulate VPD_a and VPD_l and account for potentially

confounding factors such as $g_{cuticular}$, circadian rhythms and plant xylem pressure potential recovery.

We assessed variation in g_{night} and E_{night} across entire leaf lifetime. In contrast to Cechin and Fumis (2004), who found that g_{day} declined as *Helianthus* leaves aged, and Blom-Zandstra et al. (1995) who found that g_{night} declined as rose leaves aged, we found no decline in *H. annuus* g_{night} and E_{night} as recently matured (i.e. fully expanded) leaves aged over the following 4 weeks. Measures on the same plants indicated that leaf lifespan (number of days from 1 cm leaf blade length to 50% of leaf senesced) averaged 40 d (5.7 weeks). Thus, our gas exchange measurements captured the majority of leaf lifespan. The lack of decline in nighttime gas exchange rates over leaf lifespan suggests that for *Helianthus*, instantaneous g_{night} on a recently matured leaf may be used to scale up to instantaneous g_{night} for whole plant leaf area, provided that there is an open canopy structure.

To generalize across plant life stages we investigated variation in g_{night} and E_{night} across plant reproductive stages, controlling for leaf age. Pre-reproductive *H. annuus* showed significantly higher g_{night} and E_{night} than individuals that were flowering or setting seeds. Our results are consistent with those of Grulke et al. (2004) who found g_{night} to be higher in large saplings compared to mature ponderosa pine.

Young plants, during rapid vegetative growth, expend a large portion of respiratory energy on nutrient uptake, and this proportion generally declines as plants age (Marschner 1995). Thus, although *Helianthus* species appear unable to regulate nighttime water loss in response to soil nutrient conditions, an inherently higher E_{night} for younger plants may be beneficial if it reduces formation of a nutrient depletion zone around roots at night, as suggested by results with the Barber-Cushman model (Barber and Cushman 1981). Nutrient depletion zones may be more

pronounced around roots of prereproductive plants due to a significantly lower root mass ($F_{2,46}$=6.89, P<0.01). Whether or not increased E_{night} represents a nutrient uptake benefit for pre-reproductive phase plants, it is possible that estimates of total water flux in mixed aged stands or integrated over the life of a crop are underestimated when based on a combination of E_{day} and E_{night} measured only on reproductive aged individuals.

The contribution of $g_{cuticular}$ to g_{night}

Measures of g_{night} and E_{night} include cuticular and stomatal pathways in parallel, yet only water loss though stomata, at an aperture greater than maximal possible closure, may be subject to guard cell regulation. For all wild *Helianthus* species except for *H. deserticola,* $g_{stomata}$ was five times greater than $g_{cuticular}$, suggesting that most nighttime water loss can be regulated. With the exception of the extremely high $g_{cuticular}$ for *H. deserticola*, which deserves further investigation, the remaining $g_{cuticular}$ for *Helianthus* were in the upper range of those reported in the literature using comparable techniques (Rawson and Clarke 1988, Kerstiens 1995, Boyer et al. 1997, Burghardt and Riederer 2003, Nobel 2005). More characterizations are needed of inter- and intra-specific variation in $g_{cuticular}$, including the extent to which growth conditions and atmospheric humidity can change $g_{cuticular}$ components (Schreiber et al. 2001, Kerstiens 2006, Kock et al. 2006).

Variation among studies in magnitude of g_{night}

Although our tests of g_{night} responses to nutrients and water occurred within each study, and cross study comparisons were not preplanned, the study differences in maximum g_{night} deserve some comments. For wild *H. annuus* in the nutrient and water manipulation studies, g_{night} of sufficiently-watered plants ranged from 0.04 to 0.12 mol m^{-2} s^{-1} (Figure 2.1, 2.2, Table 2.2). Because studies were conducted in different seasons and years, some of the variation may

have been due to differences in the growth environment, and to VPD_l differences during the nights and days of gas exchange measurements. However, the study with the lowest g_{night} (Fall 2005-1) did not stand out as having the highest VPD_l on the night or accompanying day of gas exchange measurements, or an unusual VPD_a across the growth interval of the study. It is possible that using study means obscures a specific time interval where VPD_a affected leaf development and maximum g_{night}, but there are many other potential contributing factors. We recommend more exploration of growth environment (temperature, humidity, CO_2 levels, light quantity and quality, plant nutritional status, growth medium, etc.) on leaf structure, stomatal density and size, cuticular properties, and maximum g_{night} (Hetherington and Woodward 2003, Bergman et al. 2006, Kock et al. 2006). Additionally, the effects of VPD_a and VPD_l prior to and during the gas exchange measurements deserve more attention (Franks and Farquhar 1999, Schreiber et al. 2001).

Across multiple studies, we demonstrate substantial g_{night} and E_{night} in *Helianthus* wild species and domesticates. For *Helianthus*, nighttime water loss occurs largely through stomata, and is regulated in response to plant water stress but not soil nutrient availability. Additionally, *Helianthus* g_{night} varies nocturnally and across plant reproductive stages, but does not vary for individual leaves as they age. More research is needed to test the commonality of these findings in plants of various life histories and native to diverse habitats. Building generalities for variation and regulation of g_{night} and E_{night} is necessary for predicting the conditions under which nighttime water loss will be biologically significant.

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Table 2.1: Overview of nine studies including *Helianthus* species, water and nutrient treatments, and experimental design. The *H. annuus* was wild, except where designated: *H. annuus* dom. is a commercial domesticate and *H. annuus* Hopi is an early domesticate. See methods for composition of high and low nitrate modified Hoagland solution. For experimental design, RCBD is randomized complete block and CR is completely randomized.

Study	Species	Nutrient treatment	Water stress treatment	Additional tests	Experimental design
Fall 2003-1	*H. annuus*, *H. anomalus*, *H. deserticola*, *H. petiolaris*	**Yes**: 40g or 4g Osmocote	**No**	---	RCBD: 4 species * 2 NPK trts * 3 blocks * 3 replicates = 72 plants (gas exchange measures taken on each species separately)
Fall 2003-2	*H. annuus* dom.	**Yes**: Hydrosol, then 10 or 1 g Osmocote	**Yes:** sustained	---	RCBD: 2 NPK trts * 2 water trts * 3 blocks * 4 replicates = 48 plants
Fall 2004-1	*H. annuus*, *H. anomalus*, *H. deserticola*, *H. petiolaris*	**Yes**: 140 or 7 $\mu g\ mL^{-1}$ N (as nitrate) Hoagland	**No**	Species Cuticular - wilting Nocturnal time course	RCBD: 4 species * 2 nitrogen trts * 3 blocks * 3 replicates =72 plants.
Fall 2004-2	*H. annuus*, *H. annuus* Hopi	**No**: 20:10:20 NPK soluble fertilizer	**Yes:** before measurements	Accession (wild vs. Hopi)	RCBD: 2 accessions * 2 water trts * 3 blocks * 3 replicates = 36 plants.
Spring 2005	*H. annuus*	**Yes**: 140 or 7 $\mu g\ mL^{-1}$ N (as nitrate) Hoagland	**No**	Leaf age Cuticular - wilting	RCBD: 2 nitrogen trts * 3 blocks * 3 replicates = 18 plants.
Summer 2005	*H. annuus*	**Yes**: 140 or 7 $\mu g\ mL^{-1}$ N (as nitrate) Hoagland	**Yes:** before measurements	---	RCBD: 2 nitrogen trts * 2 water trts * 3 blocks * 3 replicates = 36 plants.
Fall 2005-1	*H. annuus*	**Yes**: 140 or 7 $\mu g\ mL^{-1}$ N (as nitrate) Hoagland	**No**	Plant age Leaf age	RCBD: 3 ages * 2 nitrogen trts * 3 blocks * 3 replicates = 54 plants.
Fall 2005-2	*H. annuus*	**No**: 140 $\mu g\ mL^{-1}$ N (as nitrate) Hoagland	**Yes:** before measurements	24-hour time course Gravimetric E	CR: 2 water trts * 13-14 replicates (10 for gas exchange & 3-4 for xylem pressure potential) = 27 plants
Spring 2006	*H. annuus*, *H, annuus* dom.	**No**: 140 $\mu g\ mL^{-1}$ N (as nitrate) Hoagland	**No**	Cuticular - ABA	CR: 2 accessions * 2 trts (ABA or control) * 3-7 replicates = 19 plants

Table 2.2: Gas exchange traits from studies that assessed a nutrient limitation treatment, a water limitation treatment, or both. If no treatment is designated (i.e. "---") then all plants in that study received sufficient levels of that resource. Trait values are lsmeans ± 1 SE (presented as – SE and then + SE when unequal due to back transformation after log). F-values and associated degrees of freedom ($F_{df\ num,\ df\ denom.}$) are presented for each trait and model effect (PROC MIXED ANOVA, block as random). F-values in bold indicate statistical significance (* P<0.05, ** P<0.01, *** P<0.001).

Study and *Species*	**Nutrient treatment**	**Water treatment**	**g_{night} (mol m^{-2} s^{-1})**	**E_{night} (mmol m^{-2} s^{-1})**	**g_{day} (mol m^{-2} s^{-1})**	**E_{day} (mmol m^{-2} s^{-1})**	**Photosynthesis (μmol m^{-2} s^{-1})**
	Model effects						
Fall 2003-1							
H. annuus	sufficient	---	0.102 ± 0.017	1.36 ± 0.17			
	limiting	---	0.102 ± 0.017	1.42± 0.17			
	Nutrient effect		0.00 $_{1,10}$	0.06 $_{1,10}$			
H. anomalus	sufficient	---	0.166 ± 0.021	1.69 ± 0.16			
	limiting	---	0.130 ± 0.021	1.47 ± 0.16			
	Nutrient effect		2.8 $_{1,10}$	1.85 $_{1,10}$			
H. deserticola	sufficient	---	0.225 ± 0.052	2.46 ± 0.45			
	limiting	---	0.134 ± 0.062	1.84 ± 0.53			
	Nutrient effect		1.27 $_{1, 8}$	0.82 $_{1, 8}$			
H. petiolaris							
	sufficient	---	0.121 ± 0.022	1.57 ± 0.25			
	limiting	---	0.066 ± 0.024	1.01 ± 0.27			
	Nutrient effect		3.78 $_{1, 9}$	3.54 $_{1, 9}$			
Fall 2003-2							
H. annuus dom.	sufficient	sufficient	0.035 ± 0.004	0.42 –0.07, +0.09	1.272 ± 0.161	7.79 ± 0.80	27.8 ± 2.5
	sufficient	limiting	0.012 ± 0.004	0.15 –0.03, +0.03	0.460 ± 0.161	4.04 ± 0.80	12.8 ± 2.5
	limiting	sufficient	0.023 ± 0.004	0.29 –0.05, +0.06	1.201 ± 0.161	8.44 ± 0.80	26.0 ± 2.5
	limiting	limiting	0.014 ± 0.004	0.20 –0.03, +0.04	1.086 ± 0.161	8.12 ± 0.80	24.5 ± 2.5
	Water effect		**20.81 $_{1, 30}$ *** **	**14.86 $_{1, 30}$ *** **	**8.25 $_{1, 30}$ ** **	**6.84 $_{1, 30}$ * **	**10.66 $_{1, 30}$ ** **
	Nutrient effect		1.74 $_{1, 30}$	0.05 $_{1, 30}$	2.97 $_{1, 30}$	**9.24 $_{1, 30}$ ** **	3.92 $_{1, 30}$
	*Water*nutrient*		4.00 $_{1, 30}$	3.50 $_{1, 30}$	**4.66 $_{1, 30}$ * **	**4.91 $_{1, 30}$ * **	**7.28 $_{1, 30}$ * **
Fall 2004-1							
H. annuus	sufficient	---	0.124 ± 0.020	0.61 ± 0.24	0.924 ± 0.151	18.86 ± 1.525	19.9 ± 1.8
	limiting	---	0.104 ± 0.019	1.35 ± 0.22	0.645 ± 0.141	14.53 ± 1.426	18.4 ± 1.7
H. anomalus	sufficient	---	0.119 ± 0.019	1.65 ± 0.23	0.771 ± 0.144	18.11 ± 1.426	18.9 ± 1.7
	limiting	---	0.134 ± 0.021	1.86 ± 0.25	0.635 ± 0.157	15.36 ± 1.647	21.2 ± 1.9
H. deserticola	sufficient	---	0.148 ± 0.019	1.88 ± 0.23	0.837 ± 0.145	16.57 ± 1.525	18.2 ± 1.8

	limiting	---	0.135 ± 0.018	1.80 ± 0.21	1.026 ± 0.136	18.66 ± 1.345	21.1 ± 1.6
H. petiolaris	sufficient	---	0.086 ± 0.019	1.14 ± 0.23	0.929 ± 0.144	18.30 ± 1.426	20.5 ± 1.7
	limiting	---	0.105 ± 0.021	1.44 ± 0.24	0.756 ± 0.155	16.23 ± 1.525	17.8 ± 1.8
	Nitrate effect		0.00 $_{1, 51}$	0.09 $_{1, 51}$	1.41 $_{1, 51}$	2.83 $_{1, 51}$	0.05 $_{1, 50}$
	Species effect		**3.08 $_{3, 51}$***	**3.03 $_{3, 51}$***	1.24 $_{3, 51}$	0.18 $_{3, 51}$	0.12 $_{3, 50}$
	*Nitrate*species*		0.76 $_{3, 51}$	0.79 $_{3, 51}$	1.46 $_{3, 51}$	1.77 $_{3, 51}$	1.28 $_{3, 50}$
	PAR (covariate)		-	-	-	-	**24.18 $_{1, 50}$*****
Fall 2004-2							
H. annuus	---	sufficient	0.060 ± 0.007	0.74 ± 0.08	1.489 ± 0.121	18.46 ± 1.37	32.04 ± 2.1
	---	limiting	0.022 ± 0.007	0.32 ± 0.08	0.365 ± 0.121	8.34 ± 1.37	18.5 ± 2.1
H. annuus Hopi	---	sufficient	0.053 ± 0.007	0.68 ± 0.08	1.029 ± 0.121	16.40 ± 1.37	25.2 ± 2.1
	---	limiting	0.029 ± 0.007	0.41 ± 0.09	0.243 ± 0.128	6.54 ± 1.45	14.2 ± 2.2
	Water effect		**17.64 $_{29, 1}$ ***	**16.77 $_{29, 1}$***	**60.28 $_{29, 1}$***	**51.82 $_{29, 1}$***	**32.73 $_{29, 1}$***
	Accession effect		0.0 $_{29, 1}$	0.02 $_{29, 1}$	5.57 $_{29, 1}$*	1.29 $_{29, 1}$	**6.83 $_{29, 1}$***
	*Water*accession*		0.92 $_{29, 1}$	0.81 $_{29, 1}$	1.88 $_{29, 1}$	0.01 $_{29, 1}$	0.34 $_{29, 1}$
Spring 2005							
H. annuus	sufficient	---	0.104 -0.012, +0.013	1.20 –0.14, +0.16			
	limiting	---	0.082 -0.009, +0.01	0.98 –0.11, +0.13			
	Nitrate effect		3.96 $_{1, 11}$	3.92 $_{1, 11}$			
	Leaf age effect		0.83 $_{1, 321}$	0.57 $_{1, 321}$			
Summer 2005							
H. annuus	sufficient	sufficient	0.079 -0.008, +0.009	0.55 -0.04, +0.05			
	sufficient	limiting	0.027 ± 0.003	0.22 ± 0.02			
	limiting	sufficient	0.096 -0.010, +0.011	0.61 ± 0.05			
	limiting	limiting	0.034 -0.003, +0.004	0.27 ± 0.02			
	Water effect		**93.88 $_{1, 31}$***	**101.3 $_{1, 31}$***			
	Nitrate effect		3.57 $_{1, 31}$	2.98 $_{1, 31}$			
	*Water*nitrate*		0.02 $_{1, 31}$	0.28 $_{1, 31}$			
Fall 2005-1	sufficient;						
H. annuus	15.5 wks age sufficient;	---	0.037 -0.009, +0.011	0.50 -0.11, +0.14			
	10 wks age sufficient;	---	0.037 -0.009, +0.012	0.49 -0.11, +0.15			
	5.5 wks age limiting;	---	0.098 -0.023, +0.03	1.19 -0.26, +0.33			
	15.5 wks age limiting;	---	0.054 -0.013, +0.017	0.72 -0.16, +0.20			
	10 wks age limiting;	---	0.037 -0.009, +0.011	0.49 -0.11, +0.14			
	5.5 wks age	---	0.153 -0.036, +0.047	1.76 -0.39, +0.50			

		Nitrate effect	$2.39_{1,46}$	$2.25_{1,46}$			
		Plant age effect	$\mathbf{17.45_{2,46}}$***	$\mathbf{15.96_{2,46}}$***			
		*Nitrate*plant age*	$0.55_{2,46}$	$0.58_{2,46}$			
Fall 2005-2							
H. annuus	---	sufficient	0.072 -0.021, +0.030	0.58 -0.16, +0.23	1.854 -0.345, +0.423	15.15 ± 0.29	34.9 ± 1.0
	---	limiting	0.003 ± 0.001	0.03 ± 0.01	0.019 -0.004, +0.005	0.60 ± 0.30	3.8 ± 1.1
		Water effect	$\mathbf{38.55_{1,19}}$***	$\mathbf{38.01_{1,19}}$***	$\mathbf{236.96_{1,19}}$***	$\mathbf{1211.89_{1,19}}$***	$\mathbf{443.65_{1,19}}$***

Table 2.3: Vegetative shoot biomass at harvest and total leaf nitrogen (N) content of gas exchange leaves for studies that included a nutrient limitation treatment. If no treatment is designated (i.e. "---") then all plants in that study received sufficient levels of that resource. Values are lsmeans ± 1 SE. F-values and associated degrees of freedom ($F_{df\ num,\ df\ denom.}$) are presented for each model effect (PROC MIXED ANOVA, block as random). F-values in bold indicate statistical significance (* P<0.05, ** P<0.01, *** P<0.001).

Study and *species*	**Nutrient treatment**	**Water treatment**	**shoot (g)**	**N (mg g^{-1}) for gas exchange leaf**
	Model Effects			
Fall 2003-1				
H. annuus	sufficient	---	7.3 ± 3.3	55.4 ± 3.9
	limited	---	1.8 ± 3.3	41.7 ± 4.1
	Nutrient effect		**29.57** $_{1, 14}$*******	**26.57** $_{1, 6}$******
H. anomalus	sufficient	---	14.5 ± 3.0	43.0 ± 2.2
	limited	---	5.1 ± 3.0	41.3 ± 2.2
	Nutrient effect		**10.46** $_{1, 14}$******	1.24 $_{1, 6}$
H. deserticola	sufficient	---	15.4 ± 3.3	Not assessed
	limited	---	3.3 ± 3.9	Not assessed
	Nutrient effect		**5.6** $_{1, 8}$*****	Not assessed
H. petiolaris	sufficient	---	10.4 ± 2.9	58.5 ± 3.6
	limited	---	4.7 ± 2.9	48.9 ± 3.2
	Nutrient effect		**7.01** $_{1, 12}$*****	4.10 $_{1, 5}$
Fall 2003-2				
H. annuus dom.	sufficient	sufficient	8.0 ± 0.4	Not assessed
	limited	sufficient	4.2 ± 0.4	Not assessed
	sufficient	limited	2.8 ± 0.4	Not assessed
	limited	limited	1.7 ± 0.4	Not assessed
	Water effect		**78.33** $_{1, 42}$*******	
	Nutrient effect		**30.69** $_{1, 42}$*******	
	*Water*nutrient*		**9.83** $_{1, 42}$******	
Fall 2004-1				
H. annuus	sufficient	---	4.5 -1.0, +1.2	28.6 ± 2.3
	limited	---	1.6 -0.3, +0.4	28.2 ± 2.2
H. anomalus	sufficient	---	7.3 -1.6, +2.1	37.0 ± 2.4
	limited	---	1.0 -0.3, +0.3	34.4 ± 2.6
H. deserticola	sufficient	---	7.8 -1.7, +2.2	35.3 ± 2.4
	limited	---	2.9 -0.6, +0.8	31.9 ± 2.3
H. petiolaris	sufficient	---	7.9 -1.7, +2.1	41.7 ± 2.3
	limited	---	1.2 -0.3, +0.4	34.4 ± 2.6
	Nitrate effect		**70.56** $_{1, 56}$*******	**6.78** $_{1, 56}$*****
	Species effect		2.45 $_{3, 56}$	**10.16** $_{3, 56}$*******
	*Nitrate*species*		2.38 $_{3, 56}$	1.21 $_{3, 56}$
Spring 2005				
H. annuus	sufficient	---	81.7 -5.5, +5.9	Not assessed
	limited	---	7.8 -0.5, +0.6	Not assessed
	Nitrate effect		**561.23** $_{1,33}$*******	Not assessed

Summer 2005				
H. annuus	sufficient	sufficient	114.0 -10.5, +11.6	38.3 ± 1.0
	sufficient	limited	71.6 -6.3, +6.8	Not assessed
	limited	sufficient	6.2 -0.6, +0.6	21.7 ± 1.0
	limited	limited	4.6 -0.4, +0.4	Not assessed
	Water effect		**38.4** $_{1, 29}$***	-
	Nitrate effect		**2065.79** $_{1, 29}$***	**130.65** $_{1, 32}$***
	*Water*nitrate*		1.62 $_{1, 29}$	-
Fall 2005-1				
H. annuus	sufficient; 15.5 wk age	---	87.2 -16.6, +20.5	27.5 ± 1.8
	sufficient; 10 wk age	---	10.2 - 2.0, +2.5	38.7 ± 1.8
	sufficient; 5.5 wk age	---	0.6 ± 0.1	51.7 ± 1.8
	limited; 15.5 wk age	---	7.6 -1.4, +1.8	18.2 ± 1.8
	limited; 10 wk age	---	1.3 -0.2, +0.3	28.1 ± 1.8
	limited; 5.5 wk age	---	0.3 ± 0.1	42.7 ± 1.8
	Nitrate effect		**180.16** $_{1,46}$ ***	**42.81** $_{1,46}$ ***
	Plant age effect		**351.87** $_{2,46}$ ***	**91.46** $_{2,46}$ ***
	*Nitrate*plant age*		**18.49** $_{2,46}$ ***	0.11 $_{2,46}$

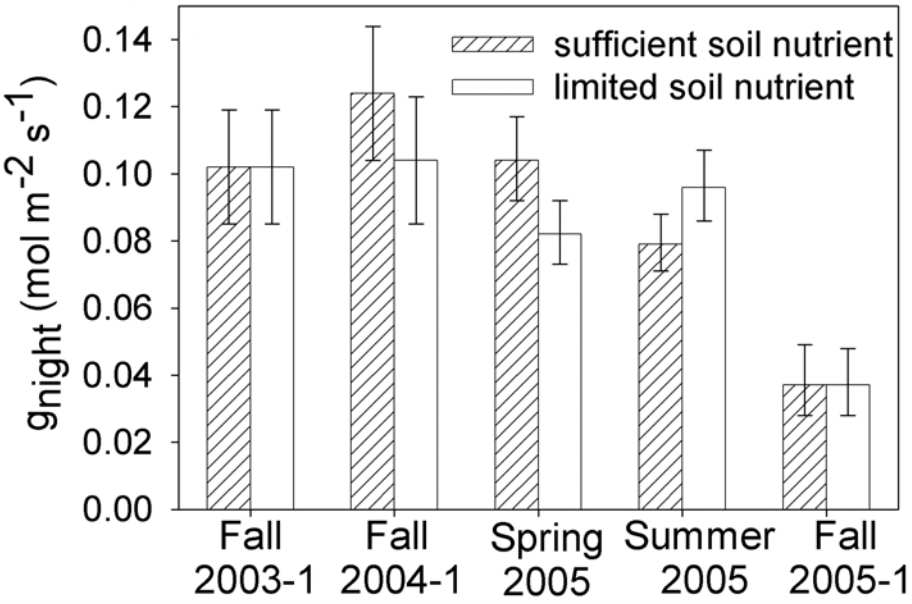

Figure 2.1: Effect of manipulating soil nutrient availability on nighttime leaf conductance (g_{night}) showing all of the tests for wild *Helianthus annuus*. In Fall 2003-1 availability of all macro- and micronutrients were manipulated, whereas only nitrogen, available as nitrate, was manipulated in the additional four studies. Bars are lsmeans ± 1 SE. See Table S1 for nutrient treatment comparisons for *H. annuus* domesticate, *H. annuus* Hopi, and other *Helianthus* species.

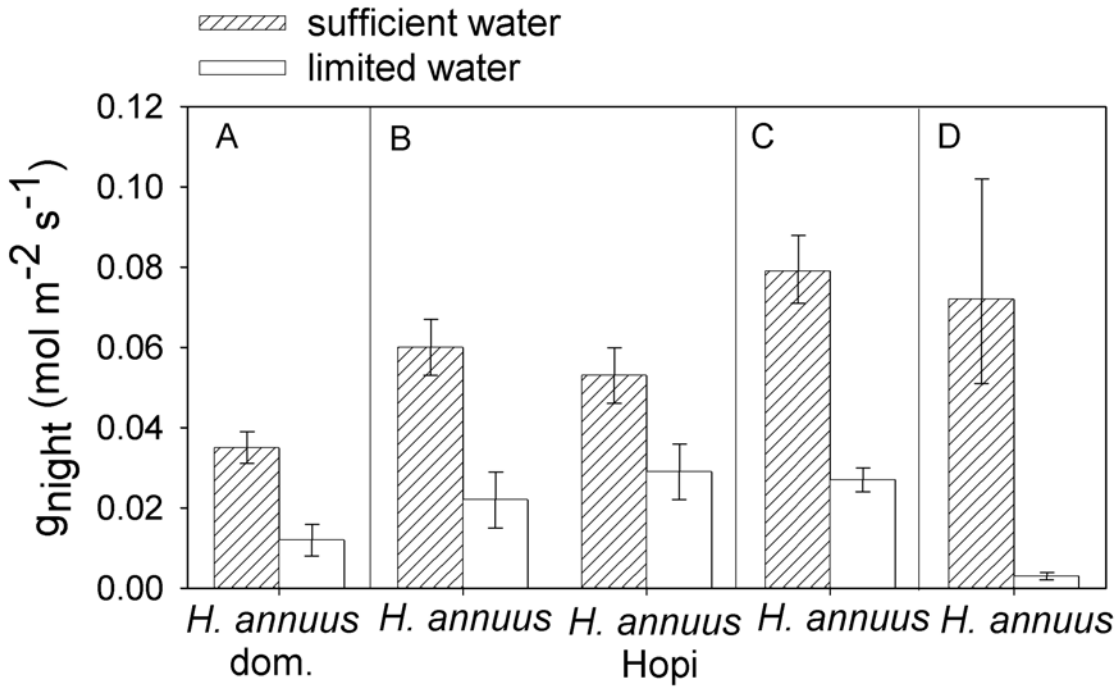

Figure 2.2: Effect of manipulation soil water availability on nighttime leaf conductance (g_{night}) during Fall 2003-2 (A), Fall 2004-2 (B), Summer 2005 (C) and Fall 2005-2 (D). In studies where both a water and nutrient treatment were applied (A, C) bars represent data from the high nutrient treatment only. Bars are lsmeans ± 1 SE. $g_{cuticular}$ for *H. annuus* and *H. annuus* dom., measured in Fall 2004-1, Spring 2005 and Spring 2006, ranged from 0.013 to 0.023 mol m^{-2} s^{-1}.

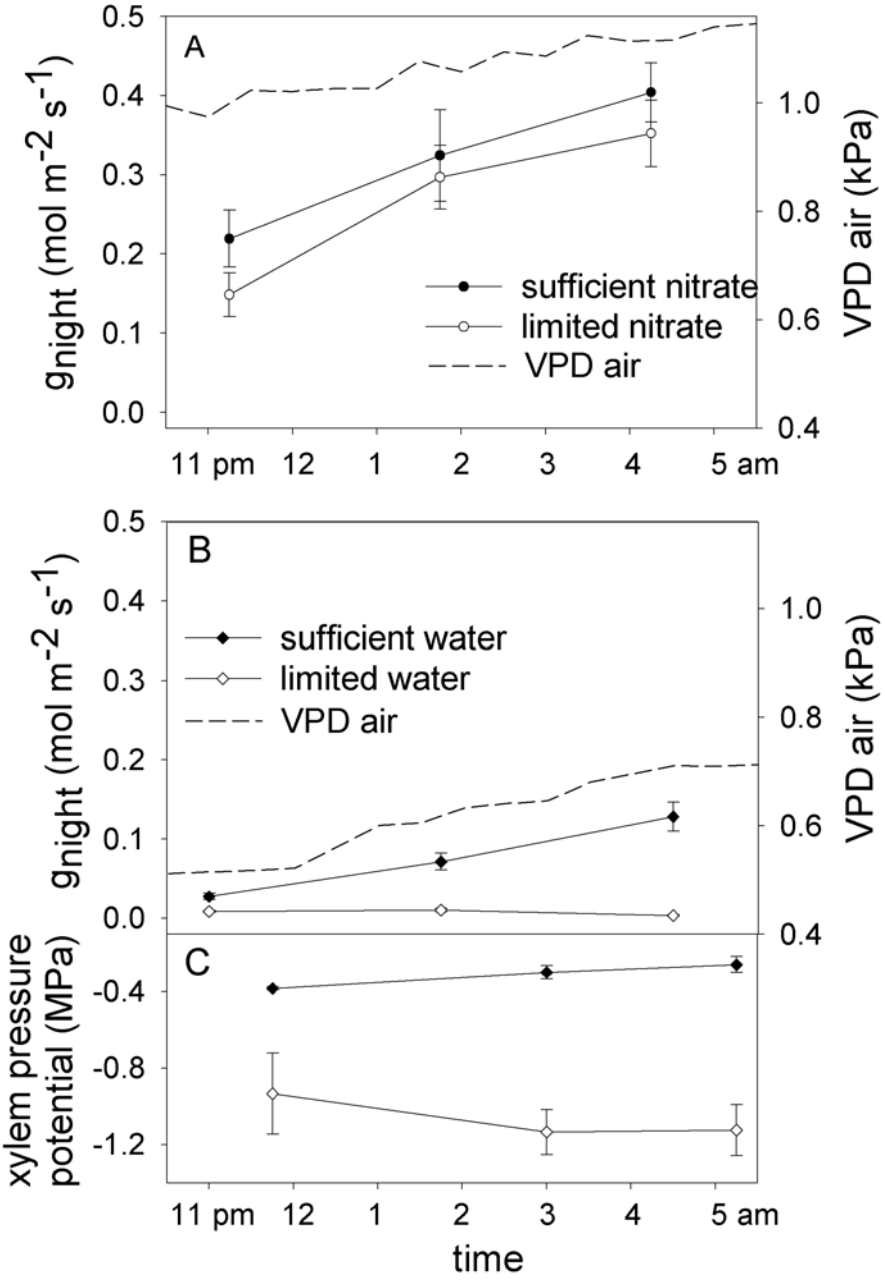

Figure 2.3: Variation in *Helianthus annuus* nighttime leaf conductance (g_{night}) and transpiration (E_{night}) across a single night during Fall 2004-1 (A) and Fall 2005-2 (B) studies. Included are independent measurements of atmospheric VPD (VPD air). Fall 2005-2 included measurements of xylem pressure potential (C) made on separate, randomly chosen plants from each treatment level. Points represent means ± 1 SE, n=5-6 for g_{night} and E_{night} and n=3-4 for xylem pressure potential. $g_{cuticular}$ for *H. annuus*, measured in Fall 2004-1, Spring 2005 and Spring 2006, ranged from 0.013 to 0.023 mol m^{-2} s^{-1}.

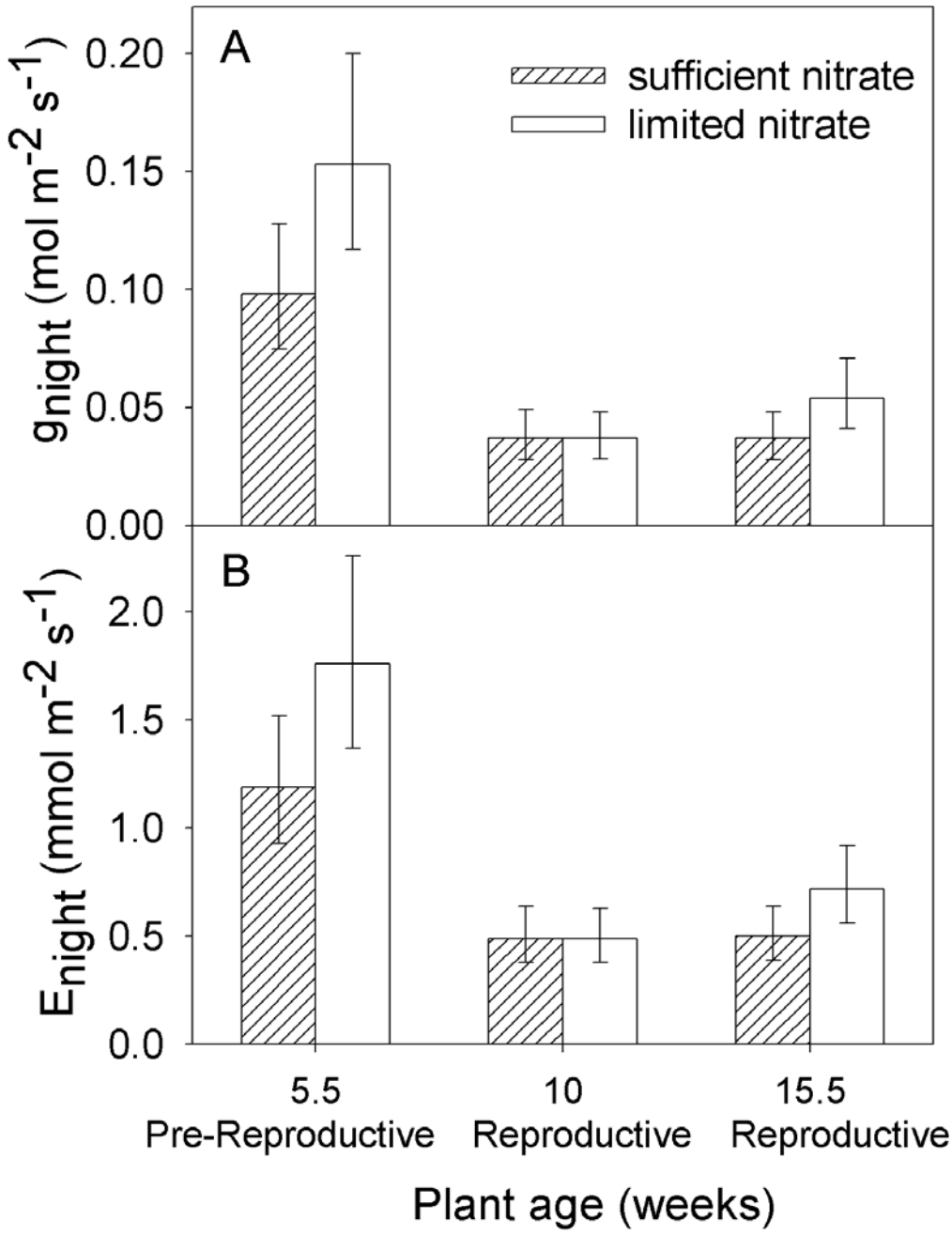

Figure 2.4: Effect of plant reproductive stage on nighttime leaf conductance (g_{night}) (A) and transpiration (E_{night}) (B) in *Helianthus annuus* taken during the Fall 2005-1 study. Measurements were made on most recently fully mature leaves produced concurrently. Five and a half week old plants were pre-reproductive while 10 and 15.5 week old plants were both reproductive. Bars are lsmeans ± 1 SE, n=8-9.

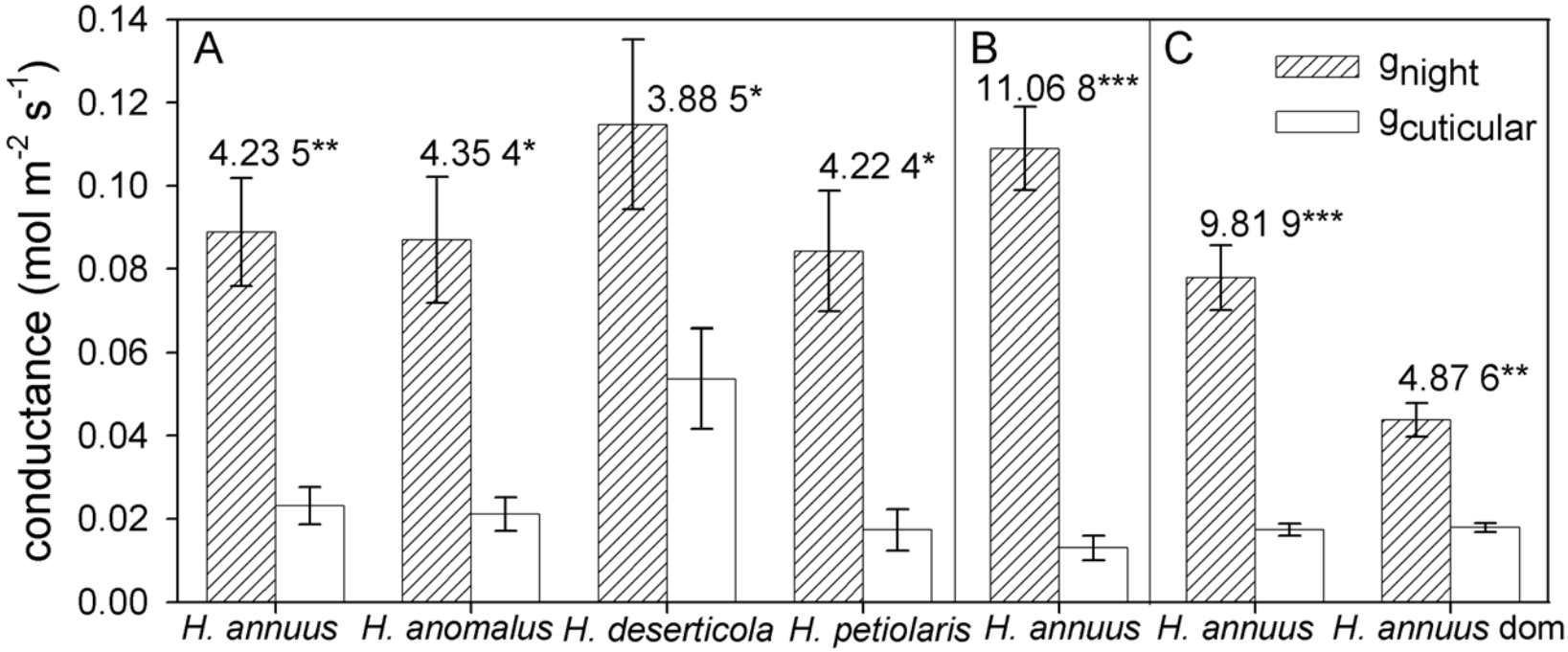

Figure 2.5: Instantaneous measures of nighttime leaf conductance (g_{night}) and cuticular conductance ($g_{cuticular}$), functionally defined as conductance though both the cuticle and stomata at maximal closure. Measures of $g_{cuticular}$ were made on excised, wilted leaves during the Fall 2004-1 study (A) and Spring 2005 study (B) and on intact leaves of plants infused with exogenous ABA during the Spring 2006 study (C). Bars are means ± 1 SE, n=5-6 bulked across nitrate treatment in Fall 2004-1, n=9 bulked across nitrate treatment in Spring 2005, and n=3-7 high nitrate treated plants during Spring 2006. Measures were made on different leaves of the same plant in Fall 2004-1and Spring 2005 and made on separate control or ABA treatment plants during one night during Spring 2006. t-values and associated degrees of freedom are presented from a paired t-test in Fall 2004-1 and Spring 2005, and from an independent t-test in Summer 2006 (* $P<0.05$, ** $P<0.01$, *** $P<0.001$).

CHAPTER 3

RESPONSE OF NIGHTTIME WATER LOSS IN *POPULUS* TO SOIL WATER AND NITROGEN MANIPULATION.[2]

[2] Howard, A.R. and L.A. Donovan. To be submitted to *Tree Physiology*.

ABSTRACT

Nighttime water loss from C_3 trees occurs without carbon gain and is both common and substantial. The magnitude of this water loss varies across time and better understanding of the environmental factors that may be driving this variation is needed. We investigated the response of nighttime conductance (g_{night}) and transpiration (E_{night}) to water and nitrogen manipulation. We used instantaneous gas exchange measures in a greenhouse study of *Populus angustifolia* (narrowleaf cottonwood) and *P. balsamifera spp. trichocarpa* (black cottonwood). Nighttime conductance ranged from 0.045 – 0.308 mol m^{-2} s^{-1} for *P. balsamifera* and 0.037 – 0.118 mol m^{-2} s^{-1} for *P. angustifolia* which was much larger than our measures of minimal leaf conductance (g_{min}; 0 – 0.005 mol m^{-2} s^{-1}) at night. Based on known daytime responses to water limitation, we hypothesized that g_{night} and E_{night} would decrease when soil water availability was limited. Water limitation reduced g_{night} to values indistinguishable from g_{min} and E_{night} was reduced to 2 to 3 % of well-watered controls. Based on the potential for transpiration to increase mass flow of mobile nutrients to roots, we hypothesized that g_{night} and E_{night} would increase under limiting soil nitrogen. Nitrogen limitation substantially reduced biomass in both nitrogen treatment studies, but did not affect g_{night} or E_{night} when potentially confounding water stress effects were eliminated. We find that that nighttime water loss from two *Populus* species is large and is regulated in response to soil water availability. E_{night} and its variability in response to soil water availability should be incorporated into models of stand and ecosystem water flux.

INTRODUCTION

Water availability is considered a major limiter of plant productivity in natural and agricultural systems (Lambers et al. 1998). Yet, water loss without concomitant carbon gain occurs at night in a wide range of C_3 plants species (Musselman and Minnick 2000, Caird et al.

2007, Dawson et al. 2007). A recent review by Caird et al. (2007) suggests that approx 63% of plants with reported nighttime conductance (g_{night}) are woody trees and shrubs. Since then, at least 15 additional woody species have been reported to have significant g_{night} (Cavender-Bares et al. 2007; Dawson et al. 2007, Scholz et al. 2007, Seibt et al. 2007). Reported rates of g_{night} in woody species range from 0.001 – 0.450 mol m^{-2} s^{-1}. Although low compared to daytime rates, these nighttime rates are often considerably higher than estimates of minimum leaf conductance (g_{min}) (Rawson and Clarke 1988, Howard and Donovan 2007, Marks and Lechowicz 2007). This suggests g_{night} is largely due to stomatal opening and should be under guard cell regulation.

Continued water loss at night from plants has important implications for plant, ecosystem and catchment water balance studies (Dawson et al. 2007) and substantial nighttime stomatal opening impacts uptake of pollutants (Musselman and Minnick 2000, Seibt et al. 2007). Important methodological concerns also arise from nighttime plant water loss such as underestimation of soil water potential measured from plant predawn water potentials and underestimation of transpiration by eddy covariance towers (Donovan et al. 2003, Fisher et al. 2007). The commonality and implications of g_{night} and nighttime transpiration (E_{night}) underscores the importance of understanding the conditions that result in substantial nighttime water loss.

Most studies quantify nighttime water loss in natural populations and report correlations with environmental and other physiological variables (Benyon et al. 1999, Oren et al. 2001, Kavanagh et al. 2007, Marks and Lechowicz 2007, Dawson et al. 2007). These studies have demonstrated that g_{night} varies between species and within individuals across hours, nights, and seasons. Environmental factors contributing to these differences have been suggested based mostly on correlations and a few manipulative experiments. These environmental conditions

include changing soil moisture (Grulke et al. 2004, Kavanagh et al. 2007), air temperature (Hubbart et al. 2007), and atmospheric vapor pressure deficit (Oren et al. 2001, Bucci et al 2004, Barbour and Buckley 2007). Variation in soil nutrient availability has also been suggested as a factor that may impact regulation of g_{night} and E_{night}, based on the potential for continued flux of soil solution to roots to enhance nutrient uptake (Snyder et al. 2003, Caird et al. 2007, Howard and Donovan 2007, Scholz et al. 2007). However, more manipulative studies are needed to directly test these correlations and assertions regarding the effect of environmental conditions on g_{night} and E_{night}.

Low rates of water loss at night may not reduce productivity when water is abundant in the environment, but could limit productivity when water becomes scarce. Studies of natural populations suggest that g_{night} in woody species may be sensitive to seasonal changes in soil water availability (Donovan et al. 2003, Grulke et al. 2004, Dawson et al. 2007). Manipulative greenhouse studies with wheat (Rawson and Clarke, 1988), sunflowers (Howard and Donovan 2007) and live oaks (Cavender-Bares et al. 2007) found that g_{night} and E_{night} decreased in response to decreased soil water availability. We provide a manipulative test of this relationship for deciduous riparian tree species.

If maintaining a flux of water towards roots at night can decrease the formation of nutrient depletion zones, then increased E_{night} could be beneficial when water is plentiful and mobile nutrients such as nitrate are scarce. This idea is supported theoretically by the Barber Cushman model of root nutrient uptake which predicts increased water flux to the root rhizoplane will decrease nitrate depletion zones around roots (Barber and Cushman 1981, Barber 1995). No consensus has been found among studies assessing the effect of E_{night} manipulations on plant nitrogen uptake (Christman et al. *submitted,* McDonald et al. 2002, Snyder et al. 2008).

A complementary approach is to test whether nutrient limitations result in up-regulation of g_{night} to promote higher E_{night} when nutrients are limiting. No regulation of g_{night} in response to nitrogen limitation was found for *Helianthus* and *Arabidopsis* (Howard and Donovan 2007, Christman et al. *submitted),* but g_{night} declined in response to long term nitrogen fertilization in a tree species, *Ouratea hexasperma* (Scholz et al. 2007). Here we determine the potential for regulation of g_{night} in response to soil nitrogen availability in two woody *Populus* species. Carefully controlled experiments that sufficiently account for potentially confounding secondary effects on plant water status are needed to resolve the seemingly contradictory results reported thus far.

Our objective was to assess the effect of controlled manipulations of soil water and nitrogen availability on the rate of g_{night} and E_{night} in C_3, fast-growing, riparian tree species, *Populus angustifolia* (narrowleaf cottonwood) and *P. balsamifera spp. trichocarpa* (black cottonwood). We also measured g_{min} for these species to determine baseline conductance above which stomatal regulation of g_{night} should be possible.

MATERIALS AND METHODS

Plant material and growth conditions

Cuttings for *Populus angustifolia* and *P. balsamifera* were obtained from Mono County, CA, U.S., in November and December of 2004 respectively. Stems were transported to the University of Georgia, Athens, GA and used in three studies: Nitrogen'05, Water'07 and Nitrogen'08 (Table 3.1). All studies included both species except the Nitrogen'08 study, which only included *P. balsamifera*.

All studies were conducted in greenhouses at the University of Georgia Biological Science Plant Growth Facilities. Natural daylight was supplemented during winter months to 12

to 14 hours with metal-halide lamps and temperatures were set to be at or above 26ºC (day) and 16ºC (night). Pruning of plants ceased at least 2 months prior to gas exchange measures. Plants in the Water'07 study were kept in an outdoor shade house after establishment and were returned while leafless and dormant to the greenhouse in February 2007 (2 months prior to use in gas exchange measures to assess minimum conductance). In all three studies leaves used in gas exchange measures were produced in the greenhouse under similar environmental conditions.

Nitrogen and water treatments

Treatment in the Water'07 study consisted of supplying plants with ample water to maintain soils near field capacity (sufficient), and limiting the soil water availability (limited) for five day just prior to gas exchange measures until visual signs of wilting occurred. Prior to treatment all plants were watered to field capacity daily.

Plants in the Nitrogen'05 and Nitrogen'08 studies received modified Hoagland nutrient solution, sufficient to bring the soil moisture to field capacity, three times per week. The nutrient solutions contained sufficient (140) or limiting (7 $\mu g\ mL^{-1}$) nitrogen as nitrate. Nitrate was used because it is a soil mobile ion and its availability to plants is more likely to be affected by variation in transpiration rate than ammonium. Both solutions contained equal amounts potassium (332 $\mu g\ mL^{-1}$ K). All other macronutrients were supplied at sufficient levels not expected to limit plant growth, although there were small differences between the sufficient and limiting nitrogen solutions because of the need to balance cations with the different levels of nitrate anions. The additional macronutrient concentrations were as follows: phosphorus 62 $\mu g\ mL^{-1}$ in high, 46 $\mu g\ mL^{-1}$ in low; calcium 160 $\mu g\ mL^{-1}$ in high, 10 $\mu g\ mL^{-1}$ in low; sulfur 96 $\mu g\ mL^{-1}$ in high, 88 $\mu g\ mL^{-1}$ in low) and magnesium (73 $\mu g\ mL^{-1}$ in high; 6 $\mu g\ mL^{-1}$ in low). The micronutrient concentrations did not differ between the sufficient and limiting nitrogen solutions:

chlorine 160 μg mL^{-1}), boron (0.068 μg mL^{-1}), manganese (0.027 μg mL^{-1}), zinc (0.033 μg mL^{-1}), copper (0.032 μg mL^{-1}), molybdenum (0.012 μg mL^{-1}) and iron (0.698 μg mL^{-1} as FeEDTA). Electrical conductivity of the sufficient and limiting nitrogen solutions was -2.47 ± 0.08 and -1.72 ± 0.02 mS cm^{-1}, respectively.

Plants in the Water'07 and Stomata'07 study were fertilized with weekly applications of 20:10:20 NPK soluble fertilizer (Peter's Peat-Lite Special, Scotts-Sierra Horticultural Products) when not dormant and application once to twice yearly of a slow release fertilizer (Osmocote Plus, Scotts-Sierra Horticultural Products) containing micronutrients. Plants in all studies were irrigated at least once daily between fertilizer applications to raise soil water to field capacity, except during the water limitation in the Water'07 study.

Leaf xylem pressure potentials (Ψ_{leaf}) were sampled to accompany nighttime gas exchange measurements in the Water'07 and Nitrogen'08 studies using a pressure chamber (Soil Moisture Equipment Corp., Santa Barbara, CA, USA). Measures were taken predawn in the Water'07 study and the July sampling of the Nitrogen'08 study and at sunrise during the September sampling of the Nitrogen'08 study. Although measures were taken later in the Nitrogen'08 study, plants were sampled in the order in which they were randomly assigned to the experimental design. Therefore leaf xylem pressure potentials in the Nitrogen'08 study provide a reliable comparison of plant water stress between sufficient and limited nitrogen plants in this study. Additionally, soil volumetric water content (SWC) was sampled immediately following nighttime gas exchange measures in July and September in the Nitrogen'08 study using a theta probe and hand-held meter (Dynamax Inc., Houston, TX, USA).

Gas exchange procedures

Leaf level measurements of daytime and nighttime gas exchange were made with a portable photosynthesis system (LI-6400, LI-COR Inc., Lincoln, NE, USA). Nighttime measures were made between 12:00am and a half hour before dawn. Daytime measures were made between 10:00am and 5:00pm. Measurements were made on a young fully expanded leaf of each plant. The chamber red/blue light source was turned off at night and was set to 2000 μmol m^{-2} s^{-1} during the day. Flow was 125 to 300 μmol s^{-1} at night and 500-700 μmol s^{-1} during the day. Block temperature was set equal to ambient and CO_2 was supplied at 360 to 400 μmol m^{-2} s^{-1}. Averaged within a study (mean ± 1 standard error), the range of leaf to air vapor pressure deficit (VPDl) across the four studies was 0.44 to 1.29 kPa (± 0.01) during nighttime measures and 1.27 (± 0.02) to 2.68 (± 0.04) kPa during daytime measures.

Instrument error was measured every four to six leaf measures with the chamber empty or clamped onto dry paper. Sample and reference infrared gas analyzers were matched after each empty or paper measurement. Averaged within a study, error for g_{night} ranged from 0 ± 0.002 to 0.012 ± 0.002 mol m^{-2} s^{-1}, which was always substantially less than rates for sufficiently watered plants. Before every set of measurements CO_2 and water zeros were checked and the leaf thermocouple was checked to ensure it was reading accurately to within 0.1 ^{o}C. To view equipment and plants at night without triggering stomatal opening we used a green safety headlamp with intensity below the detection of a LI-6400 external PAR sensor (0 μmol m^{-2} s^{-1} photosynthetic photon flux density; LiCor Inc.). When leaves did not fill the standard chamber (2 by 3 cm) they were marked to indicate chamber position, cut to remove area not inside the chamber and scanned (Winfolia, Regent Instruments Inc., Quebec, Canada) to determine area.

Adaxial and abaxial stomatal conductance was assessed on sufficiently fertilized plants using an Arabidopsis (6400-15 LiCor) chamber with air flow to the lower chamber blocked. The stomatal conductance of the adaxial surface was measured, then leaves were flipped over and conductance of the abaxial surface was measured. The mean stomatal conductance ratio for *P. angustifolia* and *P. balsamifera* were 0.286 and 0.158, respectively. These values were used as the stomatal ratio in all gas exchange computations to replace the default value of 1.

Biomass measures and Leaf nitrogen analysis

Biomass and leaf nitrogen content were assessed in the two nitrogen manipulation studies. Twelve of the 54 plants from the Nitrogen'05 study were harvested for above ground biomass. The twelve plants were each a unique genotype and represented three replicates of each species by nitrogen treatment combination. All plants in the Nitrogen'08 study were harvested for above and below ground biomass. Plants were harvested within three weeks of completing gas exchange measures. Biomass was divided into leaf, stem and root measures, dried at 60°C, and weighed. Leaves used for gas exchange measures were sampled within 48 hours of measurement, dried, ground and analyzed for nitrogen content (Carbo Era NA 1500 CN analyzer).

Assessment of g_{min}

We define minimum leaf conductance (g_{min}) as conductance through the cuticle and stomata at maximum closure induced by either leaf wilting (water stress) or exogenous ABA application. As such it includes both water loss through the cuticle and water loss through stomata at minimum aperture. The conductance measured by the LI-6400 (g_{night} or g_{day} in this study) is a measure of total leaf conductance. The portion of g_{day} and g_{night} that is due to stomatal

opening and is therefore subject to guard cell regulation can be calculated as total leaf conductance (g_{night} or g_{day}) minus g_{min} (Nobel 2005).

Assessment of g_{min} was made on the 16 plants in the Water'07 study prior to water manipulation. Four branches from a similar area of the canopy of each plant were selected and a recently mature leaf on each was chosen for measurements. The four leaves were used to measure the following: (1) g_{night} on sufficiently watered plants (intact treatment), (2) g_{min} assessed after ABA treatment (ABA treatment), (3) g_{night} as a control for the ABA procedure (ABA control), (4) g_{min} assessed after wilting (wilting treatment). For the intact treatment one chosen branch was left on each tree and measures were made concurrent with the ABA treatment and ABA control. For the ABA treatment and ABA control, branches were cut prior to dawn on 20 April 2007, put into bottles containing 130 ml of degassed solution (0.4 mol m^{-3} $Ca(NO_3)_2$ and 2.0 mol m^{-3} KH_2PO_4) (Borel et al. 2001) and immediately re-cut under solution. Solutions contained either 0 (ABA control) or 0.8 mol m^{-3} synthetic (±)-ABA (ABA treatment). Stems were sealed into the bottles with parafilm and bottles were covered with aluminum foil. Gas exchange measures for the intact treatment, ABA treatment and ABA control were made four to nine hours (daytime) and 17.5 to 21.5 hour (nighttime) after branches were cut and put into solution. Plants were watered amply throughout the period of experimentation. For the wilting treatment a leaf was excised from the remaining chosen branch on 27 April 2007 and was allowed to wilt in the dark. After leaves were wilted minimum leaf conductance was measured with the LI-6400.

Experimental design and statistical analysis

The Nitrogen'05 study was an unbalanced randomized block design with three blocks containing 54 plants (2 species *2 nitrogen treatments*9 to 17 replicates). The experimental

design was unbalanced due to plant death that occurred during the 12 months of growth prior to measurements. Among the replicates, there were seven genotypes of *P. angustifolia* and eight genotypes of *P. balsamifera,* and one to four individuals of each genotype (i.e. cuttings from the same tree). Gas exchange characteristics and leaf nitrogen content of the gas exchange leaf were measured for all 54 plants. These data were analyzed with a mixed-model ANOVA (proc MIXED; SAS Institute, 2004, version 9.1) with nitrogen treatment, species and nitrogen treatment*species as a fixed effects and block and genotype within species as random effects. One outlier was apparent in the residuals plot from the analysis of g_{day}, E_{day} and leaf nitrogen content. For each of these analyses the outlier was removed because it affected the results of the analysis of nitrogen treatment. The biomass data were analyzed with a general liner model ANOVA (proc GLM; SAS Institute, 2004, version 9.1) with species, nitrogen treatment and species by treatment as fixed effects. Genotype was not included in this analysis because biomass was measured on a subset of 12 plants, each of a unique genotype. In all analyses variables were log transferred when needed to meet the assumptions of normality.

In the Nitrogen'05 study, variation in g_{night} through the course of a night was assessed at 11:30pm, 1:15am and 3:30am on a single night. Repeated measures were made at these times on six sufficient nitrogen *P. angustifolia* and four sufficient nitrogen *P. balsamifera*. For this analysis g_{night} was log transformed to meet assumptions of normality and analyzed with a repeated-measurement mixed-model in PROC MIXED with an unstructured covariance matrix.

The Water'07 study was a balanced completely randomized design containing 16 plants (2 species*2 water treatments*4 replicates). Data did not meet assumptions of normality and treatment effects were analyzed separately for each species using a Wilcoxon rank-sum test (proc NPAR1WAY wilcoxon; SAS Institute, 2004, version 9.1). Data to assess g_{min} was taken on the

Water'07 plants prior to water treatment and were analyzed separately for each species. The analyses were repeated measures mixed-models (PROC MIXED) with treatment as a fixed effect, genotype as a random effect and plant as subject. One outlier was present in the analysis of nighttime data for *P. balsamifera* and was removed in order to meet assumptions of normality.

The Nitrogen'08 study was a randomized block design with 2 blocks containing 64 *P. balsamifera* (8 genotypes * 2 nitrogen treatments * 4 replicates). Measures were made on block 1 in July 2008 and on block 2 in September 2008 and data from each block were analyzed separately. Gas exchange, leaf nitrogen content, biomass data, leaf pressure potential and soil water content were analyzed with a mixed-model ANOVA (proc MIXED) with nitrogen treatment as a fixed effect and genotype and genotype*treatment as random effects. Three plants from block 1 and two plants from block 2 died prior to measurement. One large outlier was apparent in the analyses of soil water content from each block (1 and 2) and these plants were removed from the soil water content analyses.

RESULTS

Across all studies, g_{night} for sufficiently watered greenhouse grown *Populus* ranged from 0.045 to 0.308 mol m^{-2} s^{-1} for *P. balsamifera* and 0.037 to 0.118 mol m^{-2} s^{-1} for *P. angustifolia*. This represented a range of 7.5 to 31.3% of g_{day} measured the day preceding or following nighttime measures. g_{night} values for sufficiently watered plants were always substantially larger than measures of g_{min} or instrument error.

Response to soil water and nitrogen manipulation:

Withholding water from plants in the Water'07 study resulted in reduced daytime and nighttime gas exchange (Table 3.2). Daytime and nighttime conductance and transpiration were similarly reduced under limiting water to 1 to 3% of sufficiently watered controls. g_{night} and

E_{night} under limited water conditions were not significantly different from instrument error as measured with empty chamber measurements ($P>0.4$ for both species). Ψ_{leaf} was measured the night of gas exchange to confirm the success of the water limitation treatment. For both species Ψ_{leaf} were substantially lower in the limited water treatment plants (Table 3.2).

Soil nitrogen limitation had different effects on gas exchange measures in the Nitrogen'05 and Nitrogen'08 studies. In the Nitrogen'05 study limitation significantly increased both nighttime and daytime conductance and transpiration (Table 3.3). In this study g_{night} for *P. angustifolia* and *P. balsamifera* increased 1.5 and 2.3 fold, respectively, when nitrogen was limiting compared to sufficient. This resulted in a 1.4 and 2.0 fold increase in E_{night}, respectively. SWC and Ψ_{leaf} were not measured in this study.

In contrast, in the Nitrogen'08 study nitrogen limitation had no effect on nighttime or daytime conductance or transpiration in *P. balsamifera*. This held true for both measures of block 1 plants in July and block 2 plants in September when average g_{night} for *P. balsamifera* was lower than and higher than, respectively, measures of *P. balsamifera* in the Nitrogen'05 study. In this study SWC and Ψ_{leaf} were taken to accompany nighttime gas exchange measures and test for potentially confounding water stress effects. SWC measured during or immediately following nighttime gas exchange measures was not significantly different between sufficient and limiting nitrogen treatments both for block 1 and block 2 plants ($P>0.1$). Averaged across all plants, SWC was 0.257 $m^3 m^{-3}$ ($\pm$ 0.01) and 0.201 $m^3 m^{-3}$ ($\pm$ 0.003) for block 1 and 2 plants, respectively. Ψ_{leaf} measured following nighttime gas exchange measures did not differ between nitrogen treatments for block 1 or block 2 plants ($P>0.2$). Averaged across all plants, Ψ_{leaf} was -0.16 ($\pm$ 0.01) MPa and -0.38 ($\pm$ 0.03) MPa for block 1 and block 2 plants, respectively.

In both the Nitrogen'05 and Nitrogen'08 studies biomass, leaf N, and photosynthesis were measured to confirm the effectiveness of the soil nitrogen limitation treatment. The limited nitrogen treatment reduced shoot biomass in both studies (Table 3.3). Root biomass, which was additionally harvested in the Nitrogen'08 study, was reduced for the limited nitrogen plants. Comparing *P. balsamifera* given sufficient nitrogen across the two studies, at time of harvest sufficient nitrogen plants in the Nitrogen'05 study were 8.6 and 2.9 times larger than bock 1 and block 2 plants, respectively, in the Nitrogen'08 study. Leaf nitrogen concentration of gas exchange leaves was either unaffected or significantly reduced for the limited nitrogen plants (Table 3.3). A significant decrease was found in the Nitrogen'05 study for *P. balsamifera*, but not for *P. angustifolia*, and in block 1 in the Nitrogen'08 study. In both studies there was a trend toward lower photosynthesis in limited nitrogen plants and these differences were significant for block 1 plants in the Nitrogen'08 study.

Comparison of total and minimum leaf conductance

g_{night} (total leaf conductance to water at night) was substantially greater than g_{min} assessed either by exogenous ABA application or wilting (Table 3.4). We found no difference between g_{min} at night estimated by the ABA or wilting methods. Both methods produced g_{min} rates that were indistinguishable from instrument error assessed with empty chamber readings ($P>0.1$). In contrast, during the day g_{min} in both species measured with ABA treatment was greater than instrument error ($t_{16.4}=7.97$, $P<0.001$) but was substantially less than g_{day} in the intact treatment (leaves attached to the plant) or the ABA control (leaves on branches put in a solution lacking ABA).

Variation in g_{night} across a single night

Variation in g_{night} assessed across a single night showed an overall pattern of increasing in *P. angustifolia* while remaining fairly large and constant in *P. balsamifera* (species*time effect: $F_{2,8}$=36.35, P<0.001, Figure 3.1). During this time period atmospheric VPD in the greenhouse remained between 0.5 to 0.3 kPa. VPDl for the measured leaf declined from an average of 1.1 kPa at 11:30pm to 0.6 kPa at 3:30 am.

DISCUSSION

The g_{night} reported here for sufficiently watered greenhouse grown *Populus* (0.043 to 0.308 mol m^{-2} s^{-1}) are within the range reported for other woody species (Caird et al. 2007). Our range for g_{night} also encompasses the rates reported for *P. balsamifera* in naturally established populations in California (Snyder et al. 2003) and for a field-grown cross of *P. koreana* x *P. trichocarpa* (Furukawa et al. 1990). This suggests that effects on g_{night} seen in our greenhouse studies will be relevant to *Populus* in their natural habitats. Additionally, g_{night} for *P. angustifolia* and *P. balsamifera* was up to 37 and 154 times larger, respectively, than the highest g_{min} measured for each species. This indicates that most of the observed variation in g_{night} and E_{night} is due to guard cell regulation.

Our results with *P. angustifolia* and *P. balsamifera* show that known daytime stomatal responses to water limitation persist at night. In *P. angustifolia* and *P. balsamifera* g_{night} and E_{night} decreased when soil water availability was limited (Table 3.2). The decline of g_{night} and E_{night} under soil water deficit has also been demonstrated in other controlled experimental manipulations (Rawson and Clarke 1988, Cavender-Bares et al. 2007, Howard and Donovan 2007). Here, as in these other studies, g_{night} recorded for the plants with limiting water were

within the range of g_{min} for those species. In Cavender-Bares et al. (2007) live oaks were exposed to a prolonged drought and declines in g_{night} were at least in part due to a decline in the length and density of stomata. Here, as in our previous study with *Helianthus* (Howard and Donovan 2007), the limited water treatment was imposed just prior to measurements and measurements were made on leaves produced prior to the drought. Thus, the observed declines in g_{night} cannot be attributed to changes in leaf structure, stomatal size and density, or cuticle. Drought reduced g_{night} by stomatal regulation. The mechanism of this regulation is not known but likely involves the same signals as in daytime regulation including pH and ABA (Dodd 2003, Davies et al. 2005, Li et al. 2006).

Our results with *P. angustifolia* and *P. balsamifera* suggest that while decreased g_{night} and E_{night} can be found in response to differences in fertilization (Table 3.3), the response is likely due to secondary affects on water availability. This contradicts our hypothesis that regulation at night might occur for increased g_{night} and E_{night} when plants were grown under limiting soil nitrogen availability. Several authors have suggested that continuing bulk flow of soil solution towards roots at night might reduce formation of nutrient depletion zones and that this effect would be most pronounced for soil mobile nutrients such as nitrate (Snyder et al. 2003, Caird et al. 2007, Scholz et al. 2007). In support of this we found g_{night} and E_{night} to be increased by limiting soil nitrogen during the Nitrogen'05 study. However, a repeat of the experiment in 2008 with *P. balsamifera* did not yield significant effects of the fertilizer treatment on either of two sampling dates.

Careful examination of the differences between the Nitrogen'05 and Nitrgen'08 study suggests that the different results are not due to differences in success of imposing a nitrogen limitation. In both the Nitrogen'05 and Nitrogen'08 studies the low nitrogen treatment resulted

in substantially reduced biomass suggesting treatment was successful in causing a nitrogen limitation (Table 3.3). However, the Nitrogen'05 plants were grown for approximately twice as long as the Nitrogen'08 plants (Table 3.1) and the sufficient nitrogen treated plants in 2005 were much larger than those in 2008. With substantial E_{night}, the sufficient nitrogen plants in 2005 may have decreased soil water availability, despite evening watering, enough to experience slight water stress by 1:45am when nighttime gas exchange measures began. This would explain reduced g_{night} and E_{night} and also reduced g_{day} and E_{day} when compared to the small nitrogen limited plants. Alternatively, root restriction occurring in the large nitrogen sufficient plants may have caused reduced day and night gas exchange. In the Nitrogen'08 study, the sufficient nitrogen plants were much smaller than in 2005. Root growth did not appear to be restricted and we confirmed that there were no water stress effects between the nitrogen treatments using measures of Ψ_{leaf} and SWC. These results demonstrate that some nutrient manipulation effects on g_{night} and E_{night} may actually be secondary effects mediated though plant size, root restriction and/or water stress.

Our results suggest a broader interpretation of a previous study that altered soil nutrient availability in a Brazil Cerrado forest. Scholz et al. (2007) showed that long-term fertilization of *Ouratea hexasperma* with nitrogen resulted in reduced g_{night}. A secondary water stress effect could explain the findings of Scholz et al. (2007). Of the three Cerrado tree species included in the long-term fertilization experiment, only *Ouratea hexasperma* showed an effect of fertilization decreasing g_{night} and this was of marginal significance ($P<0.1$). To assess water stress Ψ_{leaf} was measured during the night period using leaves that had been covered to prevent E_{night}. With E_{night} minimized, Ψ_{leaf} can be a good estimator of soil water potential. Although Ψ_{leaf} was not significantly different between plants in control plots and fertilized plots, there was

a trend towards more negative Ψ_{leaf} in fertilized plots and this discrepancy was largest for *O. hexasperma* (-0.19 MPa in control plots versus -0.31 MPa in nitrogen fertilized plots). Manipulating soil nutrients in studies with four *Helianthus* species and *Arabidopsis* also found no effect on g_{night} and E_{night} (Howard and Donovan 2007, Christman et al. *submitted*). Together with our results these studies suggest that g_{night} and E_{night} in C_3 species both herbaceous and woody are not regulated in direct response to soil nutrient availability, but are highly sensitive to changes in water availability.

An important distinction must be made between regulation of g_{night} and E_{night} in response to soil nitrogen availability and whether or not substantial E_{night} results in increased nitrogen acquisition. Our study only examined the potential for regulation of g_{night} in response to soil nitrogen content, but other studies have manipulated E_{night} and found conflicting results on plant nutrition. Support for a passive role of E_{night} in nitrogen acquisition was found for *Populus deltoides* (McDonald et al. 2002) and *Sarcobatus vermiculatus* shrubs (Snyder et al. 2008). However, contrary evidence has also been found. Reducing E_{night} in *Arabidopsis thaliana* by 50 to 80% every night had no effect on shoot biomass, root biomass or leaf nitrogen content. This held true both when plants were grown with sufficient nitrogen and when grown with limiting nitrogen (Christman et al. *submitted*).

Another important distinction can be made between total soil nitrogen and distribution of soil nitrogen. Our plants were provided with a fairly uniform nitrogen distribution by watering a porous sandy soil past field capacity with fertilizer solutions thrice weekly. A recent study with *Ehrharta calycina* showed that g_{day} and E_{day} increased beyond what was needed to maintain photosynthesis levels when nutrients were applied to a localized area not accessed by plant roots (Cramer et al. 2008). This would appear to be an adaptation to increase mass flow of nutrients

towards roots. The relative benefit of nutrient delivery per amount of water transpired increases with increasing concentration of mobile nutrients in the soil (Marschner 2002). Thus, g_{night} and E_{night} might change in response to heterogeneous distribution of sufficient nutrients and ease of direct interception by roots even while homogenous changes in total nitrogen availability did not drive changes in g_{night} and E_{night}. This has yet to be tested.

We record a fairly broad range of g_{night} and E_{night} with rates varying between species, across the studies and even within a single night for *P. angustifolia*. This is likely due to inherent differences in g_{night} between the species, differences in environmental conditions on the different dates of measurement and differences in species response to environmental variables. When we assessed variation across a single night we found g_{night} was fairly constant in *P. balsamifera*, but increased though the night in *P. angustifolia* (Figure 3.1). The increase may have been a response to declining VPDl (Oren et al. 2001, Bucci et al 2004, Barbour and Buckley 2007) or part of a circadian rhythm (Lasceve et al. 1997, Bucci et al. 2004, Dodd et al. 2005) or both. In either case, our measures of g_{night} and E_{night} taken in the latter part of the night likely captured the period when g_{night} and E_{night} were highest for both species.

The higher g_{night} recorded here are in the upper range reported in the literature (see Donovan et al. 1999 and Barbour and Buckley 2007 for reports of higher g_{night}). It has recently been suggested that g_{night} and E_{night} may be most prominent in fast growing shade intolerant tree species (Marks and Lechowicz 2007, Daley and Phillips 2006) that experience high water availability (Dawson et al. 2007). The *Populus* studied here fit this description. They are classified by the USDA as having rapid growth and being shade intolerant and except for the five days leading up to measurements in the Water'07 study all plants were provided daily with ample water. However, while our data support a trend for higher g_{night} and E_{night} in fast growing

species, differing growth rates within a species did not support this trend. Supplying plants with limiting soil nitrogen greatly decreased growth as seen by our biomass measured. The larger and faster growing sufficient nitrogen plants did not show increased g_{night} and E_{night} compared to limited nitrogen plants in the same study.

The large and variable g_{night} and E_{night} we found in both *P. angustifoila* and *P. balsamifera* suggests that nighttime water loss is an important component of the diurnal water budget. Estimates of stand and ecosystem water flux would be improved by inclusion of E_{night} in models and by inclusion of factors allowing for down-regulation of g_{night} and E_{night} in response to soil drying. We find, however, that g_{night} and E_{night} does not vary in response to soil nitrogen availability when secondary water stress effects and root restriction are avoided.

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Table 3.1: Overview of three studies, growth conditions and tests performed. Planting date is the date when rooted cuttings were transferred to pots and fertilization began. GE stands for gas exchange measures taken with the LI-6400 (Licor Inc., NE USA). The following products were used in the studies: Turface (fritted clay, Profile Products LLC, Buffalo Grove, IL, USA); liquid 20:10:20 NPK fertilizer (Peter's Peat-Lite Special, Scotts-Siera Horticultural Products); Osmocote Plus (slow-release fertilizer with micronutrients, Scotts-Siera Horticultural Products); micronutrient mix (Soluble trace element mix, Scotts-Siera Horticultural Products).

Study	Tests	Planting Date	Growth Media	Nutrients	Date of GE measures.
Nitrogen'05	Soil nitrogen availability Time of night	January 2005	$^1/_4$ sand and $^3/_4$ Turface	140 or 7 μg mL N (as nitrate) Hoagland solution	1-2 December (Nitrogen) 29-30 September (Time)
Water'07	Soil water availability Minimum leaf conductance	January 2005 (*P. balsamifera* were high N plants reserved from Nitrogen'05 study)	$^5/_7$ pine bark mulch and $^2/_7$ vermiculite	20:10:20 NPK and Osmocote Plus	14-15 May (Water) 20-27 April (minimum g)
Nitrogen'08	Soil nitrogen availability	April 2008	$^1/_4$ sand and $^3/_4$ Turface	140 or 7 μg mL N (as nitrate) Hoagland solution and additional micronutrient mix	1-2 July (block 1) 3-4 September (block 2)

Table 3.2: Gas exchange traits and leaf predawn water potential (Ψ_{leaf}) for two *Populus* species kept well watered (sufficient) or after experiencing a five-day drought (limiting). Gas exchange traits include nighttime conductance (g_{night}) and transpiration (E_{night}) and daytime conductance (g_{day}), transpiration (E_{day}) and photosynthesis. Trait values are arithmetic means (n=4) ± 1 SE. Wilcoxon two-sample test statistic (S) is presented for the effect of water treatment (*Water*). Bold indicates statistical significance.

Species	water treatment	g_{night} (mol m^{-2} s^{-1})	E_{night} (mmol m^{-2} s^{-1})	g_{day} (mol m^{-2} s^{-1})	E_{day} (mmol m^{-2} s^{-1})	Photo (μmol m^{-2} s^{-1})	Ψ_{leaf} (MPa)
P. angustifolia	sufficient	0.188 ± 0.078	0.72 ± 0.28	0.900 ± 0.152	11.83 ± 1.01	24.60 ± 1.39	-0.16 ± 0.03
	limiting	0.004 ± 0.004	0.02 ± 0.02	0.014 ± 0.005	0.32 ± 0.10	0.78 ± 0.81	-1.12 ± 0.26
	Water	**S=10, P<0.05**	**S=26, P<0.05**	**S=26, P<0.05**	**S=26, P<0.05**	**S=26, P<0.05**	**S=10, P<0.05**
P. balsamifera	sufficient	0.115 ± 0.033	0.48 ± 0.14	1.074 ± 0.263	11.59 ± 1.26	20.75 ± 2.18	-0.17 ± 0.03
	limiting	0.001 ± 0.002	0.01 ± 0.01	0.014 ± 0.004	0.31 ± 0.09	0.02 ± 0.72	-1.26 ± 0.16
	Water	**S=10, P<0.05**	**S=10, P<0.05**	**S=10, P<0.05**	**S=10, P<0.05**	**S=10, P<0.05**	**S=10, P<0.05**

Table 3.3: Gas exchange traits, leaf nitrogen (N) content of gas exchange leaves and biomass at harvest for studies that included a nitrogen limitation treatment. Trait values are model estimates ± 1 SE. F-values and associated degrees of freedom ($F_{\text{df num, df denom.}}$) are presented for the fixed model effect of nitrogen treatment *(Nitrogen*) and additionally in the Nitrogen'05 study for the fixed effects of species (*Species*) and the interaction term (*N x Spp*). Bold indicate statistical significance (* P<0.05, **P<0.01, ***P<0.001) and "-" indicates data were not collected.

Study & Species	Nitrogen treatment	g_{night} (mol $m^{-2}s^{-1}$)	E_{night} (mmol $m^{-2}s^{-1}$)	g_{day} (mol $m^{-2}s^{-1}$)	E_{day} (mmol $m^{-2}s^{-1}$)	Photo (μmol $m^{-2}s^{-1}$)	Leaf N (mg g^{-1})	Shoot mass (g)	Root mass (g)
Nitrogen'05									
P. angustifolia	sufficient	0.037 -0.007, +0.009	0.49 -0.08, +0.10	0.299 -0.043, +0.051	6.97 ± 0.86	18.4 ± 1.3	24.31 ± 1.35	224.1 -46.6, +58.5	-
	limiting	0.054 -0.012, +0.016	0.70 -0.14, +0.18	0.422 -0.073, +0.088	8.81 ± 1.02	18.0 ± 1.6	25.67 ± 1.71	28.8 -6.0, +7.6	-
P. balsamifera	sufficient	0.098 -0.023, +0.029	1.23 -0.25, +0.31	0.598 -0.078, +0.090	10.25 ± 0.89	22.6 ± 1.1	28.51 ± 1.19	96.9 -20.1, +25.4	-
	limiting	0.226 -0.056, +0.074	2.48 -0.54, +0.68	0.723 -0.102, +0.119	11.45 ± 0.95	20.0 ± 1.2	18.07 ± 1.33	17.4 -3.6, +4.6	-
	Nitrogen	**9.36** $_{1,35}$ **	**8.00** $_{1,35}$ **	**4.31** $_{1,34}$ *	**4.96** $_{1,34}$ *	2.00 $_{1,35}$	**15.60** $_{1,34}$ ***	**65.47** $_{1,8}$ ***	-
	Species	**26.01** $_{1,13}$ ***	**25.82** $_{1,13}$ ***	**15.61** $_{1,13}$ **	**11.48** $_{1,13}$ **	4.43 $_{1,13}$	1.17 $_{1,13}$	**8.30** $_{1,8}$ *	-
	*N*Spp*	1.28 $_{1,35}$	0.85 $_{1,35}$	0.38 $_{1,34}$	0.22 $_{1,34}$	1.02 $_{1,35}$	**26.41** $_{1,34}$ ***	0.51 $_{1,8}$	-
Nitrogen'08 - Block 1 (July)									
P. balsamifera	sufficient	0.055 ± 0.012	0.33 ± 0.06	0.685 ± 0.048	10.86 ± 0.61	25.9 ± 1.2	27.29 -1.78, +1.91	11.3 -1.5, +1.7	5.8 -0.9, +1.1
	limiting	0.045 ± 0.011	0.28 ± 0.06	0.598 ± 0.046	9.90 ± 0.59	22.0 ± 1.2	18.20 -1.16, +1.24	1.3 ± 0.2	1.1 ± 0.2
	Nitrogen	0.52 $_{1,7}$	0.68 $_{1,7}$	2.04 $_{1,7}$	1.53 $_{1,7}$	**8.80** $_{1,7}$ *	**18.40** $_{1,7}$ **	**255.65** $_{1,7}$ ***	**98.24** $_{1,7}$ ***
Nitrogen'08 – Block 2 (Sept)									
P. balsamifera	sufficient	0.308 ± 0.037	1.18 ± 0.10	1.323 ± 0.098	9.87 ± 0.42	28.8 ± 1.5	21.20 ± 1.25	35.9 -3.6, +4.0	27.9 -2.6, +2.9
	limiting	0.258 ± 0.038	1.11 ± 0.10	1.116 ± 0.098	8.87 ± 0.42	26.4 ± 1.5	21.35 ± 1.25	2.2 -0.2, +0.3	2.0 ± 0.2
	Nitrogen	1.92 $_{1,7}$	0.50 $_{1,7}$	2.24 $_{1,7}$	2.84 $_{1,7}$	1.34 $_{1,7}$	0.01 $_{1,7}$	**459.15** $_{1,7}$ ***	**338.26** $_{1,7}$ ***

Table 3.4: Leaf conductance (g_{night} or g_{day}) in two *Populus* species subjected to control treatments or treated to minimize stomatal conductance (g_{min}). Trait values are model estimates ± 1 SE. For means, different superscript letters in one row indicate significant differences according to Tukey's LSD. F-values and associated degrees of freedom ($F_{df\ num,\ df\ denom.}$) are presented for the fixed model effect (PROC MIXED ANOVA, treatment as fixed, genotype as random and plant as subject). F-values in bold indicate statistical significance (*** P<0.001).

Species	Time	Intact plant $g_{day\ /night}$ (mol m^{-2} s^{-1})	Branch in water $g_{day\ /night}$ (mol m^{-2} s^{-1})	Branch in ABA g_{min} (mol m^{-2} s^{-1})	Wilted leaf g_{min} (mol m^{-2} s^{-1})	Model effect
P. angustifolia						
	Night	$0.043^{a} \pm 0.011$	$0.065^{a} \pm 0.011$	$-0.001^{b} \pm 0.011$	$0.005^{b} \pm 0.011$	**$11.06_{3,\ 21}$ *** **
	Day	$0.573^{a} \pm 0.065$	$0.582^{a} \pm 0.065$	$0.010^{b} \pm 0.065$		**$37.10_{2,\ 14}$ *** **
P. balsamifera						
	Night	$0.059^{a} \pm 0.008$	$0.073^{a} \pm 0.007$	$0.000^{b} \pm 0.007$	$0.002^{b} \pm 0.007$	**$36.17_{3,\ 20}$ *** **
	Day	$0.682^{a} \pm 0.073$	$0.554^{a} \pm 0.073$	$0.010^{b} \pm 0.073$		**$23.76_{2,\ 14}$ *** **

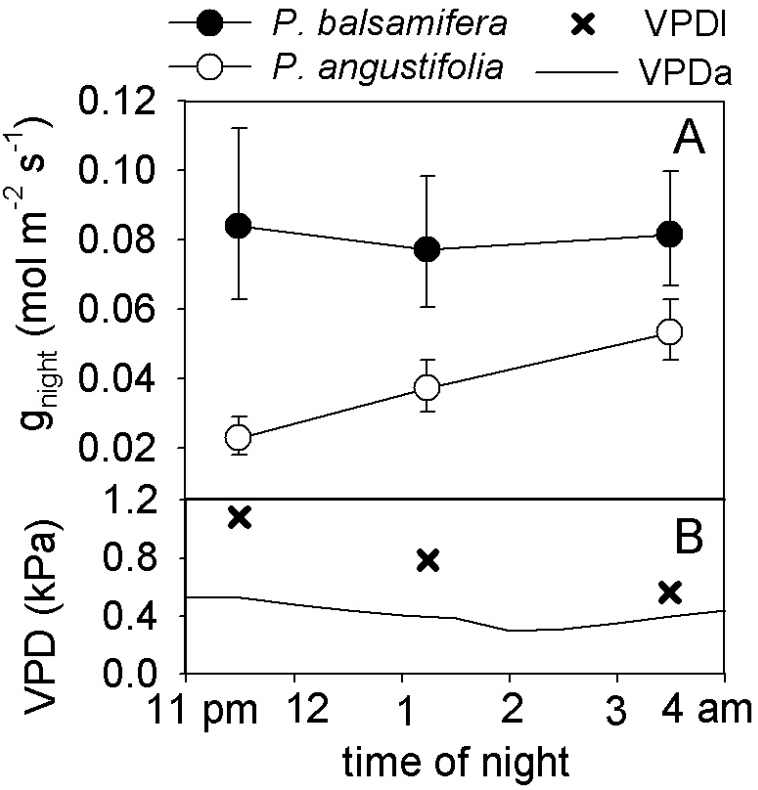

Figure 3.1: Variation in *Populus angustifolia* and *P. balsamifera* nighttime leaf conductance (g_{night}) across a single night during the Nitrogen'05 study. Included are measures of leaf-to-air vapor pressure deficit (VPDl) and independent measurements of atmospheric vapor pressure deficit (VPDa). Points represent means ± 1 SE, n=6 for *P. angustifolia* and n=4 for *P. balsamifera*.

CHAPTER 4

NIGHTTIME TRANSPIRATION CAN DECREASE HYDRAULIC REDISTRIBUTION[3]

[3] Howard, A.R., M.W. van Iersel, J.H. Richards and L.A. Donovan. To be submitted to *Plant, Cell and Environment*.

ABSTRACT

C_3 plants dominate many landscapes and play a critical role in ecosystem water cycling. At night, water losses from these plants can include both transpiration (E_{night}) from the canopy and hydraulic redistribution (HR) from roots. We tested the hypothesis that the magnitude of HR is limited by the magnitude of E_{night} in a greenhouse study using *Artemisia tridentata, Helianthus anomalus* and *Quercus laevis*. Plants were grown with their roots split between two compartments. HR was initiated by briefly withholding all water, followed by watering only one rooting compartment. HR was defined as the difference between lowest and highest soil water potential during a specified diel time period in the compartment from which water was withheld, as measured by screen cage psychrometers. All species showed substantial HR and E_{night} under our study conditions. Bagging plant canopies over night to suppress E_{night} increased the magnitude of HR during the treatment period (HR_N) for *A. tridentata* and *H. anomalus* by 73% and 33% respectively, but had no effect on HR_N by *Q. laevis*. Total daily HR (HR_T) was positively correlated with the soil water potential gradient between the rooting compartments. This gradient was in turn correlated with light and/or atmospheric vapor pressure deficit (VPDa) the prior day. For *A. tridentata* a negative correlation was found between HR_T and nighttime VPDa. Ecological implications of the impact of E_{ngiht} on HR may include decreased plant productivity during dry seasons, altered ecosystem water flux patterns and reduced nutrient cycling in drying soils.

KEY WORDS: transpiration, nighttime, nocturnal, hydraulic redistribution, hydraulic lift, *Helianthus*, *Artemisia*, *Quercus*

INTRODUCTION

At night, plants facilitate translocation of water in the environment though two major pathways: (1) movement from the roots to the canopy and subsequent loss to air (nighttime transpiration; E_{night}) and (2) movement from one part of the root system to another and subsequent loss to the soil (hydraulic redistribution; HR). Separately, the processes of E_{night} and HR have been demonstrated to be common in a wide range of plant species across many ecosystems (Caldwell et al. 1998; Caird et al. 2007a, Dawson et al. 2007). It has also been shown that HR and E_{night} can co-occur in the same plant in natural populations (Donovan et al. 2003). Several authors have suggested that naturally occurring E_{night} may limit HR (Hultine et al. 2003, Caird et al. 2007a, Dawson et al. 2007, Scholz et al. 2008). Previous studies have shown that when "E_{night}" is experimentally increased by nighttime lighting, HR is diminished (Caldwell and Richards 1989, Caldwell 1990, Bauerle et al. 2008), suggesting that these two pathways are competitive. However, nighttime lighting likely has unintended impacts to processes other than E_{night} and HR. We provide the first controlled test of the impact of naturally occurring E_{night} on the magnitude of HR.

Hydraulic redistribution occurs when a gradient in soil moisture exists across the root system of a plant. Water moves passively from higher soil water potential (Ψ_s) to lower (more negative) Ψ_s using roots as a conduit. Water flow in the root xylem can occur in an upward, downward or lateral direction (Richards and Caldwell 1987; Brooks et al. 2002; Scott et al. 2008). Traditionally, it was thought that HR occurred only when transpiration was minimal, such as at night or when the canopy was bagged. However there is now evidence that HR can occur at the same time as substantial transpiration both during the day and night as long as the

necessary environmental conditions are present to drive each process (Scholz et al. 2002, Donovan et al. 2003).

Substantial E_{night} occurs when stomata remain partially open at night and a vapor pressure difference between the leaf and air (VPDl) drives water loss. Water lost via transpiration is replaced by proximal water flow in the xylem of roots and upward flow in stem xylem towards leaves. Recent studies demonstrate that E_{night} can be a large fraction of daytime transpiration (5-30%, Benyon 1999; Snyder et al. 2003; Bucci et al. 2004, 2005; Daley and Phillips 2006; Caird et al. 2007b; Cavender-Bares et al. 2007; Dawson et al. 2007; Fisher et al. 2007; Howard and Donovan 2007; Scholz et al. 2007). Such substantial E_{night} can decrease predawn leaf water potential (Donovan et al. 2001, 2003; Bucci et al. 2005; Kavanaugh et al. 2007). Since HR is a passive process driven by water potential gradients, reduced canopy water potential, due to E_{night}, may increase the portion of water moving towards the canopy and decrease flow towards areas of dry soil.

Several consequences of HR and E_{night} to plants have been proposed. Evidence suggests that HR reduces surface soil evaporative loss (when redistribution is downward), increases next-day transpiration (when redistribution is upward), prolongs fine root lifespan, increases microbial and mycorrhizal activity in the rhizosphere, and overall improves carbon, water and nutrient acquisition (Caldwell et al. 1998; Domec et al. 2004; Lee et al. 2005; Querejeta et al. 2003; Bauerle et al. 2008; Scott et al. 2008; Aanderud and Richards *submitted*). The consequences of E_{night} for plants remain less clear. Water loss at night without concomitant carbon gain could decrease productivity in water-limited environments where HR often occurs, but a fitness cost of E_{night} has not yet been documented. A proposed benefit of E_{night} is delivery of soil-mobile nutrients to roots for uptake (McDonald et al. 2002; Snyder et al 2008; but see also Christman et

al. *submitted*). It seems likely that a competitive interaction between E_{night} and HR could result in significant impacts to plant productivity, plant water use, and nutrient cycling.

Our study species, *Artemisia tridentata*, *Quercus laevis* and *Helianthus anomalus*, were chosen because substantial E_{night} and/or HR have been documented for each (Caldwell et al. 1998; Espeleta et al. 2004, Caird et al. 2007a), and they represent a diversity of growth forms. The species are native to habitats in which the necessary environmental conditions for E_{night} (substantial nighttime VPDl) and HR (substantial Ψ_s gradient) often exist concurrently. *Artemisia tridentata* and *H. anomalus* occur in the Western United States. *Artemisia tridentata* is a dominant shrub over much of the Great Basin while *H. anomalus* is a large annual endemic to desert sand dunes in Utah and Arizona. *Quercus laevis* is a tree native to the sand-hills of the S.E. United States. Our objective was to use controlled manipulations to test the effect of E_{night} on the magnitude of HR of soil water by plant roots. Additionally, we assessed the impact of environmental variables on the magnitude of HR. These included the magnitude of the Ψ_s gradient, light, and atmospheric vapor pressure deficit (VPDa) during the day preceding HR and VPDa during the night of HR.

MATERIALS AND METHODS

Artemisia tridentata ssp. *tridentata* seedlings were collected near Eureka in Juab County, UT, USA and grown for 1.5 years at the University of Georgia Greenhouse facilities, Athens GA, USA prior to this study. *Quercus laevis* acorns were obtained from Sheffield Seeds (Sheffield Seed Co. Inc., Locke NY, USA) and grown for two years prior to study. *Helianthus anomalus* seeds were collected from Little Sahara Dunes Recreational Area in Juab County, UT, germinated in Petri dishes, and seedlings transferred to pots four months prior to experimentation.

All plants were grown in a soil mix consisting of 3/4 Turface (fritted clay, Profile Products LLC., Buffalo Grove, IL, USA) and 1/4 sand. After initial root development plants were removed from original pots and soil was carefully washed away from roots. Two tree pots ("Tall-one" Treepot, Stuewe and Sons Inc., Corvallis, OR, USA) measuring 35 x 10 x 10 cm were placed side-by-side and a notch approximately six cm deep was cut in the top of the adjoining sides. Roots were split approximately 50:50 between the two pots with the central stem resting above the notch (Figure 4.1). Soil was filled up to the base of the stem and 15 g of a slow release fertilizer (Osmocote Plus, Scotts-Sierra Horticultural Products) containing micronutrients was applied to each rooting compartment. Prior to experimentation both rooting compartments were watered daily and fertilized weekly with 20:10:20 NPK soluble fertilizer (Peter's Peat-Lite Special, Scotts-Sierra Horticultural Products). Upon initiation of HR the height of soil in each rooting compartment was checked and surface soil was removed as needed to ensure that the soil volumes in the two compartments were not in contact with each other.

Experiments were conducted in a heated and lighted greenhouse during April 2006 (*A. tridentata*), June 2006 (*Q. laevis*) and May-June 2007 (*H. anomalus*). Photosynthetically active radiation (PAR), air temperature and air humidity in the greenhouse were logged hourly with an LI-190 PAR sensor (LiCor Inc., Lincoln NE, USA) and a Vaisala humidity and temperature sensor (Vaisala, Vantaa, Finland) both interfaced with a CR23X data logger (Campbell Scientific, Logan, UT, USA). Light conditions and atmospheric vapor pressure deficit (VPDa) in the greenhouse (calculated from temperature and humidity measures) during the period of study for each species are given in Table 4.1. During the study periods nighttime temperature in the greenhouse was maintained above 20 ^{o}C and daytime temperature was maintained below 33 ^{o}C.

Instrumentation

Ψ_s was assessed with individually calibrated (Brown and Bartos 1982) screen-cage psychrometers (Merrill Specialty Equipment, Logan, UT, USA). Every plant was instrumented with three psychrometers (Figure 4.1). The single psychrometer in the center of the rooting compartment that was watered prior to and during experimentation (wet compartment; readings of Ψ_{sW}) confirmed that soil in this rooting compartment stayed near 0 MPa during HR measurements. The other two psychrometers were placed one-third and two-thirds up from the base of the rooting compartment from which water was withheld during experimentation (un-watered, drier compartment; readings of Ψ_{sD}) and were used to assess HR. Psychrometers were inserted into the soil though holes drilled in the side of each pot. After insertion, holes were sealed closed around the insulated psychrometer wire using silicone gel. Plants were given a minimum of one week for roots to re-colonize the disturbed soil before initiation of HR. To minimize the effect of temperature fluctuations on measurements, plant pots and psychrometers were placed in a 0.6 x 1.2 x 2.4 meter Styrofoam box and surrounded with foam packing chips. Psychrometers were interfaced with CR7 data loggers (Campbell Scientific, Logan, UT, USA) and Ψ_s was logged every half hour for *A. tridentata* and *Q. laevis* and every hour for *H. anomalus*. To further minimize the effect of temperature fluctuation on measurements, psychrometer wires running between pots and the data loggers were bundled into pipe insulation and coated with reflective silver tape and the data loggers were kept under a canopy that allowed air circulation but blocked direct sunlight. These precautions allowed water potential measurements with minimal zero offset values (within 10 μV of zero). Water potentials were corrected for the zero offset following Brown and Bartos (1982).

HR and E_{night} treatments

To initiate HR water was withheld from plants until Ψ_{sW} and Ψ_{sD} (i.e. both root compartments) declined to approximately –1.0 MPa (two to four days). When this was achieved HR was initiated by applying water to the rooting compartment with a single psychrometer until field capacity was reached (Ψ_{sW} raised to approximately 0 MPa). From this time on, no water was applied to the rooting compartment with two psychrometers. Watering of the compartment with the single psychrometer continued one to three times daily to maintain the soil near field capacity and provide a Ψ_s gradient between the two rooting compartments to drive HR. Mean Ψ_{sW} (± 1 SE), logged every half-hour to hour during the nine to twelve days/nights of E_{night} manipulation (HR_N dates, Table 4.1), was -0.028 (±0.0003), -0.025 (±0.001) and -0.023 (±0.002) MPa for *A. tridentata*, *H. anomalus* and *Q. laevis* respectively.

E_{night} treatments occurred on nine to twelve consecutive nights (HR_N dates; Table 4.2). For each species plants were randomly divided into two groups. On each night one group had E_{night} suppressed and the other group was a control. Groups alternated daily between control and suppression treatments. E_{night} suppression consisted of enclosing a plant canopy and a wet paper towel in a plastic bag and securing the bag around the plant stem. Bagging minimizes E_{night} by trapping water vapor transpired from leaves and evaporated from the wet towel. The trapped water vapor causes air humidity in the bag to rise, thereby minimizing the gradient needed to drive transpiration. Bags were secured up to one hour before lights-off in the evening and were removed during the half-hour before lights came on in the morning. The E_{night} treatment period was 9 to 10.5 hours long.

The criteria for identifying diel fluctuations of Ψ_{sD} as HR were that they did not mirror temperature fluctuations and included a pattern of increasing Ψ_{sD} in the evening and night but

decreasing Ψ_{sD} in the morning (Espeleta et al. 2004). HR was calculated in two different ways: total daily HR (HR_T) and HR during E_{night} treatments (HR_N). HR_T was calculated as the highest diel Ψ_{sD} – the lowest diel Ψ_{sD} occurring with offsets +/- 10 μV during the 24 hour period from 10:00 am to 9:00 am the following day (Table 4.1). The broad time interval allowed us to find the 24 hour highest and lowest Ψ_{sD} and the offset allowance was narrow enough to exclude obviously incorrect data while not so narrow as to omit a large amount of daytime data. HR_T was calculated for all nights of the experiment (HR_T dates; Table 4.1) and included only control (unbagged) plants during the subset of nights used for E_{night} manipulation. Daily HR_T was compared to daily PAR and VPDa (mean of hourly logs from 7:00 am to 7:00 pm) and nightly VPDa (mean of hourly logs from 8:00 pm to 6:00 am).

HR_N was calculated as the highest Ψ_{sD} minus the lowest Ψ_{sD} occurring with offsets of ± 5 μV (Brown and Bartos 1982). HR_N only included measures of Ψ_{sD} during the E_{night} treatment period (ie. after all bags used to minimize E_{night} were secured and before any bags were removed)(HR_N time constraints; Table 4.1). Ambient greenhouse temperature at night was relatively constant allowing a more stringent zero offset cut-off for HR_N than for HR_T.

Gas exchange procedures

Leaf-level measurements of daytime and nighttime gas exchange were made with a portable photosynthesis system (LI-6400, LI-COR Inc., Lincoln, NE, USA) as described in Howard and Donovan (2007). Nighttime measures were taken between 12:00 am and 3:00 am North America eastern standard time (EST) and daytime measures were taken between 12:00 pm and 3:00 pm EST. Measurements were made on a mature leaf of each *H. anomalus* and *Q. laevis* and on a branch of each *A. tridentata*.

For *A. tridentata* nighttime gas exchange measures were taken on control plants nightly from 9 to 17 April 2006 (the nine nights of E_{night} treatments). Daytime measures were taken on 13 April 2006. For *H. anomalus* daytime and nighttime measures were taken on all plants on 8 June 2007 (six nights after completion of E_{night} treatments). For *Q. laevis* nighttime measures were taken on all plants on 1 July 2006 (four nights after completion of E_{night} treatments). Daytime measures for *Q. laevis* were also taken after completion of E_{night} treatments but they have been omitted due to an irrigation failure the morning of measurements.

Leaves of *H. anomalus* did not fill the standard LI-6400 chamber (2 by 3 cm) and were marked and scanned (Winfolia, Regent Instruments Inc., Quebec, Canada) to obtain leaf area within the chamber. Leaves on the branches of *A. tridentata* were detached, spread out and scanned to obtain total area within the chamber. This provided a very conservative estimate of gas exchange measures for this species since the natural bunching of leaves on branches increases boundary resistance and results in shading of many leaves during daytime measurements. The chamber red/blue LED (6400-02B, LI-COR Inc., Lincoln, NE, USA) light source was turned off at night and was set to 1500 or 2000 μmol m^{-2} s^{-1} during the day. Airflow was 100 to 200 μmol s^{-1} at night and 500 to 700 μmol s^{-1} during the day. The block temperature surrounding the IRGAs in the chamber head was set to match ambient and CO_2 was supplied at 400 μmol mol^{-1}. Chamber relative humidity was manually manipulated to a target 5% to 10% above ambient. Instrument error was assessed after every three to four leaf measurements with the chamber empty or clamped onto dry paper. Instrument error at night for transpiration was 0.04 ± 0.01, 0.03 ± 0.01 and 0.10± 0.03 mmol m^{-2} s^{-1} during collection of *A. tridentata*, *H. anomalus* and *Q. laevis* data, respectively, and was always substantially lower than plant measures.

Biomass

Above and below ground biomass was collected for all *H. anomalus* and *Q. laevis* within two weeks and three months, respectively, of completion of the extended study. *Artemisia tridentata* were reserved for use in a future study. Biomass was dried at 60 ^{o}C, weighed and used to calculate root weight ratio (root/total mass).

Statistics

To test the effect of E_{night} treatments on HR_N, for each species we used a repeated-measures, cross-over design (Neter et al. 1990) in which plants alternated (crossed-over) between control E_{night} and suppressed E_{night} treatments from day to day. The *A. tridentata* study contained 12 plants, the *H. anomalus* study seven and the *Q. laevis* study eight. By applying the E_{night} suppression treatment to half of the set of plants, we assessed the impact of E_{night} on HR_N within a night, which eliminated potentially confounding environmental variables that differ across nights. And by alternating which plants are bagged across many nights we accounted for inherent differences in the magnitude of HR_N by individual plants and any carry-over effects.

Magnitude of HR_N and highest and lowest Ψ_{sD} during the period of E_{night} treatments were analyzed in a repeated-measures mixed model ANOVA with compound symmetric covariance structure (PROC MIXED; SAS Institute, 2004, version 9.1). E_{night} treatment (suppressed or control) and psychrometer position (bottom or top psychrometer measuring Ψ_{sD}; Figure 4.1) were fixed effects and plant was the subject in which repeated measures occurred. An interaction between psychrometer position and E_{night} treatment was also tested but was never significant and was subsequently dropped from the analysis. The effect of environmental variables including daytime VPDa and PAR and nighttime VPDa on mean daily HR_T was

analyzed for each species using a linear regression (PROC REG; SAS Institute, 2004, version 9.1).

RESULTS

Substantial hydraulic redistribution occurred in all species, irrespective of E_{night} manipulation. For all species Ψ_{sD} declined through the morning and into the afternoon, then began to increase mid to late afternoon and continued increasing through the night (Figure 4.2). HR_T averaged across all control nights was similar for all species (0.43 ± 0.02 MPa in *A. tridentata*, 0.45 ± 0.02 MPa in *H. anomalus*, 0.42 ± 0.06 MPa in *Q. laevis*). For all species, the magnitude of HR_T was much larger than HR_N since rapid increase in Ψ_{sD} often occurred during late afternoon (Figure 4.2).

All species exhibited significant E_{night} and nighttime stomatal conductance (g_{night}) when plant canopies were not bagged to suppress E_{night} (Table 4.2). E_{night} and g_{night} were highest for *H. anomalus* and lowest for *A. tridentata*. Experimental suppression of E_{night} by canopy bagging resulted in a significant increase in HR_N in *A. tridentata* (73% increase) and *H. anomalus* (33% increase), but not in *Q. laevis* (Table 4.3, Figure 4.3). E_{night} suppression affected the highest, but not the lowest Ψ_{sD} recorded overnight for each species and used to calculate HR_N. In other words, suppressing E_{night} did not affect the Ψ_s gradient at the start of the dark period but resulted in a larger overnight increase in Ψ_{sD}.

For *Q. laevis*, HR_N was not affected by E_{night} treatments (Figure 4.3). Although there was a significant effect of E_{night} suppression on highest Ψ_{sD} during the HR_N time period, this was paralleled by a non-significant effect on the nightly low for Ψ_{sD} (Table 4.3). Thus, the result was that HR_N (highest Ψ_{sD} – lowest Ψ_{sD} between 8:30pm and 5:30am) by *Q. laevis* was not different between nights when plants transpired naturally and nights when E_{night} was suppressed (Table

4.3). Considering each species, Ψ_{sD} only differed between the psychrometer positions in the un-watered rooting compartment for *Q. laevis*. For *Q. laevis* mean Ψ_{sD} (± 1 SE) was lower in the upper portion of the rooting compartment (-0.55 ± 0.09 MPa) than in the lower portion (-0.42 ± 0.09 MPa) (Table 4.3). The root mass ratio for *Q. laevis* was 0.563, for *H. anomalus* was 0.181, and biomass was not collected for *A. tridentata*.

HR_T in control (unbagged) plants was significantly correlated with several environmental parameters. HR_T for all species was positively related to the Ψ_s gradient (Ψ_{sW}- Ψ_{sD}) between the watered and un-watered rooting compartments at the start of HR_T (Figure 4.4). Ψ_{sW}- Ψ_{sD} was in turn significantly correlated with same day mean photosynthetically active radiation (PAR) and/or mean daytime VPDa (Table 4.4). Ψ_{sW}- Ψ_{sD} was generally larger for *A. tridentata* compared to the other two species. However, a lower slope for the correlation between HR_T and $\Psi_{sW} - \Psi_{sD}$ for *A. tridentata* compared to the other species resulted in a similar magnitude of HR_T observed across all species (Figure 4.4).

For *H. anomalus* and *Q. laevis*, mean nighttime VPDa was positively correlated with Ψ_{sW}- Ψ_{sD} ($P<0.01$). Therefore an independent effect of VPDa at night on HR_T could not be tested. However, in *A. tridentata*, removal of two data points from cloudy days/nights where the Ψ_{sW} - Ψ_{sD} was lower than 0.8 MPa allowed for VPDa at night to vary independently ($P=0.22$) of the Ψ_{sW} - Ψ_{sD} gradient. On the remaining 25 nights of HR_T measurement, HR_T in *A. tridentata* was negatively correlated to mean nighttime VPDa (Figure 4.5).

DISCUSSION

Substantial naturally occurring E_{night} can reduce overnight water potential recovery by plant canopies (Donovan et al. 2001, 2003; Bucci et al. 2005; Kavanaugh et al. 2007) but its affect on the magnitude of HR was not well understood. Given the passive nature of HR, in

which water movement is driven along water potential gradients, we hypothesized and confirmed that naturally occurring E_{night} would reduce the magnitude of HR. For *A. tridentata* and *H. anomalus* the impact of E_{night} on the magnitude of HR was very large and suppression of E_{night} resulted in a 73 and 33% increase in HR_N, respectively. Scholz et al. (2002) noted that reverse sap flow in a lateral root (HR) during the daytime increased linearly as the soil-to-leaf water potential difference decreased. More recently, Scholz et al. (2008) bagged a tree canopy for 24 hours and recorded increased HR during this time period. They noted in these studies, that the plant shoot and soil seemed to act as competing sinks for hydraulically redistributed water. We provide new correlative data and the first controlled, manipulative test showing that naturally occurring E_{night} can decrease the magnitude of HR.

An effect of reducing E_{night} on HR_N was not seen in *Q. laevis*. This is not due to differences in the ability of the species to conduct HR since, in the absence of E_{night} manipulation, the magnitude of HR_T was similar among all species. It was also not due to lack of transpiration at night since *Q. laevis* neither exhibited the lowest E_{night} nor did the study occur during a period of particularly low nighttime VPD (Table 4.1, 4.2). Amongst the three species a significant effect of psychrometer position on Ψ_{sD} was only found in *Q. laevis*. Lower Ψ_{sD} recorded in the soil close to the top of the un-watered rooting compartment, could be due to evaporation of water from the soil surface, or high root density resulting in large daily decline in Ψ_{sD} in this region. These explanations do not involve competing water sinks at night, and therefore the significant effect of psychrometer position does not explain the lack of effect of E_{night} suppression on HR_N for *Q. laevis*.

The lack of an effect of reducing E_{night} on HR_N could have been caused by a relatively low canopy biomass compared to root biomass in the young *Q. laevis* used in this study. The

root weight ratio for *Q. laevis* was three times smaller than that for *H. anomalus*, which minimized the impact of whole canopy E_{night} on $HR_{N.}$ Stem and taproot water storage and the amount of water at night required for phloem sap flux might also act as competing water sinks, additionally minimizing the impact of E_{night} on HR. Although biomass measures were not collected on *A. tridentata*, branches were densely covered in leaves (personal observation) and these plants likely had a relatively high root weight ratio compared to *Q. laevis*. We think it unlikely that the lack of treatment effect for *Q. laevis* is because it is a tree as compared to the shrubby *A. tridentata* and the annual *H. anomalus*, but additional tests with other species will be needed to rule-out any effects of life form.

We expect our measured effects of E_{night} on HR_N in a greenhouse experiment to be comparable to or even underestimate what might happen in natural plant populations. Plants in this study exhibited g_{night} rates comparable to previously published estimates for these species (*A. tridentata*, *H. anomalus*) or closely related species (*Q. rubra*) (Caird et al. 2007a and references therein). The range of Ψ_s and magnitude of HR_T in this study are within the range of what has been seen in field studies for *A. tridentata* (Richards and Caldwell 1987; Caldwell and Richards 1989; Caldwell et al. 1998; Aanderud and Richards *submitted*). The range of Ψ_s and magnitude of HR_T in this study represent the drier and larger end of what has been seen for *Q. laevis* (Espeleta et al. 2004). To our knowledge this is the first study of HR in *H. anomalus*. Environmental conditions in natural plant populations might promote even greater effects because higher nighttime VPDa and increased wind speed possible in the field have both been correlated with increased E_{night} in natural populations (Benyon 1999, Oren et al. 2001, Daley and Philips et al. 2006, Dawson et al. 2007, Kavanagh et al. 2007), which could result in a larger reduction in HR.

The magnitude of HR_T was positively correlated with the Ψ_s gradient between the watered and un-watered rooting compartments (Ψ_{sW} - Ψ_{sD}), explaining from 42% to 82% of the variation in HR_T (Figure 4.4). This is logical given that HR is a passive process in which water moves through roots along a gradient from higher to lower Ψ_s. Differences in the slope of the correlation among species (Figure 4.4) could be due to differences in xylem conductivity, production and maintenance of fine roots in drying soil, differences in abundance and regulation of aquaporins, and effects of root architecture (combined with xylem anatomy) on branch conductance (Caldwell et al. 1998, Espeleta et al. 2004, Domec et al. 2004, Hultine et al 2003, Vandeleur et al. 2005; Valenzuela-Estrada *submitted*). Ψ_{sW} - Ψ_{sD} on a particular day was correlated, often quite strongly, with mean PAR and/or mean VPDa during that day (Table 4.4). Since one rooting compartment (Ψ_{sW}) was kept near field capacity, Ψ_{sW} - Ψ_{sD} was driven by changes in Ψ_{sD}. Under conditions of high PAR (and concomitant high VPDa) open stomata contribute to both high photosynthesis and high transpiration. Our findings agree with data from a natural population showing that shading by clouds, which minimizes daytime Ψ_{sW} - Ψ_{sD} gradients, limits HR the following night (Williams et al. 1993).

A relationship between E_{night} and HR can be examined using VPDa as a surrogate for E_{night}, because stomatal regulation does not fully compensate for changes in VPDa (Barbour and Buckley 2007, Caird et al. *submitted*, Howard unpublished). For *A. tridentata*, we found that as nighttime VPDa increased, the magnitude of HR_T decreased, above a Ψ_s threshold. We were only able to test for this relationship above a Ψ_s threshold of 0.8 MPa, and only for *A. tridentata*, because these data fulfilled the necessary condition that VPDa at night and Ψ_{sW} - Ψ_{sD} not be significantly correlated. Our result of a negative relationship between nighttime VPDa and the magnitude of HR is consistent with that of Hultine et al. (2003) for *Fraxinus velutina*. They

found that HR, measured as reverse sap flux in a lateral root, declined strongly with increasing VPDa at night, although only in one tree. The correlative evidence from our results and those of Hultine, in addition to the manipulative results above, supports a role of naturally occurring E_{night} in limiting the magnitude of HR.

The impact of E_{night} on HR_N was large for two of our three study species and this may have substantial impacts on both plant water-use and ecosystem hydrology where HR is common. Many studies have demonstrated that HR benefits plants by increasing next-day transpiration, prolonging the growing season and improving growth (Caldwell and Richards 1989, Emerman and Dawson 1996, Caldwell et al. 1998; Lee et al. 2005, Scott et al. 2008). Thus a negative relationship of HR with E_{night} means that E_{night} not only represents water lost without concomitant carbon gain in C_3 plants, but it may also reduce total carbon gain by reducing following day transpiration and shortening the growing season. A negative impact of E_{night} on HR may also affect ecosystem hydrology. Recent efforts to model the effect of HR on ecosystem processes suggest that HR may decrease the water table and stream flow (Jackson et al. 2000) and may even affect seasonal air temperature cycles (Lee et al. 2005). These models generally do not consider E_{night} and thus may overestimate some HR effects on ecosystem processes.

Another suggested benefit of HR is to improve nutrient acquisition by wetting the rhizosphere and increasing activity of mycorrhizae and microbes (Caldwell et al. 1998, Querejeta et al. 2003; Aanderud and Richards *submitted*). Thus substantial E_{night} may decrease nutrient uptake in drying soils by decreasing HR. However, E_{night} has itself been proposed as a mechanism to enhance uptake of mobile soil nutrients (McDonald et al. 2002; Howard and Donovan 2007; Scholz et al. 2007, Snyder et al 2008). The relative nutrient benefit or cost of

each process may depend on soil moisture level and nutrient form. E_{night} may enhance uptake of nitrate when it is readily available in the soil and soil moisture is abundant, but likely reduces the beneficial effects of HR on decomposition, microbe activity and nutrient mobilization and uptake in drying soils.

Here we provide results from a controlled, manipulative experiment, and supporting correlative data, to demonstrate that naturally occurring E_{night} can substantially reduce the magnitude of HR. This relationship may have important consequences for plant productivity, ecosystem hydrology and nutrient cycling. However, the size of the impact of E_{night} on HR differed between the species investigated here and further exploration of the relationship between multiple, potentially competitive water sinks and flows at night is still needed.

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Table 4.1: Light conditions and atmospheric vapor pressure deficit (VPDa) in the greenhouse and date and time periods used for measurement of total hydraulic redistribution (HR_T) and hydraulic redistribution during E_{night} treatments (HR_N). VPDa is a mean of hourly logs from 8pm to 6am (nighttime) and from 7am to 7pm (daytime) ± 1 SE during the HR_N date range. Approximate times are indicated by "~". Sunrise and Sunset times are for Athens, GA during the HR_N date range according to the U.S. Naval Observatory, Astronomical Applications Department, Washington, DC, USA.

Species	Mean VPDa (kPa)		Sunrise (am)	Lights-on (am)	Sunset (pm)	Lights-off (pm)	HR_N time constraints	HR_N dates	HR_T time constraints	HR_T dates
	Daytime	Nighttime								
A. tridentata	2.14 ± 0.05	0.99 ± 0.02	7:00 – 7:10	~7:00	7:59 – 8:05	~7:30	8:00 pm - 6:00 am	8 – 16 April	10 am – 9 am next day	8 April - 4 May
H. anomalus	2.19 ± 0.04	0.76 ± 0.03	6:23 – 6:28	~ 5:40	8:32 – 8:39	~7:30	8:00 pm - 5:00 am	21 May – 1 June	10 am – 9 am next day	20 May - 10 June
Q. laevis	1.54 ± 0.03	0.91 ± 0.03	6:22 – 6:24	~ 7:00	8:46 – 8:48	~8:00	8:30 pm - 5:30 am	14 – 25 June	10 am – 9 am next day	7 – 25 June

Table 4.2: Instantaneous gas exchange measures during the day and night for plants allowed to transpire naturally. Values are means ± 1 SE. Except for nighttime measures on *A. tridentata* readings were taken on a single day or night and averaged (n=7-12). For *A. tridentata* at night, the mean is an average of measures taken on control (unbagged) plants on each night of the E_{night} manipulation study (n=54).

Species	Nighttime		Daytime		
	conductance (mol m^{-2} s^{-1})	transpiration (mmol m^{-2} s^{-1})	conductance (mol m^{-2} s^{-1})	transpiration (mmol m^{-2} s^{-1})	photosynthesis (μmol m^{-2} s^{-1})
A. tridentata	0.014 ± 0.001	0.18 ± 0.01	0. 088 ± 0.010	3.12 ± 0.30	5.87 ± 0.39
H. anomalus	0.131 ± 0.023	1.01 ± 0.14	1.60 ± 0.00	18.14 ± 0.69	34.8 ± 2.7
Q. laevis	0.051 ± 0.008	0.39 ± 0.05			

Table 4.3: Effect of minimizing nighttime transpiration (E_{night}) on soil water potential (Ψ_{sD}) and magnitude of hydraulic redistribution (HR_N) between a rooting compartment maintained near field capacity and an un-watered compartment in which Ψ_{sD} readings were taken. Data are for the 9 – 12 days during the period of E_{night} treatments for each species (HR_N dates). Measures of Ψ_{sD} are based on psychrometer offsets of ± 5 μV and shown as lsmeans ± 1 SE. F-values and degrees of freedom are presented for each model effect ($F_{df\ num,,\ df\ denom.}$; PROC MIXED repeated measures, subject=plant). Bold indicates statistical significance (*P<0.05; **P<0.01; ***P<0.001).

Species	Treatment	Lowest Ψ_{sD} (MPa)	Highest Ψ_{sD} (MPa)	HR_N (MPa)
A. tridentata	Suppressed E_{night} nights	-1.12 ± 0.09	-0.86 ± 0.08	0.26 ± 0.02
	Control E_{night}	-1.13 ± 0.09	-0.97 ± 0.08	0.15 ± 0.02
	E_{night} treatment	$0.07_{1,11}$	**$14.43_{1,11}$ ****	**$80.23_{1,11}$ *****
	Psychrometer position	$2.00_{1,7}$	$0.87_{1,7}$	$1.73_{1,7}$
H. anomalus	Suppressed E_{night} nights	-0.43 ± 0.09	-0.23 ± 0.06	0.21 ± 0.04
	Control E_{night}	-0.44 ± 0.09	-0.28± 0.06	0.16 ± 0.04
	E_{night} treatment	$0.05_{1,6}$	**$10.00_{1,6}$ ***	**$18.98_{1,6}$ ****
	Psychrometer position	$1.49_{1,5}$	$0.92_{1,5}$	$2.53_{1,5}$
Q. laevis	Minimum E_{nights} nights	-0.64 ± 0.12	-0.47 ± 0.09	0.17 ± 0.03
	Control E_{night}	-0.67 ± 0.12	-0.50 ± 0.09	0.17 ± 0.03
	E_{night} treatment	$3.58_{1,7}$	**$13.13_{1,7}$ ****	$0.01_{1,7}$
	Psychrometer position	$2.98_{1,3}$	**$104.26_{1,3}$ ****	**$29.62_{1,3}$ ***

Table 4.4: Pearson correlation of photosynthetically active radiation (PAR) and daytime atmospheric vapor pressure deficit (VPDa) with soil water potential gradient ($\Psi_{sW} - \Psi_{sD}$) for each species during the dates of HR_T measurement. Data analyzed were daily means of ($\Psi_{sW} - \Psi_{sD}$) based on psychrometer offsets of ± 10 μV for control plants at time of lowest Ψ_{sD} (start of HR_T) and daily means of VPDa and PAR logged hourly between 7am and 7pm. Bold indicates statistical significance.

Species and variable	$\Psi_{sW} - \Psi_{sD}$ gradient		
	n	r	P
A. tridentata			
Daytime VPDa	**27**	**0.595**	**0.001**
PAR	**27**	**0.651**	**<0.001**
H. anomalus			
Daytime VPDa	**22**	**0.743**	**<0.001**
PAR	22	0.328	>0.1
Q. laevis			
Daytime VPDa	15	0.459	0.085
PAR	**15**	**0.632**	**0.012**

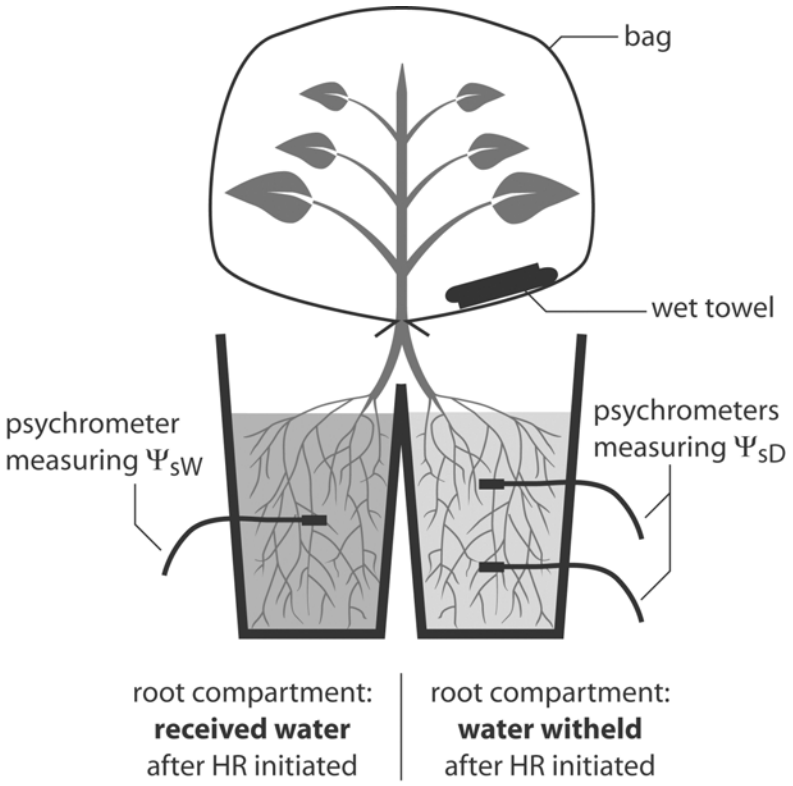
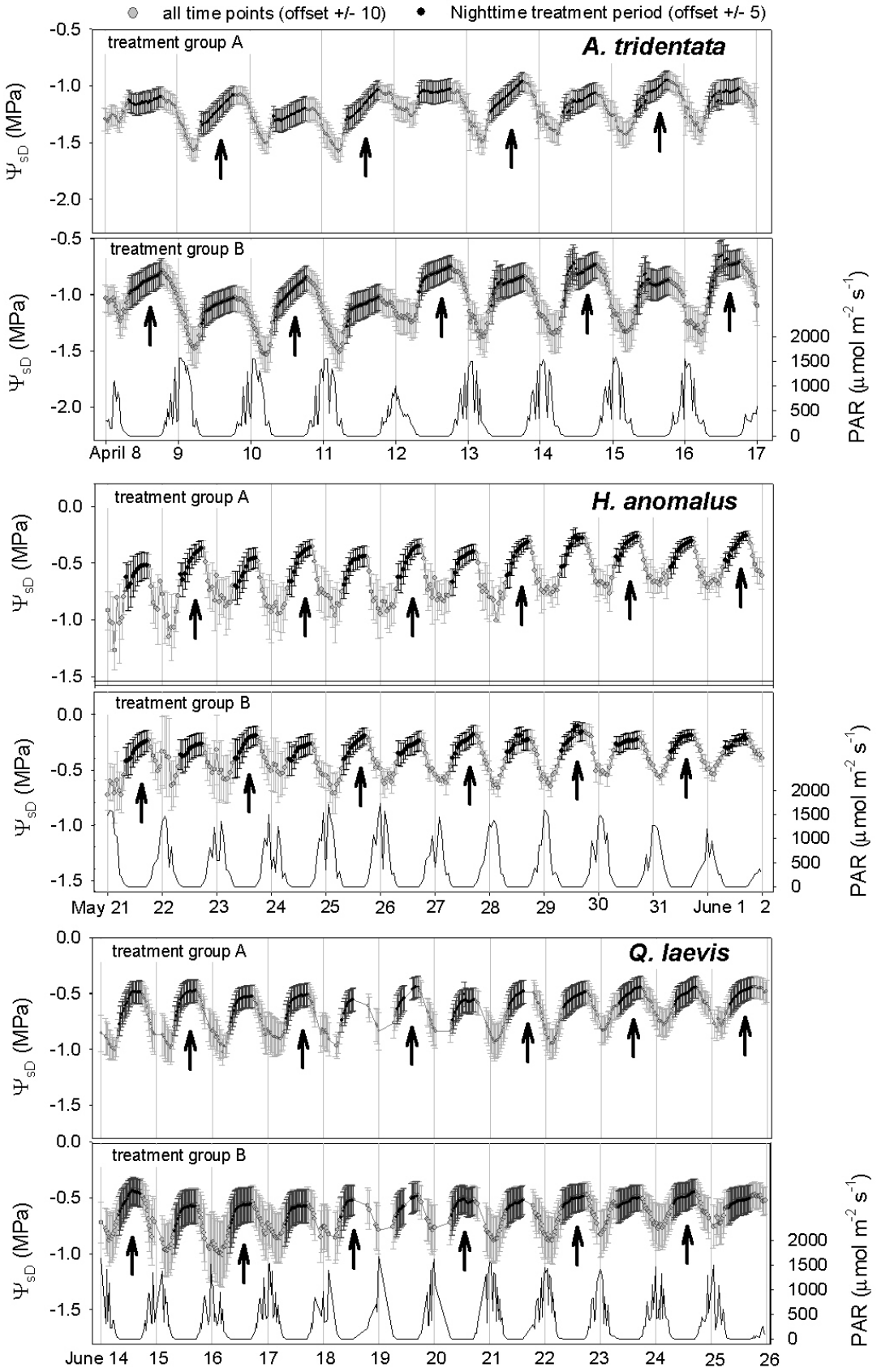


Figure 4.1: Experimental set-up of plants with two separate rooting compartments. Plant is shown "bagged" with a wet towel to minimize nighttime transpiration (E_{night}). The single psychrometer in the left-hand compartment confirmed that the water potential of the soil stayed near 0 MPa in this compartment throughout the period of E_{night} manipulation (measures of Ψ_{sW}). The two psychrometers in the right-hand compartment measured Ψ_{sD} and were used to calculate HR.

Figure 4.2: (next page) Mean soil water potential (± 1 SE) for all species in the un-watered rooting compartment (Ψ_{sD}) during the dates of HR_N measurement. For each species, data for the two E_{night} treatment groups (A and B) are presented in separate panels. Arrows indicate nights where transpiration for the indicated treatment group was minimized by canopy bagging. Vertical lines indicate midday. Note that the Ψ_{sD} axis shifts range among species but scale is constant. Missing data for *Q. laevis* was due to a logger malfunction. Photosynthetically active radiation (PAR) is presented below the Ψ_{sD} data in the lower panel for each species.


all time points (offset +/- 10)
Nighttime treatment period (offset +/- 5)
treatment group A
A. tridentata
treatment group B
H. anomalus
Q. laevis
Ψ sD (MPa)
PAR (µmol m-2 s-1)
April 8 9 10 11 12 13 14 15 16 17
May 21 22 23 24 25 26 27 28 29 30 31 June 1 2
June 14 15 16 17 18 19 20 21 22 23 24 25 26

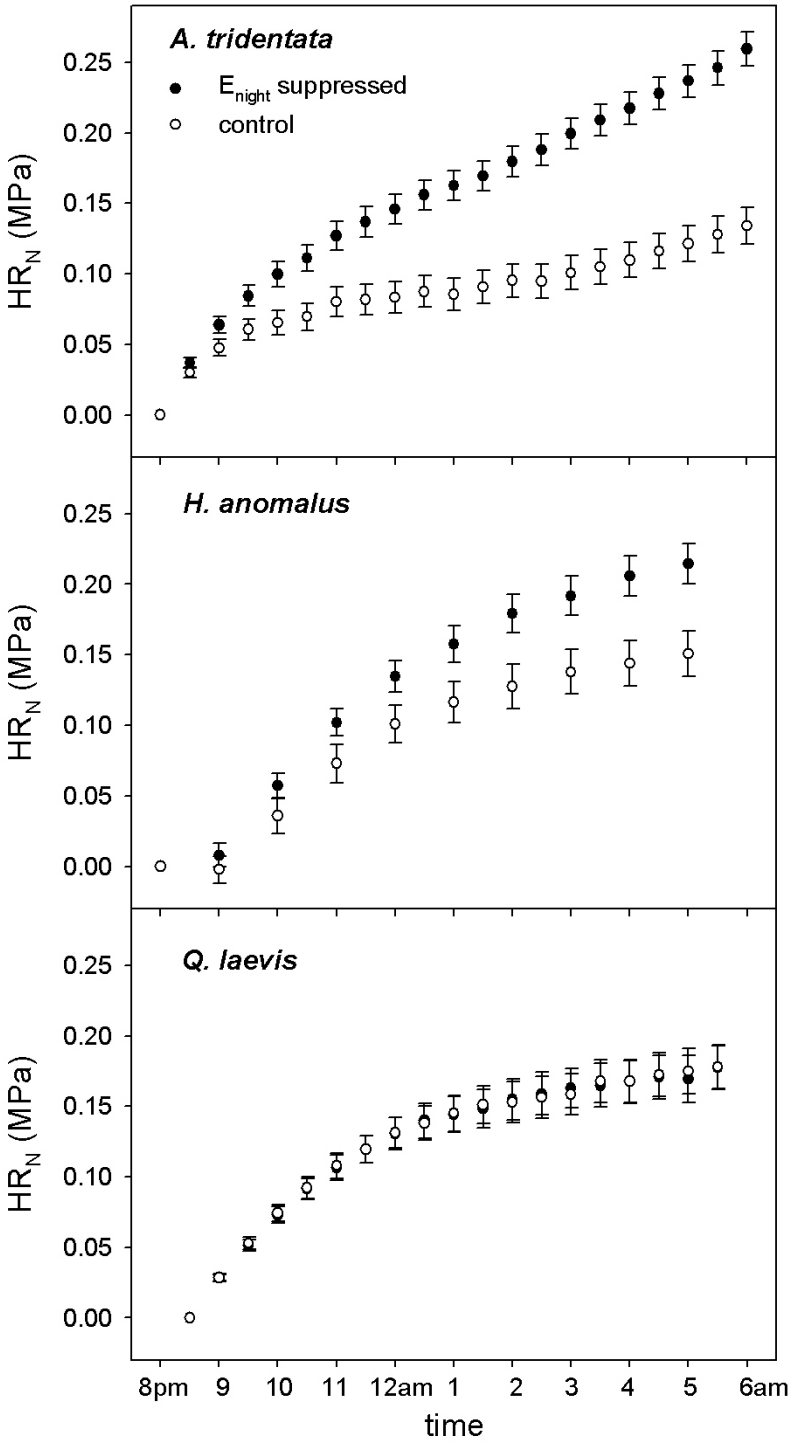


Figure 4.3: Mean hydraulic redistribution (HR_N) for each species during the period of E_{night} treatments. Plants were either allowed to transpire naturally (control) or bagged to suppress transpiration (E_{night} suppressed). HR_N was calculated as Ψ_{sD} at each time-point minus the first Ψ_{sD} recorded during the period of E_{night} treatment for that plant each night. This removes variation due to daily initial Ψ_{sD}, psychrometer position and plant. Values are means averaged across 42-95 plants*psychrometers*days ± 1 SE.

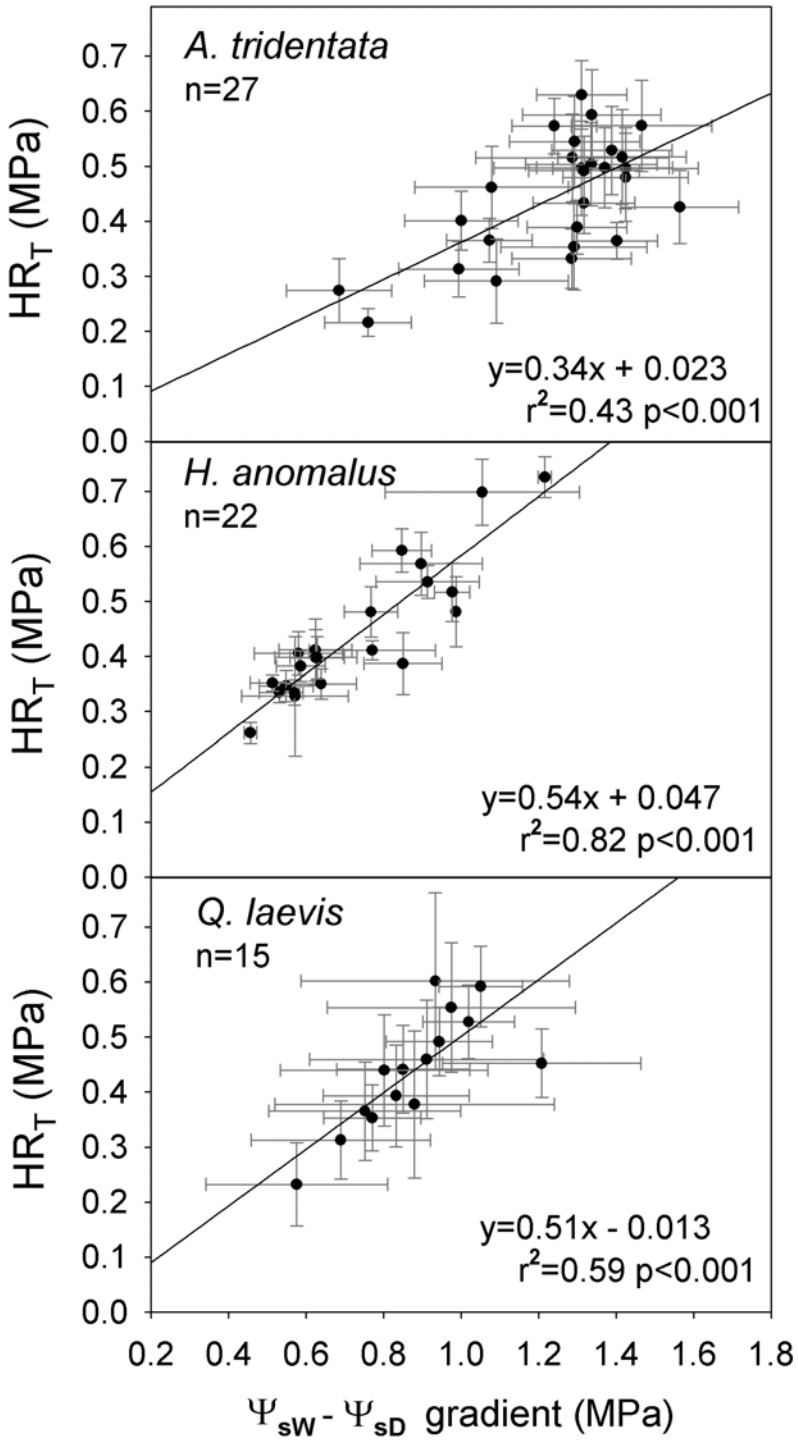


Figure 4.4: Relationship for each species, between total hydraulic redistribution (HR_T) and the soil water potential gradient from watered to un-watered rooting compartments. The gradient was measured at the time when HR_T began ($\Psi_{sW} - \Psi_{sD}$ at time of lowest Ψ_{sD} daily). Data points are means of HR_T and ($\Psi_{sW} - \Psi_{sD}$) (± 1 SE) averaged across all psychrometers (n=3-17) in the un-watered rooting compartment each day.

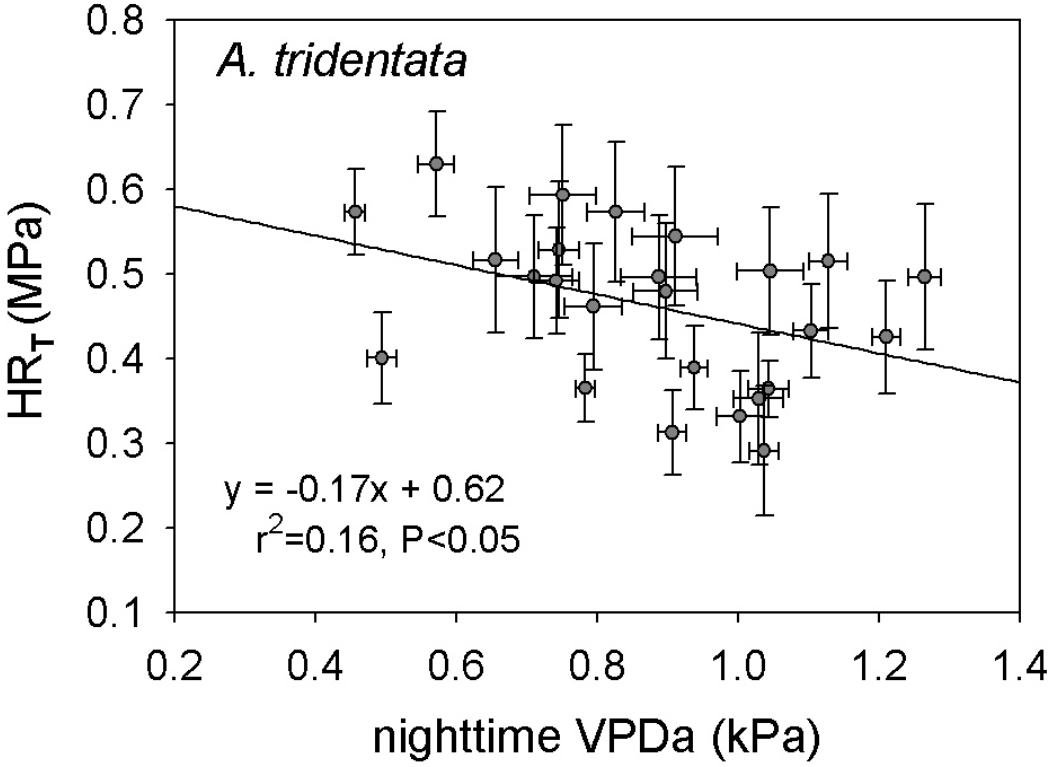


Figure 4.5: Relationship between total hydraulic redistribution (HR_T) and atmospheric vapor pressure deficit (VPDa) at night for *Artemisia tridentata*. Data points (n=25) are daily means of HR_T (±1 SE) across all psychrometers in the un-watered rooting compartment (n=8-17 psychrometers) and for VPDa measured hourly between 8pm and 6am (n=11 VPD logs daily). Two cloudy days (night of 19 April and 4 May 2006) with the lowest $\Psi_{sW} - \Psi_{sD}$ gradients (below 0.8 MPa) and small HR_T have been omitted (see two points in bottom left corner of *A. tridentata* panel Figure 4.4).

CHAPTER 5

CONCLUSIONS

Until recently it was widely accepted that C_3 plants have minimal water loss at night when carbon gain for photosynthesis is not occurring. However, an increasing number of studies show that nighttime stomatal conductance (g_{night}) and transpiration (E_{night}) are substantial and common in C_3 species (Musselman and Minnick 2000, Caird et al. 2007, Dawson et al. 2007). Studies of minimal g_{night} in Chapter 2 and 3 demonstrated that most nighttime water loss occurs through stomata and is subject to regulation in a variety of species. Excision and wilting of leaves or treatment with exogenous abscisic acid (ABA) caused stomatal closure and a large reduction in g_{night} and E_{night}. E_{night} in C_3 plants therefore does not simply represent a physical inability to further conserve water.

Water loss without carbon gain would appear to be costly for plants. In support of this, Chapters 2 and 3 demonstrated that when soil water was limited, g_{night} in *Helianthus* and *Populus* species was reduced to levels indistinguishable from minimum leaf conductance. This reduction was caused by stomatal closure rather than changes in cuticular properties or stomatal size and density. Chapters 2 and 3 demonstrated this by employing a short (1-5 day) drought treatment after measured leaves on control and treatment plants were fully matured under well-watered conditions.

Chapter 4 provided further evidence that E_{night} may be costly to plants. When *Artemisia tridentata* and *Helianthus anomalus* were provided with a soil moisture gradient plants that were allowed to transpire naturally at night had 73% and 33% lower hydraulic redistribution than

plants with minimized E_{night}. The same relationship was not found in *Quercus laevis* but this was possibly due to the small canopy biomass of our study plants rather than physiological differences between this species and the prior two species. Hydraulic redistribution has been shown to provide several benefits to plants including greater transpiration and carbon gain the next day and increased root longevity and nutrient cycling in drying soils (Caldwell et al. 1998, Querejeta et al. 2003, Domec et al. 2004, Lee et al. 2005, Bauerle et al. 2008, Scott et al. 2008, Aanderud and Richards *submitted*). Therefore the negative relationship with E_{night} demonstrated in Chapter 4 further suggests that E_{night} is costly for plants.

One potential benefit of E_{night} that could offset the costs of water loss is improved nutrient acquisition. However, despite several suggestions that such a benefit exists, our carefully controlled studies in Chapter 2 and 3 showed that plants do not regulate g_{night} and E_{night} in response to soil nutrient availability. It is still possible that a passive nutrient uptake benefit of E_{night} exists even if regulation does not occur in response to nutrient availability. Studies of the effect of E_{night} on nutrient uptake have produced contradictory results (McDonald et al. 2002, Snyder et al 2008, but see Christman et al. *submitted*) and more research is needed to fully understand the impact of E_{night} on plant nutrition.

Chapters 2, 3 and 4 demonstrate that g_{night} and E_{night} in *Helianthus*, *Populus* and several additional species is large and regulated in response to soil water availability. E_{night} also interacted with hydraulic redistribution to decrease movement of water from wet to dry soils (Chapter 4). These results have important implications for models of plant, stand and ecosystem water flux. Inclusion of E_{night}, with increased understanding of the variability in its magnitude, into estimates of total plant water use and ecosystem evapotranspiration will improve the accuracy with which plant productivity and ecosystem hydrology can be predicted.

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