

Nighttime stomatal conductance and transpiration in *Helianthus*

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C_3 plants are predicted to close their stomata at night, yet many plants exhibit significant nighttime stomatal opening. Little is known about regulation of, and temporal variation in, nighttime stomatal conductance (g_{night}) and transpiration (E_{night}).



Objectives

1. Assess the magnitude of g_{night} and E_{night} in *Helianthus*.
2. Test for regulation of g_{night} and E_{night} in response to manipulation of soil nutrient and water availability.
3. Quantify variation in g_{night} and E_{night} diurnally and across leaf lifespan and plant reproductive stages.
4. Determine the proportion of total leaf g_{night} and E_{night} potentially subject to stomatal regulation.

Objectives were addressed in nine greenhouse studies that primarily used wild sunflower, *Helianthus annuus*, but also included a commercial and a land-race (Hopi) domesticated of *H. annuus*, and three wild species (*H. petiolaris*, *H. deserticola*, and *H. anomalus*).

Methods

The nine studies occurred in a heated and lighted greenhouse at the University of Georgia between August 2003 and June 2006. Experiments were either complete randomized block designs or completely randomized. All sunflowers were grown from seed and generally planted in 3/4 sand : 1/4 fritted clay in 25cm diameter pots. Nutrient treatments consisted of manipulating total macro- and micronutrients with Osmocote slow-release fertilizer or just nitrogen by thrice weekly applications of a modified Hoagland solution containing 140 or 7 mg mL⁻¹ nitrogen as nitrate. Soil water treatments generally consisted of either supplying plants with ample water (sufficient), or of withholding water (limiting) until visual wilting and depression of daytime gas exchange measures were achieved.

Instantaneous gas exchange measures were made with an LI-6400 portable photosynthesis system (LiCor Inc). Measures were normally made on most recently fully-expanded leaves. Nighttime measurements were generally made between 1:00am and the start of astronomical twilight (sun geometrically 18 degrees below the horizon). Except when assessing response to soil water deficit, all plants were watered the evening before nighttime measurements and the morning of daytime measurements. At night we used a green safety lamp to avoid triggering stomatal responses.

Acknowledgements

This research is supported by NSF grants IBN-0131078 and IBN-0416627 to LAD and IBN-0416581 to JH Richards

Conclusions

Across multiple studies we demonstrate substantial g_{night} and E_{night} in *Helianthus* wild species and domesticates. *Helianthus*, g_{night} is regulated and is naturally variable across several temporal scales from hours to months. These results further our understanding of factors that must be considered for scaling to whole plant and ecosystem water use and for predicting conditions under which nighttime water loss will be biologically significant.

1 Averaged across all studies of sufficiently watered wild *Helianthus* species g_{night} was 9.4% of g_{day} and E_{night} was 8.8% of E_{day}

In all nine studies the four wild species of *Helianthus* and both domesticates showed substantial loss of water at night. E_{night} in domesticated *H. annuus*, averaged across all studies, was lower than in the wild species, with g_{night} 2.3% of g_{day} and E_{night} 3.8% of E_{day} . Differences between wild species were detected but were smaller than differences in maximal g_{night} and E_{night} observed for *H. annuus* across different studies.

2 Soil nutrient limitation does not affect *Helianthus* g_{night} and E_{night}

We hypothesized that regulation might occur for increased g_{night} under limiting nutrient conditions to increase bulk flow of soil solution to the roots and reduce the development of a nutrient depletion zone in the rhizosphere. Six studies applied a soil nutrient treatment, four of which only manipulated soil nitrate. Although the soil nutrient limitations decreased shoot biomass (full nutrient limitation $P < 0.05$; nitrate-only limitation $P < 0.001$) and generally decreased reproductive biomass and leaf nitrogen concentration, they did not affect g_{night} and E_{night} in any of the wild *Helianthus* species or in domesticated *H. annuus* (fig. 1).

Contrary to our *Helianthus* results, there is growing evidence that some perennial species do respond to soil nutrient limitations with higher g_{night} (*Distichlis spicata*, M. Caird unpublished; *Populus balsamifera* ssp. *trichocarpa*, A. Howard unpublished). 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.

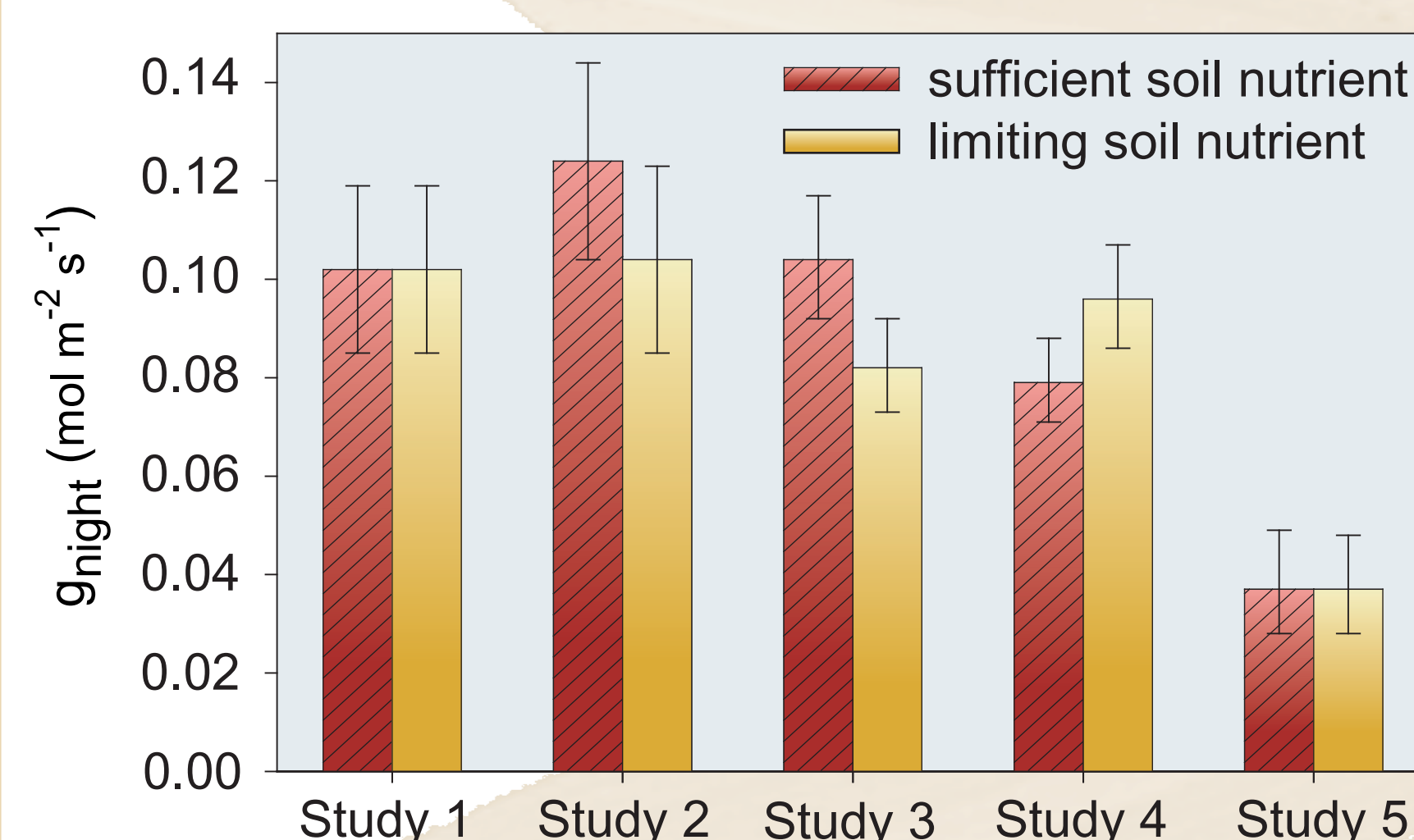


Figure 1: Effect of manipulating soil nutrient availability on nighttime leaf conductance (g_{night}) in five studies with wild *H. annuus*. All nutrients were manipulated in study 1, whereas only nitrogen was manipulated in studies 2 to 5. Bars are 1smeans \pm 1 SE.

Soil water limitation substantially reduced *Helianthus* g_{night} and E_{night}

We hypothesized that, analogous to daytime responses, regulation would occur to decrease g_{night} and E_{night} when soil moisture was limited. Four studies applied a soil water treatment. In all cases g_{night} and E_{night} declined substantially in response to a soil moisture limitation ($P < 0.001$) that was generally sufficient to decrease leaf predawn xylem pressure potential (fig. 2), g_{day} , E_{day} and photosynthesis.

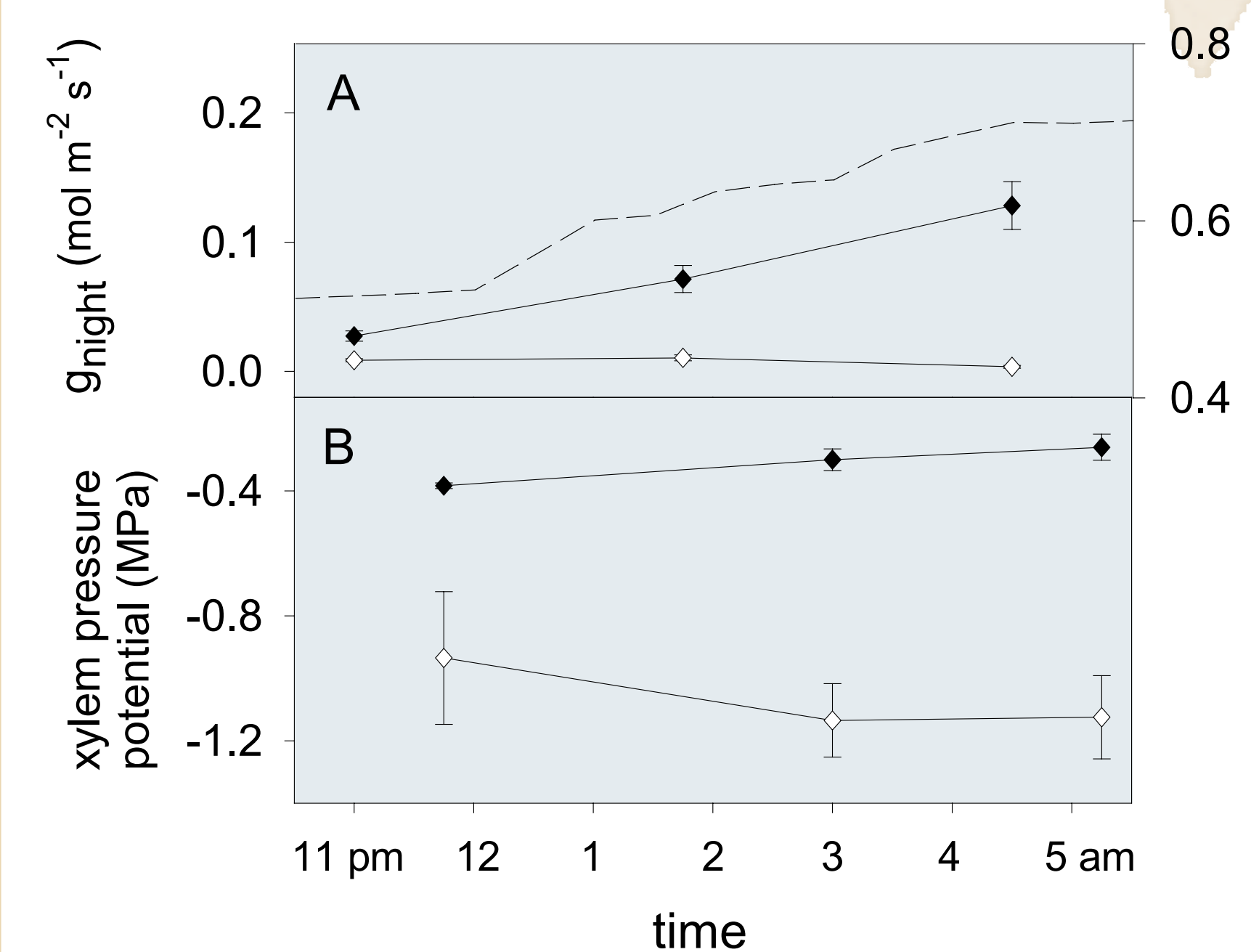


Figure 2: Variation across a night in *H. annuus* nighttime leaf conductance (g_{night}) (A) and xylem pressure potential (C) made on separate, randomly chosen plants from each treatment level. Points are means \pm 1 SE, $n=5-6$ for g_{night} and $n=3-4$ for xylem pressure potential.

3 *Helianthus annuus* g_{night} and E_{night} is naturally variable across temporal scales from hours to months

Repeated measures of g_{night} and E_{night} were made on *H. annuus* in two studies. Both studies showed g_{night} and E_{night} increased through the night in the sufficiently watered plants despite small increases in atmospheric VPD (time effect: $P < 0.001$). Variation across a night could involve a response to plant water potential recovery (fig. 2) but could also involve circadian rhythms.

The effect of leaf ageing was assessed in two studies. In both, there was no decline in *H. annuus* g_{night} and E_{night} due to leaf ageing ($P > 0.2$) from most recently matured to near the start of senescence. This suggests that for *Helianthus*, instantaneous measures on recently mature leaves may be used to scale up to whole plant leaf area provided that there is an open canopy structure.

Pre-reproductive *H. annuus* (5.5 weeks old) had significantly higher g_{night} and E_{night} than did reproductive plants (10 or 15.5 week old) ($F_{2,46}=17.45$, $P < 0.001$; $F_{2,46}=15.96$, $P < 0.001$, respectively)(fig. 3). Plant reproductive stage also affected photosynthesis, which was higher in pre-reproductive plants, but not g_{day} and E_{day} . This suggests that estimates of total water flux in mixed aged stands or integrated over the life of a crop could be underestimated when based on measured made on reproductive aged individuals.

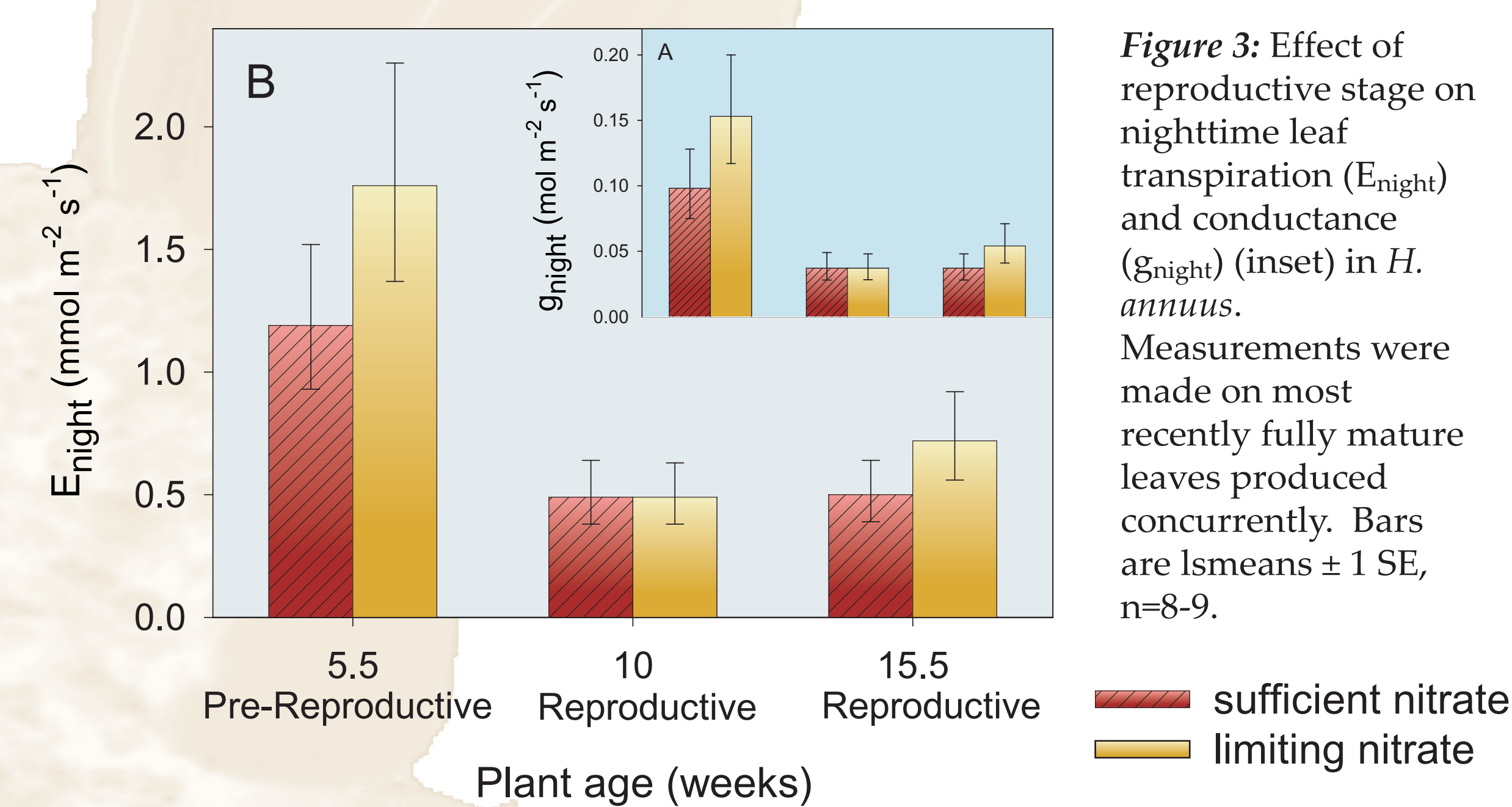


Figure 3: Effect of reproductive stage on nighttime leaf transpiration (E_{night}) and conductance (g_{night}) (inset) in *H. annuus*. Measurements were made on most recently fully mature leaves produced concurrently. Bars are 1smeans \pm 1 SE, $n=8-9$.

4 A substantial portion of total g_{night} and E_{night} occurs through stomata

Measures of g_{night} and E_{night} are typically made gravimetrically or by instantaneous gas exchange or sap flux and include cuticular and stomatal pathways operating in parallel. Three studies assessed the proportion of nighttime water loss that might be subject to stomatal regulation. In all cases g_{night} and E_{night} were substantially higher than measures on leaves with induced stomatal closure (fig. 4). Measures of instrument error, taken during two studies, were significantly lower than leaf measures ($P < 0.001$). These results suggest that a substantial portion of g_{night} and E_{night} is subject to stomatal regulation.

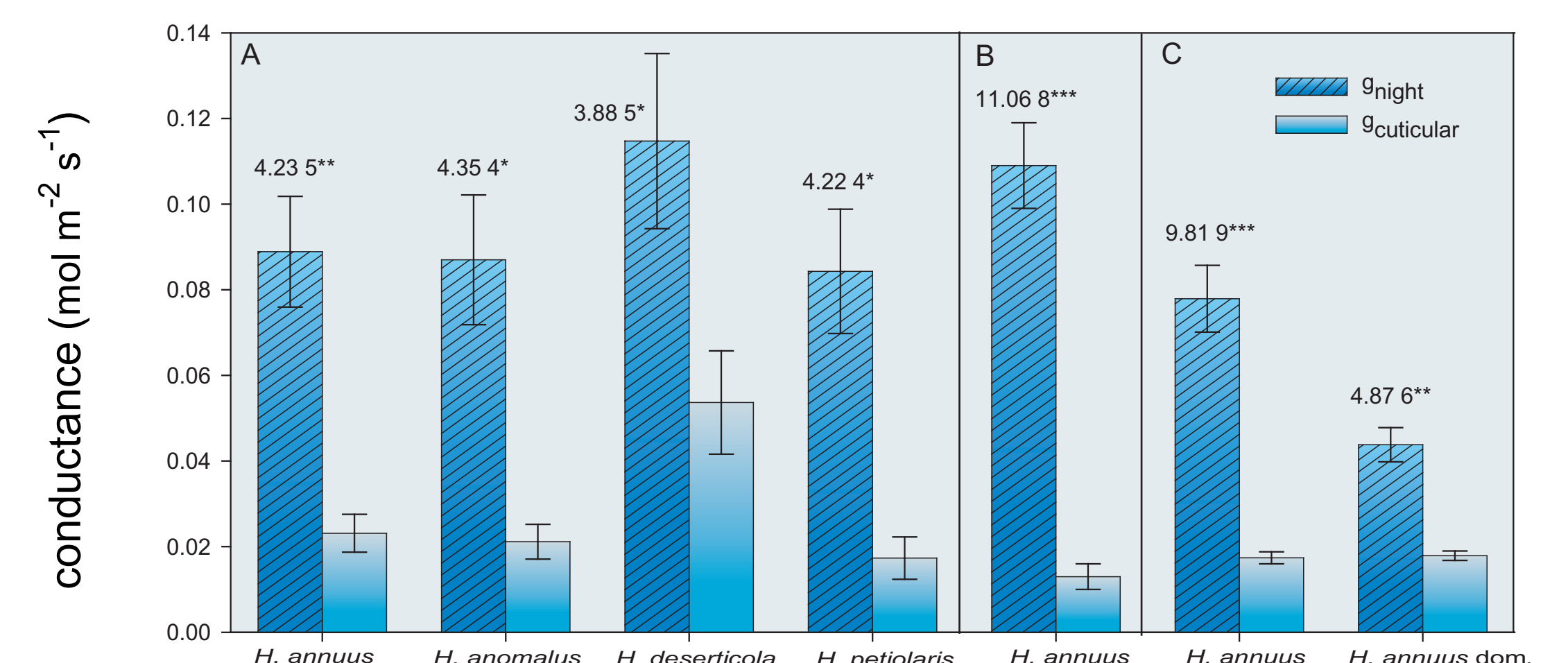


Figure 4: Total nighttime leaf conductance (g_{night}) and cuticular conductance ($g_{\text{cuticular}}$), functionally defined as conductance through both the cuticle and stomata at maximal closure. Measures of $g_{\text{cuticular}}$ were made on excised, wilted leaves during study 1 (A) and 2 (B) and on intact leaves of plants infused with exogenous ABA during study 3 (C). Bars are means \pm 1 SE. For study 1, 2 and 3 $n=5-6$ bulked across nitrate treatment, $n=9$ bulked across nitrate treatment, and $n=3-7$ high nitrate treated plants respectively. t -values and associated degrees of freedom are presented (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).