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Diurnal depression in leaf hydraulic conductance at ambient and elevated [CO_2] reveals anisohydric water management in field-grown soybean and possible involvement of aquaporins

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ABSTRACT

Diurnal cycles of photosynthesis and water use in field-grown soybean (*Glycine max*) are tied to light intensity and vapor pressure deficit (VPD). At high mid-day VPD, transpiration rates can lead to a decline in leaf water potential (Ψ_{leaf}) if leaf hydraulic conductance (K_{leaf}) is insufficient to supply water to intercellular airspaces in pace with demand. K_{leaf} is determined by leaf xylem conductivity to water, as well as extra-xylem pathways that are likely mediated by aquaporin water transport proteins. When transpiration demand exceeds the maximum capacity of K_{leaf} to supply water, high tension in the water column can cause cavitation in xylem, and these emboli-blocked xylem vessels reduce water transport and thus lower K_{leaf}. Stomatal conductance typically remains high at mid-day for soybean, suggesting either a mid-day increase in K_{leaf} or that photosynthesis may be maintained at the cost of leaf water status, indicative of an anisohydric water management strategy in soybean. This study examined diurnal fluctuations in K_{leaf} and Ψ_{leaf}, showing a mid-day depression in K_{leaf} in a pattern closely reflecting that of Ψ_{leaf}, indicating that K_{leaf} depression is the result of cavitation in leaf xylem. The diurnal depression of K_{leaf} was not prevented by growth at elevated [CO_2], which lowered stomatal conductance. Diurnal transcription patterns of aquaporin genes showed that a total of 34 genes belonging to 4 aquaporin families were expressed in soybean leaves, of which 22 were differentially expressed between at least two time points. These data suggest that mid-day K_{leaf} depression was driven primarily by cavitation at increasing xylem water tensions, but that aquaporins are also likely involved in diurnal regulation of soybean leaf water status. It is further concluded that because soybean photosynthesis is typically sustained at mid-day, K_{leaf} even at the depressed level was in excess of that needed to sustain a stomatal conductance sufficient to prevent depression of photosynthesis in soybean.

1. Introduction

Leaves must contend with dramatic environmental changes over the course of even a single day. Light and air temperature both tend to peak around the middle of the day, and vapor pressure deficit (VPD) typically peaks with leaf temperature, coinciding with maximum light and air temperature. For plants in temperate climates during the peak growing season, this means that transpiration demand is very high while the potential for maximum light-driven carbon acquisition requires fully open stomata. Maintenance of open stomata is only possible if the leaf interior can remain sufficiently hydrated, maintaining leaf water potential (Ψ_{leaf}), even as high VPD drives rapid evaporation of water from the intercellular air spaces. Two water management strategies have been described in response to high mid-day VPD: isohydric, in which stomatal conductance declines to maintain constant Ψ_{leaf}, or anisohydric, in which stomata remain open at the cost of a drop in Ψ_{leaf}. Thus, the anisohydric strategy allows a more variable Ψ_{leaf} in order to maintain open stomata open and higher photosynthetic rates for longer periods, even as leaf water potential declines. This strategy allows anisohydric plants to attain higher carbon gain than isohydric plants when water is

Abbreviations: K_{leaf}, leaf hydraulic conductance; Ψ_{leaf}, leaf water potential; *A*, photosynthesis; PPFD, photosynthetic photon flux density; VPD, vapor pressure deficit.

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abundant and even when moderately limiting (Sade et al., 2012). However, under conditions of intense drought, this risk-taking behavior could lead to a persistent collapse in carbon gain that the more conservative behavior of isohydric plants would avoid.

Leaf hydraulic conductance (K_{leaf}) determines the capacity for water transport through the leaf, and leaves are often the hydraulic bottleneck in the whole-plant transpiration stream (Sack and Holbrook, 2006). K_{leaf} can be dynamic and is determined both by xylem conductivity as well as the resistance to water transport in the leaf mesophyll. If g_s does not decrease, high VPD creates a steep water potential gradient through the leaf when K_{leaf} is insufficient to match evaporative demand. As Ψ_{leaf} decreases, resulting high tensions in the water column can cause cavitation, allowing an air embolism to fill the vessel. Cavitation renders the vessel temporarily unusable for water transport, decreasing K_{leaf} (Tyree and Sperry, 1989). Although it was originally thought that emboli could only be refilled under positive root pressure, after transpiration demand abates, embolism refilling under negative xylem pressure has now been demonstrated in several species (Salleo, 1996; Canny, 1997; Hacke and Sperry, 2003; Zwieniecki and Holbrook, 2009). However, negative-pressure refilling must come at an energetic cost; both the release of osmotically active solutes and production of transport proteins may be involved in the negative-pressure embolism repair mechanism, although the mechanism of negative-pressure refilling is uncertain (Alves et al., 2004; Salleo et al., 2004, 2009; Secchi and Zwieniecki, 2011).

K_{leaf} is known to decline over the course of the day in several species, with peak K_{leaf} ranging from early to late morning then decreasing throughout the afternoon (Brodribb and Holbrook, 2004; Lo Gullo et al., 2005; Yang et al., 2012), and mid-day decreases of K_{leaf} in other species are also expected based on xylem vulnerability curves and *in situ* mid-day Ψ_{leaf} values (Woodruff et al., 2007; Bucci et al., 2012). These diurnal depressions in conductance are interpreted to be the result of cavitation in the xylem at high tensions (McCully et al., 1998; Brodribb and Holbrook, 2004; Woodruff et al., 2007; Bucci et al., 2012). Light environment and circadian rhythms may also play a role in diurnal fluctuations of K_{leaf} (Sack et al., 2002; Tyree et al., 2005), and light-driven diurnal cycles of K_{leaf} have been linked to PIP aquaporin expression and activity (Nardini et al., 2005; Cochard et al., 2007; Hachez et al., 2008). Diurnal aquaporin expression cycles also correlated with cycles of root hydraulic conductance in *Vitis vinifera* and *Lotus japonicus* (Clarkson et al., 2000; Moshelion et al., 2002; Siefritz et al., 2004; Vandeleur et al., 2009). Increased expression or activation of aquaporins likely controls the bundle sheath- or mesophyll-based component of K_{leaf} (Clarkson et al., 2000; Moshelion et al., 2002; Sack et al., 2004; Nardini et al., 2005; Hachez et al., 2008; Chaumont and Tyerman, 2014), and they also may play a role in vessel refilling following cavitation (Secchi and Zwieniecki, 2011). As PIPs primarily localize to the plasma membrane and are known to increase plasma membrane water permeability (Kaldenhoff and Fischer, 2006), this aquaporin subfamily likely has the most direct control on the transpiration stream, but aquaporins from other subfamilies may play a role in regulating cell water status and in embolism refill mechanisms. The contribution of aquaporins to overall K_{leaf} likely varies among species, but chemical inhibition of aquaporin function reduces rosette hydraulic conductance in *Arabidopsis* by 21–23% (Postaire et al., 2010), and in soybean, chemical aquaporin inhibitors reduced the transpiration rate by 42–82% (Sadok and Sinclair, 2010).

Diurnal patterns of K_{leaf} have yet to be examined in any herbaceous crop species such as soybean (*Glycine max*), despite this crop covering over 100 million hectares worldwide. In field-grown soybean, photosynthesis (A) typically peaks at mid-day, closely following the pattern of photosynthetic photon flux density (PPFD) (Rogers et al., 2004; Bernacchi et al., 2005). Despite high leaf temperatures, and thus VPD, throughout most of the growing season in soybean-growing regions, soybean stomata typically remain open during the middle of the day, thereby maximizing carbon gain. This suggests either compensatory diurnal increases in K_{leaf} or that soybean is an anisohydric regulator of leaf water status, thereby leaving K_{leaf} highly vulnerable to cavitation at mid-day and through the afternoon especially on warm, sunny days.

Elevated [CO_2] decreases stomatal conductance and transpiration on a leaf-area basis in virtually all plant species (Ainsworth and Long, 2005), and in field-grown soybean elevated [CO_2] caused seasonal transpiration to decrease between 9% and 16%, depending upon inter-annual variation in weather conditions (Bernacchi et al., 2007). Reduced transpiration demand decreases hydrostatic tension in the water column, reducing the risk of cavitation. Growth at elevated [CO_2] has previously been shown to not affect maximum K_{leaf} in soybean (Locke et al., 2013). Thus, because water supply does not change at elevated [CO_2] while Ψ_{leaf} is less likely to decrease during transpiration due to lower stomatal conductance, we predicted that a mid-day K_{leaf} decrease due to cavitation would be smaller for plants grown at elevated [CO_2]. This study examined the fluctuation of soybean leaf water status and K_{leaf} at ambient and elevated [CO_2] over the course of the day to test the hypothesis that K_{leaf} does not increase with increasing VPD and limits soybean photosynthesis on a daily basis.

2. Materials and methods

2.1. Plant material and growth conditions

Soybean cultivar 93B15 (Pioneer Hi-Bred, Johnston, IA) was planted on 27 May 2010 at the SoyFACE research facility in Champaign, Illinois. This field site is managed according to standard agricultural practices in central Illinois, including yearly rotation with *Z. mays* (corn) and no irrigation. CO_2 was fumigated in open-air, 20 m diameter octagonal plots, with a computer-controlled target elevated [CO_2] of 585 ppm. Elevated [CO_2] was within 10% of the target 75% of the time. A detailed description of the SoyFACE fumigation procedure has been published previously (Rogers et al., 2004). CO_2 fumigation began 13 days after planting and continued throughout the growing season, so soybeans experienced their assigned CO_2 treatment for the almost their entire life cycle.

2.2. Diurnal measurements

Two diurnal sets of K_{leaf} and Ψ_{leaf} measurements were made in 2010, the first between 10 July and 22 July and the second between 14 August and 24 August. Leaves were sampled in the field at four time points: 8:00, 11:00, 14:00, and 17:00. Three leaves (sub-samples) were sampled from each plot (ambient or elevated [CO_2]) at each time point. Due to throughput limitations with K_{leaf}, measurements could only be made for one SoyFACE block (one ambient CO_2 plot and one elevated CO_2 plot) per day. Thus, each diurnal data set contains measurements taken on four days. This design allowed environmental variation among sampling days to be accounted for with the block term in the statistical model, distributed equally across treatments and time points.

2.3. Leaf hydraulic conductance

K_{leaf} was measured with the evaporative flux method, in which the flow rate of water through the leaf is measured while the leaves are placed in an environment favorable to transpiration (Sack et al., 2002; Locke et al., 2013). Leaves in the field were cut at