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Photosynthetic parameters of *Juglans nigra* trees are linked to cumulative water stress

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2 **cumulative water stress**

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Abstract

The influence of water deficits and drought on tree physiological processes, growth, and survival has been the focus of substantial research efforts and debate over the past decades, but there is still a need to quantitatively link finer-scale mechanistic explanations of the influence of water status with the physiological responses of trees, particularly for those past the sapling stage. Hence, the objective of the study was to link accumulated water stress during the growing season to leaf physiological response mechanisms of *Juglans nigra* trees. Results showed that trees subjected to higher cumulative water stress had lower maximum light-saturated photosynthesis ($A_{\max}$), initiated net photosynthesis at higher light levels (I_c), and displayed reduced effectiveness of CO_2 fixation per photons absorbed (Q_c) at the bottom and upper positions along the vertical canopy gradient. Results suggest that water stress integral (S_Ψ), a variable that takes into account accumulated water deficits, would be useful to help future research efforts aimed at investigating responses to drought in trees past the sapling stage.

Key words: climate change; drought; gas exchange; walnut; water stress

33 **Introduction**

34 The influence of water deficits and drought on tree physiological processes, growth, and
35 survival has been the focus of substantial research efforts and debate over the past
36 decades (e.g., Pataki et al. 1998; Allen et al. 2010; Duan et al. 2018). Particularly,
37 contrasting perspectives have been reported on the relative vulnerability of forests to tree
38 mortality from hotter drought in the context of climate change (Allen et al. 2015), with
39 several studies reporting lesser or greater potential vulnerability (e.g., Breshears et al.
40 2009; Bauweraerts et al. 2014; Klein et al. 2014; Adams et al. 2015; Teskey et al. 2015).
41 Conducting experiments on extreme heat effects in the field is critical toward
42 understanding tree responses to such climatic threats, but often challenging due to the
43 difficulty in predicting their occurrence and lack of control of interacting climatic
44 variables (Teskey et al. 2015). Hence, most studies that investigated physiological
45 responses to drought were carried out for seedlings under controlled conditions. Results
46 from these studies showed substantial reductions in photosynthetic capacity under
47 drought conditions for many tree species, including *Quercus rubra* and *Juglans nigra*
48 (e.g., Davies and Kozlowski 1977; Ni and Pallardy 1991; Bauweraerts et al. 2014).

49 Several methods have been used to study water relations in trees and forests,
50 including eddy covariance (Wullschleger and Hanson 2006), stable isotopes (Sun et al.
51 2011), sap flow (Meinzer et al. 2013), soil moisture retention curves, and leaf pressure–
52 volume curves. The latter two have been used for decades (e.g., Hinckley and
53 Bruckerhoff 1975; Parker et al. 1982; Kleiner et al. 1992). Such curves were often
54 developed to study drought effects on seedlings or saplings under controlled conditions
55 (e.g., Lewis et al. 2013; Lüpke et al. 2016; Drake et al. 2017). Fewer studies developed

soil moisture retention curves in field experimentation under non-controlled conditions for trees past the sapling stage, particularly for deciduous angiosperms (e.g., Hinckley and Bruckerhoff 1975; Meinzer et al. 2013; Roman et al. 2015; Yi et al. 2017). Combining data from soil moisture retention curves, soil moisture sensors, and gas exchange may help provide finer-scale mechanistic explanations of the influence of water status on tree physiological processes.

Recent findings from a field experimentation showed that under non-limiting light conditions, diameter growth of plantation-grown *Juglans nigra*, *Quercus rubra*, and *Quercus alba* trees past the sapling stage was most strongly influenced by water stress as measured by leaf water potential (Gauthier and Jacobs 2018a). Water stress was more important in influencing growth than nutrient or temperature variables. Development of soil moisture retention curves for *Juglans nigra* allowed us to revisit this experiment by combining precipitation data, time-course data of volumetric soil water content (θ_v) and corresponding soil water potential (Ψ_s) for the growing season. Hence, the objective of this study was to provide a quantitative, finer-scale mechanistic explanation of the influence of water status on gas exchange of trees past the sapling stage under field conditions. We hypothesized that finer-scale phenomena, i.e., the cumulative effect of water stress events during the growing season, would be quantitatively linked to physiological responses of 14-year-old *Juglans nigra* trees as measured by gas exchange.

Materials and methods

Study site

The study site was a mixed hardwood plantation established in 1994 and located in Lafayette, Indiana, USA. Mean annual temperature (1981–2010) for this area was 10.0 °C and mean annual precipitation was 991 mm (NCEI 2017). Billett loam was the dominant soil type on this former agricultural land, characterized by well-drained soils on less than 2% slope. *Juglans nigra*, *Quercus alba*, and *Quercus rubra* 1+0 bareroot seedlings of random, unimproved genetic sources from Indiana provenances, were planted at 2.4 × 2.4 m spacing. Mowing and herbicide were applied for the first few years after planting and for the duration of the experiment to reduce herbaceous competition. Mean stand density at study initiation was 1324 trees ha⁻¹ and basal area was 10.8 m² ha⁻¹. Daily summaries of precipitation data for the 2007 growing season (1 May–31 July) were obtained from a weather station located 2.5 km north of the study site (Purdue University Airport, NCEI 2017).

Experimental design

Ecophysiological studies of trees past the sapling stage often require the use of ladders, towers, or lifts to reach the canopy and make *in situ* gas exchange measurements. Such efforts are time-consuming and quite often limit sample size to a few study trees (e.g., Lucier and Hinckley 1982; Kuhns et al. 1985; Loewenstein and Pallardy 1998; Yi et al. 2017). Eight *Juglans nigra* trees were used for this field experimentation. The tree represents the experimental unit (replicate). Trees were selected based on similar form, height, and diameter at breast height (dbh; 1.3 m above ground). Mean (± SE) dbh was 14.1 ± 0.2 cm and mean height was 9.6 ± 0.2 m at time of study initiation. All trees were

measured for dbh in March and late September 2007. Relative diameter growth (%) was calculated as the percentage of growth of initial dbh.

Soil water content

Monitoring of θ_v was carried out by installing one soil moisture sensor (ECH₂O dielectric aquameter; Decagon Devices, Inc., Pullman, WA) within a 2-m radius of the bole of each measurement tree. Sensors were inserted to vertically monitor average water content in the top 20 cm of the soil profile. This portion of the soil profile covers the main root distribution zone (Sun et al. 2011) and water acquisition zone during the growing season period (Link et al. 2015) in *Juglans* species. Sensors were connected to HOBO data loggers (Onset Computers, Bourne, MA). For six trees, measurements were taken every hour for 57 consecutive days, from 14 May to 9 July 2007. For the two remaining trees, measurements were taken for 38 consecutive days, from 2 June to 9 July 2007. Under relatively dry conditions, such as those found in our study, the mid-May to mid-July period covers the entire radial expansion of *Juglans nigra*, which is usually completed by the beginning of July (Carpenter and Hanover 1974; Kuhns et al. 1985). This short growing season is due to later spring bud break (Lechowicz 1984) and earlier leaf fall senescence (Lucier and Hinckley 1982; Gauthier and Jacobs 2009) compared to associated tree species.

Soil moisture retention curves

Three soil samples were collected in the same area as soil moisture sensors to estimate Ψ_s near each tree. Curves were developed using a pressure plate apparatus (Soilmoisture

Equipment Corp.; Santa Barbara, CA) according to methods described by Klute (1986). Undisturbed soil samples were used for saturated, -0.01 , -0.03 , and -0.1 MPa tensions while disturbed soil samples were used for higher tensions (-0.5 and -1.5 MPa). For tensions between -0.01 and -0.1 MPa, water is held both in pores and on surfaces of clay material and organic matter. Therefore, undisturbed soil cores were used. For tensions of -0.5 and -1.5 MPa, most pores have already been drained, and water is held by adsorptive forces on surfaces of clay and organic matter. At these high tensions, ground, air-dried and disturbed soil samples were used. Gravimetric water content was multiplied with bulk density measured from undisturbed samples to calculate θ_v . SigmaPlot (Systat Software, Inc.; San Jose, CA) was used to develop relationships between θ_v and soil Ψ_s near each measurement using third-order hyperbolic decay curves (Equation 1, Table 1, and Fig. 2):

$$\theta_v = \theta_0 + \frac{ab}{b + \Psi_s}$$

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Where θ_0 is the y-intercept, and a , b are model parameters.

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Thus, solving for Ψ_s (Equation 2):

$$\Psi_s = \frac{ab}{\theta_v - \theta_0} - b$$

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To ensure that estimates of θ_v from sensors were similar to those of soil samples used to generate the curve, three additional soil samples were collected near each *Juglans nigra* tree a few days after sensors were inserted. Volumetric water content was determined

from these 24 samples and estimates were compared to estimates of θ_v based on soil sensors using a paired t -test. Adjustment for soil sensor readings was necessary because their estimates of θ_v ($0.100 \pm 0.007 \text{ m}^3 \text{ m}^{-3}$) were lower than those derived from soil samples ($0.131 \pm 0.006 \text{ m}^3 \text{ m}^{-3}$, $t = 4.72$, $p < 0.01$). Hence, the difference was added to readings from soil sensors as a constant to estimate Ψ_s . The final model equation was as follows (Equation 3):

$$\Psi_s = \frac{ab}{(\theta_v + 0.031) - \theta_0} - b$$

Values of θ_v below those measured to develop the soil moisture retention curves were given the maximum Ψ_s value of -1.5 MPa .

Water stress integral

Myers (1988) defined water stress integral (S_Ψ) as the summation of t measurements of predawn leaf water potential (Ψ_1) at intervals of n days (Equation 4):

$$S_\Psi = \sum_{i=0}^{i=t} (\Psi_{i,i+1} - c)n$$

Where $\Psi_{i,i+1}$ is the mean Ψ_1 for any interval i , $i+1$, and c is the maximum Ψ_1 measured during the entire study. We determined S_Ψ in a similar manner. First, we summed all hourly values of Ψ_s because our measurements were continuous instead of being taken at intervals. Our c value was -0.01 MPa . Second, we divided S_Ψ by 24 hrs day^{-1} to obtain standard units for this variable, i.e., MPa-days . Third, we used a single monitoring period of 57 days for all trees to avoid introducing confounding factors based on unequal time of monitoring. This was done by multiplying S_Ψ of trees 1 and 2 by 1.5 ($57/38 \text{ days} = 1.5$).

S_{Ψ} was calculated using Ψ_s and c in the negative notation, but it was expressed as the absolute value of the summation so that larger values represented larger amounts of accumulated water stress as per Myers (1988).

Gas exchange

Light-response curves (Fig. 3) were developed for the eight *Juglans nigra* trees during the θ_v monitoring period of the 2007 growing season (Gauthier and Jacobs 2010). Gas exchange was measured on clear days, from 10:00 to 15:00, using a portable photosynthesis system (LI-6400, Li-Cor, Inc.; Lincoln, NE). Two trees were measured each day for a total of four sampling days from mid-June to mid-July. Time elapsed between the beginning of the θ_v monitoring period and gas exchange measurements averaged 65% among replicates. This allowed sufficient time for the effects of soil water stress to be reflected in leaf gas exchange measurements. For each light-response curve, *in situ* measurements were made on one fully expanded leaf per tree on the northeast side of the bottom (5.4 ± 0.3 m), middle (7.4 ± 0.3 m), and upper (9.6 ± 0.2 m) canopy position. Gas exchange parameters were measured as a function of light (photon photosynthetic flux density) inside the cuvette according to the following steps: 1500, 2000, 1000, 500, 300, 200, 100, 50, and 0 $\text{mmol m}^{-2} \text{s}^{-1}$ (Li-Cor Inc. 2003). Based on sensors attached to the infrared gas-exchange analyzer, care was taken to ensure conditions inside the chamber reflected external conditions. Average external conditions during the measurement period were $[\text{CO}_2]$ of 380 $\mu\text{mol mol}^{-1}$, relative humidity of 55%, and air temperature of 33.8 °C. Photosynthetic parameters were derived from each light-response curve by fitting a linear regression line between the 0 and 200 $\mu\text{mol photons m}^{-2}$

s⁻¹ light range, with light compensation point (I_c , $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$) determined when $y = 0$, dark respiration rate (R_d , $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) determined when $x = 0$, and apparent quantum efficiency (Q_e , $\mu\text{mol CO}_2 \mu\text{mol}^{-1} \text{ photons}$) as the slope of the regression line. Light-saturated maximum photosynthesis (A_{max} , $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) was measured according to the light level providing the highest A along each response curve.

Statistical analyses

Plots of gas exchange variables of the eight *Juglans nigra* trees as a function of various explanatory variables suggested that most relationships were nonlinear. Hence, a nonlinear regression model of the following form was used (Equation 5):

$$y = a \times \ln(x) + b + \varepsilon$$

Where y is the dependent variable (A_{max} , I_c , Q_e , or R_d), $\ln(x)$ is the natural logarithm of the independent variable (S_Ψ), a and b are model parameters, and ε is the residual error term assumed to be normally distributed, centered around 0 and with unknown variance (σ^2). Regressions were carried out separately for each vertical canopy position using the nlstools package (Baty et al. 2015) of R statistical software, version 3.5.1 (R Core Team 2018). Homogeneity of variance was assessed using plots of residuals and normality was assessed using the Shapiro–Wilk test. Relationships were deemed significant when $p \leq 0.05$.

Results

Rainfall

For the months of May through July 2007, precipitation was 160 mm, which represents 53% of the 1981–2010 average (300 mm). Time–course readings showed considerable variation in θ_v near the eight *Juglans nigra* trees (Fig. 4A). Drought conditions, as defined by $\Psi_s \leq -0.5$ MPa (Kuhns et al. 1985; Roman et al. 2015; Yi et al. 2017), occurred for three of the eight trees (Fig. 4B), with trees 1 and 3 spending 28% and 25% of the total monitoring period under drought, respectively.

Relationships between S_Ψ and gas exchange

Several nonlinear relationships were found between photosynthetic parameters and S_Ψ . At the bottom canopy position, $A_{\max}$ decreased by 73% ($p = 0.003$, $r^2 = 0.79$, Fig. 5A) and Q_e decreased by 82% ($p < 0.001$, $r^2 = 0.95$, Fig. 5B) with increasing S_Ψ that ranged from 2.0 to 27.5 MPa-days. Conversely, a six-fold increase in I_c was found over the range of increasing S_Ψ ($p < 0.001$, $r^2 = 0.89$, Fig. 5C). Similar relationships were found at the upper canopy position between S_Ψ and $A_{\max}$ ($p = 0.05$, $r^2 = 0.49$, Fig. 5D), Q_e ($p < 0.001$, $r^2 = 0.87$, Fig. 5E) and I_c ($p = 0.04$, $r^2 = 0.53$, Fig. 5F). We did not find significant relationships between gas exchange parameters and S_Ψ at the middle canopy position.

Discussion

With only 53% of total precipitation and mean air temperature near 35 °C at gas exchange sampling, the monitoring period for this study was drier than average. It cannot be considered as an extreme drought period, however, because studies that have investigated extreme droughts reported < 10% of total rainfall (e.g., Yi et al. 2017). Nevertheless, as hypothesized, accumulated water stress during the growing season was

quantitatively linked to physiological responses of 14-year-old *Juglans nigra* trees under non-limiting light conditions.

In terms of drought tolerance (Kramer 1983), *Juglans nigra* is a desiccation avoider, i.e., comparatively sensitive to water deficits, and can resort to shedding its leaves to avoid severe drought (Davies and Kozlowski 1977; Gauthier and Jacobs 2011). One of the mechanisms underlying physiological responses of mature *Juglans nigra* to water stress is leaf stomatal control of water loss (Lucier and Hinckley 1982; Kuhns et al. 1985; Loewenstein and Pallardy 1998). Both *Juglans nigra* and *Juglans regia* have been found to limit stomatal and canopy water loss in response to high vapor pressure deficits (Lucier and Hinckley 1982; Daudet et al. 1999; Rosati et al. 2006). Our results indicate that trees subjected to higher cumulative water stress as measured by S_p had much lower maximum light-saturated photosynthesis, severely reduced effectiveness of CO₂ fixation per photons absorbed, and initiated net photosynthesis at much higher light levels at the bottom and upper positions of the vertical canopy gradient. In general, our results corroborate the stomatal control displayed by *Juglans nigra* to limit carbon uptake and water loss when water conditions are less favorable. Previous studies at the molecular level have shown that stomatal closure may regulate water tension in the leaf rachis xylem as shown for seedlings of *Juglans regia* (Cochard et al. 2002). The signaling mechanism by which stomata can sense changes in the leaf rachis xylem to regulate stomatal conductance during drought may reside in the leaf mesophyll itself (Cochard et al. 2002) or in xylem sap abscisic acid concentration (Loewenstein and Pallardy 1998). Other research carried out at the molecular level has suggested that hydraulic

conductance in stems and branches of *Juglans regia* could be modulated by putative aquaporin abundance (Cochard et al. 2007; Steppe et al. 2012).

Although results indicated *Juglans nigra* displayed physiological responses to accumulated water stress, there is some evidence to suggest that its stomatal control may be less responsive compared to associated species, such as oaks (*Quercus* spp.) (Lucier and Hinkley 1982; Kuhns et al. 1985; Gauthier and Jacobs 2018b). *Juglans nigra* has much lower leaf stomatal density compared to associated species (Carpenter and Smith 1975). At a broader scale, this may help explain why *Juglans nigra* resorts to leaf shedding under extreme drought conditions to avoid injury. Kuhns et al. (1985) showed that root growth of three mature *Juglans nigra* trees decreased sharply as soon as Ψ_s departed from 0. Nearly 60% of all root growth occurred when $\Psi_s \geq -0.1$ MPa, with an additional 37% occurring when Ψ_s ranged between -0.1 and -0.5 MPa. Water deficits appear to promote root production in deeper soil layers for juvenile *Juglans regia* (Sun et al. 2011; Contrador et al. 2015) and mature *Juglans nigra* (Kuhns et al. 1985), although this response does not necessarily eliminate water stress. Further, root production can vary considerably among growing seasons and may be lower in *Juglans nigra* compared to most other associated species (McCormack et al. 2014). Myers (1988) reported that plantation-grown, 10-year-old *Pinus radiata* trees subjected to higher S_{Ψ} showed substantial reductions in primary and secondary growth ($r^2 \sim 0.90$). Relationships were nonlinear and quite similar to the ones reported in our study between S_{Ψ} and both $A_{\max}$ and Q_e at the bottom of the vertical canopy gradient.

The methodological strengths of this study reside in the extensive characterization of tree physiology in individuals past the sapling stage, including the development of

light-response curves at three positions along the vertical canopy gradient for each tree, as well as the continuous measurement of water stress through a combination of precipitation data, θ_v monitoring, and development of soil moisture retention curves. One limitation of this study is that gas exchange measurements were carried out only once during the growing season. Collecting gas exchange measurements at several points during the growing season (e.g., Gauthier and Jacobs 2009) could have facilitated the detection of time thresholds required to elicit leaf physiological responses in *Juglans nigra* trees. Future studies should monitor water stress and gas exchange variables throughout the growing season.

The significance of our work is highlighted by the few studies that have investigated water stress in *Juglans nigra* trees past the sapling stage under field conditions (Lucier and Hinckley 1982; Kuhns et al. 1985; Loewenstein and Pallardy 1998). Our study quantitatively linked multiple layers of investigation, from the accumulation of water stress during the growing season in the soil to leaf physiological responses based on photosynthetic parameters. Hence, measuring accumulated water stress as S_Ψ appears to be a useful treatment variable that could help future research efforts aimed at understanding drought responses.

Conclusions

Results from this study indicate that accumulated water stress during the growing season was quantitatively linked to substantial variations in $A_{\max}$, I_c , and Q_e at the bottom and upper positions of the vertical canopy gradient. *Juglans nigra* displayed stomatal control to limit carbon uptake and water loss when cumulative water conditions were less

favorable. Hence, using S_{Ψ} as a treatment variable could help future research efforts aimed at investigating responses to drought in trees past the sapling stage. At a larger scale, above-ground growth is an important measure of tree health, but future research efforts should also be aimed at determining threshold drought conditions that lead to mortality under field conditions (Allen et al. 2015). Such efforts are required to help modelling efforts forecast changes in species distribution. Quantitative root dynamics appear to be understudied (Radville et al. 2016) and would help to explain response mechanisms of trees to drought.

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Figure captions

Figure 1. Soil moisture retention curves developed within a 2-m radius of the bole of eight *Juglans nigra* trees to estimate soil water potential (Ψ_s) as a function of volumetric soil water content (θ_v). Each point represents the mean value of three soil cores.

Figure 2. Precipitation events for the growing season. From May to July, total rainfall was 160 mm, 53% of the 30-year average.

Figure 3. Light-response curves in eight *Juglans nigra* trees at the bottom (A), middle (B), and upper (C) positions of the vertical canopy gradient.

Figure 4. Time-course of (A) volumetric soil water content (θ_v) and (B) soil water potential (Ψ_s) near eight *Juglans nigra* trees.

Figure 5. Relationships between cumulative water stress integral (S_Ψ) and light-saturated maximum photosynthesis ($A_{\max}$), apparent quantum efficiency (Q_e), and light compensation point (I_c) at the bottom and upper canopy positions of eight *Juglans nigra* trees past the sapling stage.

Table 1. Parameter estimates and regression equations for the relationship between volumetric soil water content (θ_v) and soil water potential (Ψ_s) near eight *Juglans nigra* trees.

Tree	<i>F</i> - value	<i>P</i> - value	Adj <i>R</i> ²	Std Err	Parameter estimates and regression equation*
1	38.9	< 0.01	0.938	0.022	$\theta_v = 0.138 + (0.225 \times -0.009) / (-0.009 + \Psi_s)$
2	87.7	< 0.01	0.972	0.015	$\theta_v = 0.138 + (0.235 \times -0.006) / (-0.006 + \Psi_s)$
3	37.0	< 0.01	0.935	0.023	$\theta_v = 0.134 + (0.235 \times -0.009) / (-0.009 + \Psi_s)$
4	31.4	< 0.01	0.924	0.029	$\theta_v = 0.126 + (0.264 \times -0.009) / (-0.009 + \Psi_s)$
5	72.4	< 0.01	0.966	0.016	$\theta_v = 0.132 + (0.219 \times -0.005) / (-0.005 + \Psi_s)$
6	82.3	< 0.01	0.970	0.014	$\theta_v = 0.146 + (0.207 \times -0.006) / (-0.006 + \Psi_s)$
7	141.7	< 0.01	0.983	0.010	$\theta_v = 0.139 + (0.204 \times -0.006) / (-0.006 + \Psi_s)$
8	60.4	< 0.01	0.960	0.020	$\theta_v = 0.118 + (0.257 \times -0.006) / (-0.006 + \Psi_s)$

* For each regression, numerator degrees of freedom = 2 and denominator degrees of freedom = 3

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