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Species selection in hardwoods research: variations in baseline physiological responses of select temperate hardwood tree species

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Abstract: Drought periods are becoming more extreme worldwide and the ability of plants to contribute towards atmospheric flux is being compromised. Properly functioning stomata provide an exit for water that has been absorbed by the roots, funneled into various cell parts, and eventually released into the atmosphere via transpiration. By observing the effects that weather conditions such as climate change may have on stomatal density, distribution, and functioning, it may be possible to elucidate a portion of the mechanisms trees use to survive longer periods of water stress. This study analyzed stomatal density (SD), stomatal conductance (g_s), CO_2 assimilation (A), instantaneous water-use efficiency (WUE_i), and transpiration (E) rates in six native tree species in the Midwestern USA and showed that trees within the same ecotype followed similar trends, but that trees within the same family did not when exposed to identical greenhouse conditions. Naturally drought tolerant tree species demonstrated lower g_s and higher WUE_i, while intolerant species had higher SD. This study showed negative or no correlation between SD and g_s, A, E, and WUE_i and positive correlations between E and A and g_s and E.

Keywords: drought, water stress, stomatal density, hardwood trees

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Introduction

Over the last two centuries, researchers noted a gradual decrease in stomatal density in a number of tree and plant species. Lammertsma et al. (2011) and de Boer et al. (2011) suggested that this decrease in density was driving a decrease in global transpiration rates. Transpiration from plants is an essential part of atmospheric cooling, and is a primary factor modulating precipitation and climate change events (Stewart et al. 2005; Westerling et al. 2006). It is widely known that plants with lengthy life-spans were able to adjust stomatal densities each growing season in response to environmental conditions (Beerling et al. 1993; Miller-Rushing et al. 2009; Lammertsma et al. 2011), and thus conserve water resources. It has been less apparent, until recently, that plants with shorter life-spans also exhibited decreased stomatal numbers as CO_2 levels were increased. Environmental and signaling factors early in development contribute to stomatal density fluctuations, but only after leaf maturation densities were fixed (Lake et al. 2002; Sekiya and Yano 2008). These data, in conjunction with other research data that involved plant leaf characteristics (Bergmann et al. 2004), indicated that stomatal density was affected primarily by abiotic factors.

Relationship between drought and stomatal density

Global climate change has long been the driving factor for many of the abnormalities in nature that have been studied in research laboratories. As weather patterns continue to change, the effects are likely to first be seen in terrestrial ecosystems (Griffiths and Parry 2002; Ward and Kelly 2004; Bauerle et al. 2006; IPCC 2007, Wang et al. 2011). Several research studies involving drought and stomatal density studies relate the potential effects of climate change and drought to stomatal density and wind (Gokbayrak et al 2008), light (Teramura and Sullivan 1994; Boeger and Poulson 2006; Poulson et al. 2006; Casson et al. 2009; Kang et al. 2009), relative humidity (Mott et al. 2008; Franks et al. 2009), and temperature (Lu et al. 2000; Talbott et al.

2003; Singh et al. 2007). Literature reviews covering the topic of stomatal conductance and plant responses to environmental influences have been written with the most all-encompassing review by Ahuja et al. (2010).

Water stressed conditions have been known to increase stomatal density in certain species of C3 grasses such as Canadian wild rye (*Elymus canadensis*) (Klooster and Palmer-Young 2004) and bluebunch wheatgrass (*Pseudoroegneria spicata*) (Fraser et al. 2008). Klooster and Palmer-Young (2004) noted that their data could have been flawed as a result of low sample size, but substantial data were collected that indicated a lack of significance between the biomass and height obtained from grasses with decreased stomatal numbers. Fraser et al. (2008) reported increased stomatal densities in response to water stress, and decreased densities in response to a water surplus as well as a parabolic response in attempts to correlate temperature and water stress. In response to long-term water stress, some species such as wild velvet grass (*Holcus lanatus*) (Pedrol et al. 2000) and a variety of other forage grasses (Klich et al. 1996) showed decreased stomatal densities.

Influence of CO_2 and ozone (O_3) on stomatal density

A variety of plant species studied adjusted stomatal densities in response to elevated CO_2 levels (Woodward and Kelly 1995). Beerling and Kelly (1997) collated stomatal data from greater than 60 plant species and determined that stomatal density decreased in response to increased CO_2. In response to a drop in environmental O_2, increased densities were noted on both the adaxial and abaxial leaf surfaces (Ramonell et al. 2001). At low CO_2 concentrations, reduced stomatal densities, hence stomatal

conductances (g_s) resulted in improved water-use efficiencies (WUE) (Beerling et al. 1998). In response to elevated CO_2 levels, *Arabidopsis* plants tend to develop decreased stomatal densities (Coupe et al. 2006). *Arabidopsis* mutants deficient in the response to elevated CO_2 levels had increased rather than decreased stomatal indices (Brownlee 2001). In a thorough review of CO_2 and its effect on *Arabidopsis* stomatal density (Ward and Kelly 2004) stated that, in addition to stomatal responses, plant biomass allocation and carbon sequestration were additional factors to consider when the overall plant response was evaluated. Elevated atmospheric CO_2 concentrations have led to a greater decrease in adaxial stomata than abaxial stomata (Woodward 1993, 1998). However, Royer (2001) noted no significant difference, but attributed the lack of variability to the uniformity on the abaxial rather than the adaxial surface in amphistomatus species.

Lammertsma et al. (2011) concluded that it was probable that many C3 plants had already developed a mechanism for reduction of stomatal conductance by targeting stomatal density when grown in elevated CO_2 environments. Deceased pore size reduces water loss while maintaining maximum CO_2 uptake (Lammertsma et al. 2011). As current CO_2 levels continue to rise, the time needed for vegetation to adjust will likely be greater than the rate of CO_2 increase.

In the last 10 years research publications examining drought tolerance and WUE have increased significantly due to increased scrutiny of the mechanisms involved in water stress management. Studies involving drought tolerant and intolerant model tree species have also increased as researchers have attempted to predict how forest dynamics and populations will respond to a changing climate regime (Table 1).

Table 1. Summary of reported drought tolerance for a selection of native North American tree species

Family	Common name	Scientific name	DT[a]	References
Fagaceae	American chestnut	*Castanea dentata*	Yes	Waldboth and Oberhuber 2009; McEwan et al. 2011
Juglandaceae	Black walnut	*Juglans nigra*	No	Gauthier and Jacobs 2011
Oleaceae	Black ash	*Fraxinus nigra*	No/Yes	Pijut 2005; Štajner et al. 2011
Oleaceae	Green ash	*Fraxinus pennsylvanica*	Yes	MacFarlane and Meyer 2003
Oleaceae	Pumpkin ash	*Fraxinus profunda*	No	Pijut 2005
Oleaceae	White ash	*Fraxinus americana*	No	MacFarlane and Meyer 2003

[a]Reported drought tolerance

The objective of this study was to observe several select physiological characteristics from various tree species: black ash (*Fraxinus nigra*), green ash (*Fraxinus pennsylvanica*), pumpkin ash (*Fraxinus profunda*), white ash (*Fraxinus americana*), black walnut (*Juglans nigra*), and American chestnut (*Castanea dentata*) grown under identical greenhouse conditions. Examinations of stomatal density (SD), stomatal conductance (g_s), CO_2 assimilation (*A*), and transpiration (*E*) rates were performed to study the differences in basic physiology among species shown to be drought-tolerant such as green ash, and tree species that were drought-sensitive such as white ash. Additional species were used to obtain range of physiological data among native species with different stomatal densities and drought tolerances. Speculations about the manipulation of these characteristics and the subsequent effects on the global water cycle are presented as a lack of sufficient data exists regarding comparisons of multiple species under controlled environmental conditions.

Materials and methods

Plant growth conditions

Eight-month-old seedlings of black ash, green ash, pumpkin ash, white ash, black walnut, and American chestnut were grown in a greenhouse under controlled conditions. The average height of each species was 30.5 cm. Three trees of each genotype were grown from locally collected seeds and all measurements were

taken in triplicate. Average daily greenhouse air temperatures ranged between 23.2°C–23.7°C during the period when data measurements were taken. The highest and lowest recorded temperatures in the greenhouse during the study were 18.4°C and 30.7°C (these numbers indicate the coolest and warmest temperatures at any time in the greenhouse itself) over the course of the growth period. Relative humidity averaged 90% during the day and 78% during the night under a long-day photoperiod (16/8-h). The light levels ranged from 300–400 $\mu mol \cdot m^{-2} \cdot s^{-1}$ based on natural light. The CO_2 levels remained relatively steady at 375 $\mu mol \cdot mol^{-1}$ (ambient). All plants were grown in standard round 20.3 cm pots for the duration of the study. The soil mix used was a 4:1 mixture of Sun Gro Redi Earth plug and seedling mix (Sun Gro Horticulture). Plants were watered every 3 days by placing individual pots onto watering trays that were constantly refilled over the course of 1 h. The pots were then removed and allowed to drain when replaced on the greenhouse bench. All plants were fertilized upon initial placement in the greenhouse and once every 3 weeks afterwards with acidified water supplemented with a combination of two water-soluble fertilizers (3:1 mixture of 21N–2.2P–16.6K and 15N–2.2P–12.5K, respectively; The Scotts Co., Marysville, OH) to provide the following (in mg/L): 200 N, 26 P, 163 K, 50 Ca, 20 Mg, 1.0 Fe, 0.5 Mn and Zn, 0.24 Cu and B, and 0.1 Mo. Nitrate form was 76% of nitrogen provided. Irrigation water was supplemented with 93% sulfuric acid (Brenntag, Reading PA) at 0.08 $mL \cdot L^{-1}$ to reduce alkalinity to 100 $mg \cdot L^{-1}$ and pH to a range of 5.8 to 6.2. (http://www.hort.purdue.edu/hort/facilities/greenhouse/soilFert.s html). The plants were placed in watering trays filled with liquid fertilizer for approximately 1 h before the trays were emptied and the plants placed back on the greenhouse benches.

Physiological Measurements

Three individuals from each sample population were grown in a randomized complete block design in the same greenhouse zone. Measurements of stomatal conductance (g_s, $mmol \cdot m^{-2} \cdot s^{-1}$), net CO_2 assimilation (A, $\mu mol \cdot m^{-2} \cdot s^{-1}$), and transpiration ($E$, $mmol \cdot m^{-2} \cdot s^{-1}$) were taken each day for three consecutive days at 11 A.M. Readings of instantaneous WUE (WUE_i) were generated from the division of A readings by E readings from each species. All data were recorded in triplicate from three separate fully-developed leaves from the upper canopy directly beneath the apical meristem in each of three representative individuals per species using the LI-6400XT portable photosynthesis system (LI-COR Biosciences).

Safranin-O staining and stomatal density

The first fully mature leaf beneath the apical meristem from plants of each species were bleached in a 9:1 solution of ethanol:acetic acid with orbital shaking at 100 rpm for 2 h. Longer incubation times resulted in extremely fragile leaves that fractured easily. Leaves were removed from the ethanol:acetic acid solution and blotted dry before being subjected to a 1% Safranin-O solution with orbital shaking 200 rpm for 35 min. Leaves were

then removed from the Safranin-O solution, blotted, and soaked in distilled water for 10 min before being placed in a fresh tube of distilled water and left to shake overnight at 100 rpm to remove background stain, or visualized immediately to ascertain stomatal density. For better viewing, images were then outlined by tracing Safranin-O stained .jpeg images for greater emphasis of the difference in stomatal morphology and density, as the Safranin-O protocol required optimization for each individual species to obtain the best images.

Statistical analysis

Stomatal density data for each species were subjected to a balanced Analysis of Variance to determine if measurements were significantly different between species (SAS Institute Inc. 2008). Average readings were presented for each species with the standard error of the mean (± SEM). A LI-6400XT portable photosynthesis system (LI-COR Biosciences) was used to obtain readings of stomatal conductance (g_s), transpiration (E), and CO_2 assimilation (A) from all seedlings. This study was repeated in triplicate. Regression analyses were calculated in Microsoft Excel.

Results

In plants, a number of different cell presentations are possible. Some of the most common subsidiary cell arrangements are anomocytic, anisocytic, paracytic, and diacytic although numerous other arrangements exist in nature (Carpenter 2005) (Fig. 1). Examination of subsidiary cells and stoma among the various species showed different morphological phenotypes were represented by several of the tree species studied here (Fig. 2). *Fraxinus spp*., and *Juglans nigra* are usually anomocytic (Fig. 1A) while *Castanea* spp. are most often cyclocytic (Fig. 2D) however anomocytic patterning has also been found (Ehrendorfer 2010). This study observed cyclocytic patterning in white ash (Fig. 2D), an unusual but not impossible occurrence.

Physiological measurements

Analysis of several tree species grown at the same temperature, light level, humidity, and vapor pressure deficit (VPD) were conducted and the rate of CO_2 assimilation, stomatal conductance, and transpiration during a period of extremely warm weather when temperatures were 36.4°C outside of the greenhouse and 30.7°C within the greenhouse were recorded (Table 2). Physiological data from the different species sampled indicated that American chestnut displayed the greatest WUE_i; however the g_s readings of this species were very low, indicative of a slower growing species (Table 2). Black ash and black walnut had the second and third greatest WUE_i readings, however their g_s readings were more than twice that of American chestnut, a pattern that was also seen in E rates (Table 2).
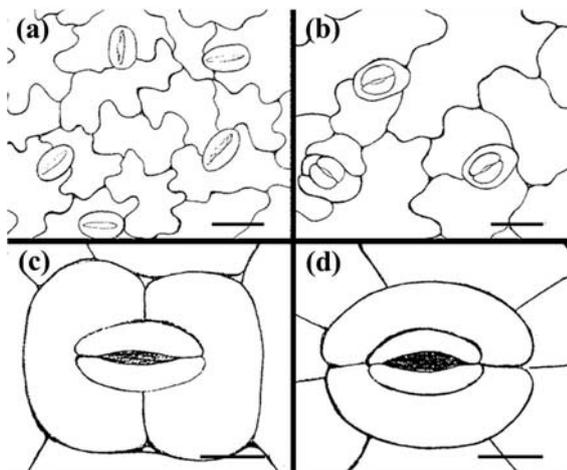
The order for A from highest to lowest was green ash followed by pumpkin, black, and white ash. The highest to lowest WUE_i in ash belonged to black, green, pumpkin, and white ash species (Table 2).

Table 2. Summary of physiological variation and intrinsic values from selected native North American tree species.

Species	SD[a] (mm^{-2})	A[b] (μmol·m^{-2}·s^{-1})	g_s[c] (mol·m^{-2}·s^{-1})	E[d] (mmol·m^{-2}·s^{-1})	WUE_i[e] (mmol·mol^{-1})
American chestnut	261.3 ± 8.3d	6.53 ± 0.36c	0.05 ± 0.01d	0.55 ± 0.01e	11.97 ± 0.19a
Black walnut	304.8 ± 12.1c	9.21 ± 0.48b	0.18 ± 0.03b	1.44 ± 0.03b	6.41 ± 0.25b
Black ash	203.2 ± 5.8e	6.23 ± 0.80d	0.12 ± 0.04c	1.01 ± 0.04d	6.16 ± 0.42c
Green ash	177.8 ± 7.4f	13.44 ± 0.44a	0.31 ± 0.03a	2.49 ± 0.03a	5.48 ± 0.23d
Pumpkin ash	406.4 ± 6.7b	6.73 ± 0.62c	0.14 ± 0.02c	1.25 ± 0.02c	5.38 ± 0.32d
White ash	482.6 ± 13.2a	5.55 ± 0.51e	0.19 ± 0.01b	1.17 ± 0.17c	4.97 ± 0.26e

[a]Abaxial stomatal density; [b]CO_2 assimilation; [c]Stomatal conductance; [d]Transpiration; [e]Instantaneous water-use efficiency.

Means with the same letter within a column were not significantly different at $p < 0.05$

It was discovered that all trees within a genetically distinct population (ecotype) and group had similar values (Table 2). Trees from the same family however did not follow the same trend. Tree species that were accustomed to longer periods without water or were reported to be drought tolerant such as green ash and American chestnut, demonstrated intrinsic values such as lower g_s and greater WUE_i that could be a result of decreased E and higher A values (Table 1, 2). Additional examination of the stomatal densities of these trees showed that stomatal density seemed to be linked, in these species, to WUE_i. Individual species drought tolerance data were based on previously published results (Table 1). Using the available drought tolerance data it was noted that trees with the highest stomatal densities, white ash and pumpkin ash, were species reported to be intolerant or mildly tolerant to drought environments, while species with lower stomatal densities such as green ash was less tolerant than other ashes (Table 1).

Comparison of native species

The A, g_s, E, and WUE_i parameters were used to show differences between and among temperate hardwood species. However, if a particular species, the one farthest from the trendline, were removed from the study, the relationship between other species changed dramatically. Greater correlation values and better trendline fits were seen with respect to all of the parameters examined. We attempt to show that study results involving physiological characteristics of trees can be dramatically different depending on tree species. Also, being within the same family or

Fig. 1. **Most common stoma morphological arrangements**. A number of pavement cell variations exist in tree species, however the most common are (A) Anomocytic, where the guard cells have no outstanding subsidiary cells (aka Ranunculaceous or agenous) and (B) Anisocytic, where the guard cells are surrounded by three subsidiary cells of unequal size (aka Cruciferous or hemimesogenous). Also prevalent in tree species are (C) Diacytic, where guard cells are surrounded by two subsidiary cells at right angles to stoma (aka Caryophyllaceous), and (D) Paracytic, where guard cells are surrounded by subsidiary cells in parallel (aka Rhubeaceous). Dozens of variations of these four arrangements exist in nature (Carpenter 2005). Bar = 50 μm
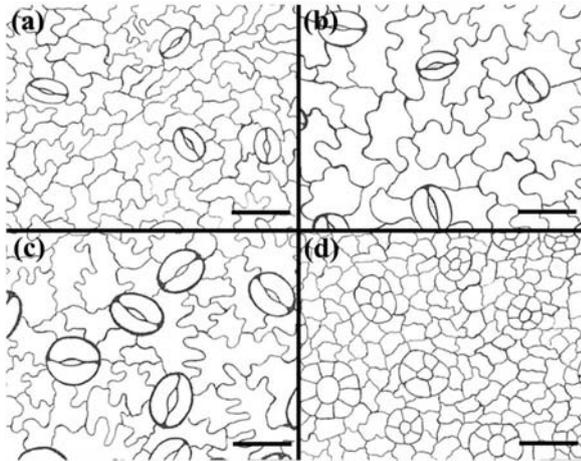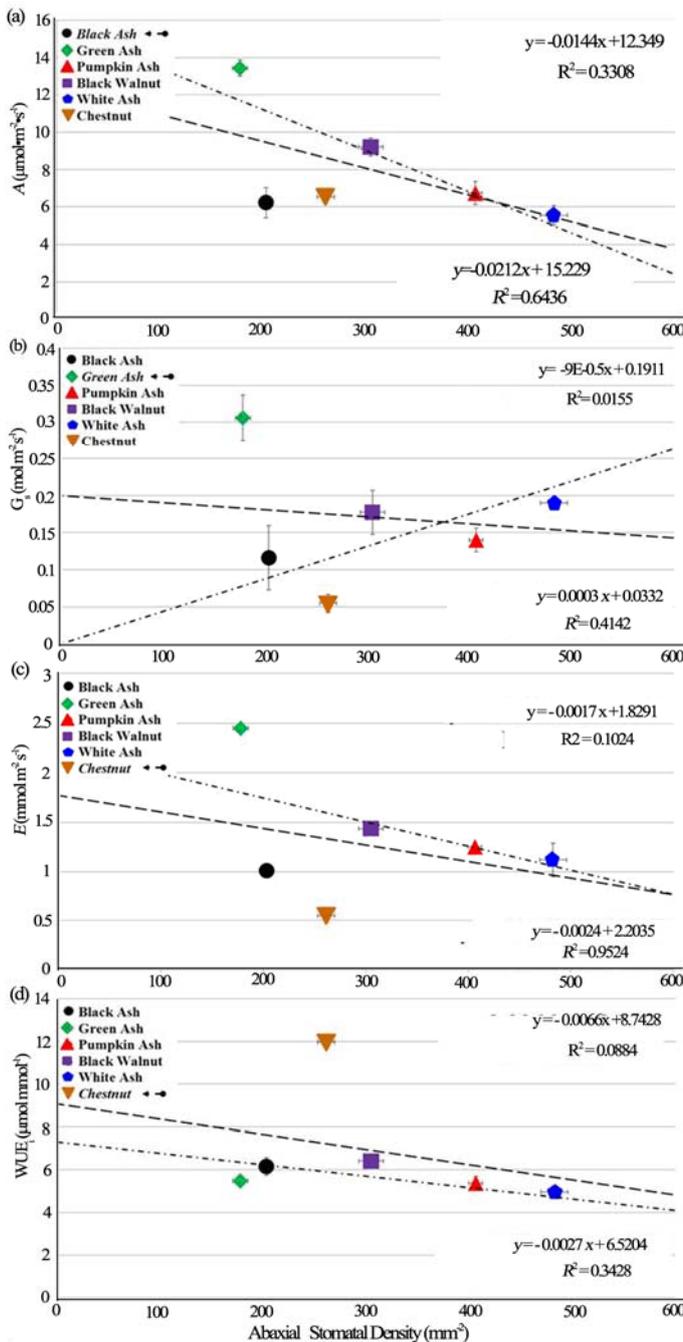

Fig. 2. **Examples of stomata, pavement, and subsidiary cell sizes and shapes**. (A) Decreased pavement cell sizes were seen in black ash, while (B) increased pavement cell sizes were seen in green ash and (C) pumpkin ash. Increases in stoma size were mainly seen in pumpkin ash. (D) White ash photos showed a cyclocytic stomatal arrangement where the stoma is surrounded by subsidiary cells organized in a ring, leading to a flower-like appearance. (A, B) Bar = 50 μm; (C, D) Bar = 25 μm

The greatest g_s rate belonged to green ash. The high A rates reflected the exceptional ability of green ash to generate biomass. The greatest E rates were found in green ash, and white ash (Table 2). When comparing only the ash species, g_s and E rates were highest in green ash followed by white, pumpkin, and black ash.

grown under the same conditions is not indicative of similarity. This study was a demonstration of the effects on the trendline and R^2 values and not an attempt to selectively improve correlations for the benefit of the study.

The A, g_s, E, and WUE_i parameters demonstrated somewhat negative correlations when compared to stomatal density (Fig. 3A–D). Analysis of A and stomatal density (SD) indicated that the removal of black ash would then increase the correlation between the remaining species from an R^2 value of 0.3308 to an R^2 value of 0.6436 (Fig. 3A). A weak correlation between stomatal density and A was observed. Examination of correlation

data for g_s indicated that the removal of green ash would improve the R^2 value more dramatically than was seen in the case of A, as the removal of green ash from the analysis would improve the R^2 value from 0.0155 (with no correlation) to an R^2 value of 0.4142, a slight positive correlation (Fig. 3B). Removal of black ash from the analysis of correlation between SD and E would improve the R^2 value from 0.1024 to 0.2857 (Fig. 3C). Examination of SD and WUE_i was resulted in the lowest R^2 value in the study (0.0884) although removal of chestnut improved the R2 value to 0.3428 (Fig. 3D).

Fig. 3. Comparison of physiological characteristics and stomatal density. (A) CO_2 assimilation (*A*) and stomatal density (SD) data from the survey species were graphed to determine if a correlation existed. The square of the correlation coefficient (R^2) indicated a slight negative correlation of 0.3308 when all of these species were evaluated together. Removal of one species, black ash (arrow, italics), from the study improved the negative correlation (dashed trendline) from 0.3308 to 0.6438 dotted trendline. (B) Stomatal conductance (g_s) and SD data were graphed and the R^2 value indicated no correlation at 0.0155, removal of green ash (arrow, italics), improved the negative correlation (dashed trendline) from 0.0155 to 0.4142 (dotted trendline).(C) Transpiration (*E*) and SD data were graphed and the R^2 value indicated a slight correlation of 0.1024 and removal of chestnut (arrow, italics), slightly improved the negative correlation (dashed trendline) from 0.1024 to 0.2857 (dotted trendline). (D) Instantaneous WUE (WUE_i) and SD data were graphed and the R^2 indicated a negative correlation of 0.0884, (dashed trendline) and removal of chestnut (arrow, italics), from the study showed a negative correlation of 0.3428 (dotted trendline).

A positive correlation was observed when A and E were compared (Fig. 4A). This comparison resulted in an R^2 value of 0.8189 (Fig. 4A) and removal of chestnut improved this R^2 value to 0.9542. A stronger, even more positive correlation and an R^2

value of 0.9149 were observed when g_s was compared to E for all species and removal of white ash improved the R^2 value to 0.9975 (Fig. 4B). The majority of the correlations examined in this study resulted in negative or no correlations among the data however, as expected, removal of the farthest species, improved the R^2 values in every case.
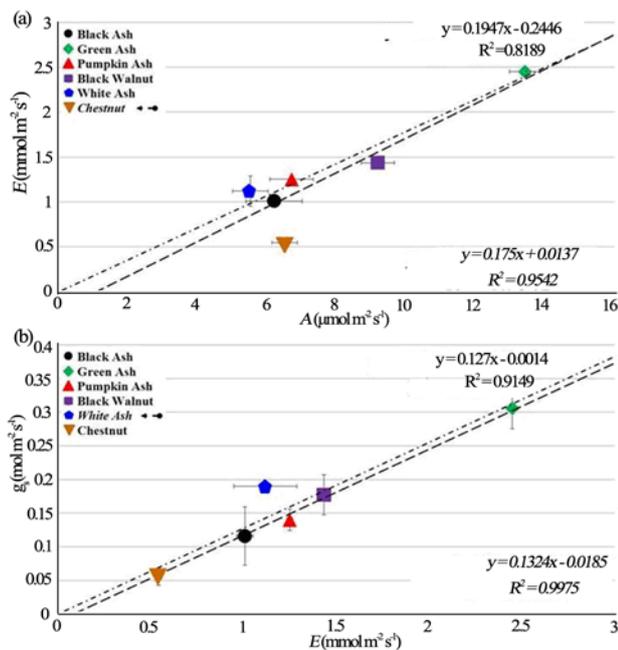

Fig. 4. A comparison of transpiration (E) with CO_2 assimilation (A) and stomatal conductance (g_s). E and A data as well as g_s and E data from the survey species were graphed to determine if a correlation existed between the parameters examined. (A) The square of the correlation coefficient (R^2) indicated a positive correlation of 0.8189 when the E and A were compared and (B) a strong positive correlation of 0.9149 when E and g_s were compared. Removal of one species, chestnut (A, arrow, italics), from the study improved the original correlation (dashed trendline) from 0.8189 for E and A to 0.9542 (dotted trendline) and removal of white ash (B, arrow, italics) from the analysis of g_s and E improved the original correlation (dashed trendline) from 0. 9149 to 0.9975 (dotted trendline). Error bars (±SEM) μ

Discussion

A number of studies conducted in recent years extol the virtues of computer-based modeling methods and the response of plants to an "artificial" drought. While these models were rigorously tested and tended to allow for a number of climate variables, the study of plant responses to an actual drought is most likely going to be the best method for evaluating plant responses. Studies that only focus on one member of a particular genotype or family could be unintentionally skewed towards a particular response when other family members could respond differently. Here, a select group of native species were used to establish baseline readings for basic physiological traits under identical conditions. This information can be used as reference information for further

drought tolerance studies.

Seedlings used in this study were not subjected to an extended drought, nor was it the intention of this study to examine drought responses however it was plausible to infer that those species with the greater WUE_i would outperform other species. Adaptive mechanisms could cause typically intolerant species to revert to a survival mode wherein stomata close for extended periods of time. This response would act to decrease g_s and the end result would be an increase in the carbon isotope ratio ($\delta^{13}C$). The carbon isotope ratio is a time-integrated measurement of WUE that indicates the ability of the plant to adjust and cope to periods of water stress over the lifetime of the leaf. During the study period it was possible that species that are not truly as drought tolerant as others, if examined, could erringly appear to be the best candidates for further evaluation of drought tolerance mechanisms. Because WUE_i was only able to provide a snapshot of how the plant was performing at that moment, $\delta^{13}C$ measurements could also be taken into account for accuracy.

Understanding the drought response

A more thorough understanding of plant drought response will only be attained by studying actual plants exposed to natural drought conditions. These plants would be consistently exposed to insects and pathogens that capitalize on the stress-weakened state of plants for colonization under little or no resistance. Multiple readings throughout the drought period would allow researchers to determine which conditions were the most damaging, and how protective mechanisms within the plant respond to sustained drought conditions. Whether a plant halts accumulation of aboveground biomass, reduces stomatal conductance, or induces premature leaf abscission would be important details to consider when examining plant responses (Ditmarová et al. 2010).

Observations of physiological characteristics of several native hardwood species provided an example of the varied responses within family members and emphasized how under identical growing conditions many species will respond in a unique manner. These data illustrate only a few of the variables that contribute to differences between tree species. Other factors to consider would be abiotic conditions, light levels, whether the study was conducted in a growth chamber, greenhouse, or the field, and seedling age are a few important factors involved in reporting results of a drought tolerance study. The use of a variety of different soils and fertilization methods would also be necessary to report as soil type and nutrient availability are vital to proper growth and maintenance of trees. Studies that only look at a single family member from a single range or USDA Zone do little to improve our understanding of that species or other members of that particular genotype as the results obtained would be extremely specialized. Time of day was an essential caveat to consider when evaluating drought responses as comparisons of results obtained from one study to another were less useful if the time-of-day for each measurement was not included. Few studies to date have incorporated a comparison between early morning, midday, and late evening drought tolerance measures, a factor which is sure to influence future evaluations of plant perform-

ance. For this survey, the inclusion of time-of-day allows other groups working with any of the individual species examined to make more accurate evaluations of experimental conditions for data comparison.

Comparative analysis of each tree species

Interestingly, some of the most drought hardy tree species, (green ash and black ash) had lower stomatal densities while the most drought intolerant species (black walnut, pumpkin ash and white ash) had higher stomatal densities. These data indicated that for these species stomatal density was not the determining factor in drought tolerance. When species with the greatest stomatal densities were examined, it was noted that the majority of them were established in the same or overlapping regions. Those species with mid- and low-range stomatal densities were also found within the same regions. This unusual finding indicated that drought tolerance mechanisms reside in regions other than leaves and should encourage other researchers to examine more than aboveground morphology when evaluating abiotic stress responses and tree drought tolerance capabilities. Therefore, rather than just counting stomatal numbers or examining physiological variables, it was postulated that stomatal morphology could also be indicative of tree species with greater drought tolerance capabilities. These observations of multiple species indicated that several stomatal characteristics were shown in the drought tolerant species such as black walnut that could contribute to heightened drought resistance. Examination of familial traits may also help to identify individuals within a group that may have more favorable abiotic stress tolerance characteristics.

Acknowledgements

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