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PLASTICITY OF *AESCULUS GLABRA* (HIPPOCASTANACEAE) LEAF TRAITS ALONG SMALL-SCALE LIGHT GRADIENTS WITHIN FOREST STANDS

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ABSTRACT. Phenotypic plasticity in response to environmental heterogeneity is an important adaptive component of plant strategies. This study addresses plasticity of *Aesculus glabra* leaf traits in response to small-scale (within-canopy, within-stand) gradients of light availability in a temperate deciduous forest. Leaf mass per area, stomatal density, stomatal length, water content, leaf life span, and chlorophyll content were measured in two populations in northern Indiana. Light availability was determined through hemispherical canopy photography. Stomatal density, leaf mass per area, and leaf life span were positively correlated with light availability ($r = 0.51$, $p < 0.001$; $r = 0.51$, $p < 0.001$; $r = 0.37$, $p = 0.02$, respectively). Chlorophyll content on an area basis and water content were negatively correlated with light availability ($r = -0.49$, $p = 0.002$; $r = -0.58$, $p < 0.001$, respectively). Most correlations of these leaf characteristics with each other were significant. Chlorophyll content on a mass basis and stomatal length did not correlate with light availability. Leaf life span was longer in branches at the top of the crown than in self-shaded lower branches. Leaf traits in this species show significant plasticity in response to small-scale gradients of light availability. The increase in leaf lifespan with increasing light is atypical, and may be due to poor carbon balance of *A. glabra* under shaded conditions. Since this species leafs out before the canopy does, it is unclear how it perceives and responds appropriately to the full-canopy light environment.

Keywords: *Aesculus glabra*, leaf life span, leaf structure, light gradient, plasticity

INTRODUCTION

Plants, as sessile organisms, must respond adaptively to variations in the environment over a range of temporal and spatial scales. Plasticity, the ability to produce different phenotypes in response to environmental variation, is a key feature of plant adaptation to heterogeneous environments (Schlichting & Pigliucci 1998; Gratani 2014). For example, plasticity has been identified as a significant adaptive property of invasive species (Schweitzer & Larson 1999; Sexton et al. 2002; Herr-Turoff & Zedler 2007). Plasticity allows plant species to occupy a wider range of light environments than they would otherwise be able to (Valladares et al. 2002; Catoni et al. 2015).

Plastic adaptation to the light environment can be accomplished by alteration of such leaf traits as anatomy (Sultan & Bazzaz 1993; Gutschick 1999; Niinemets et al. 1999; Sack et al. 2006; Poorter et al. 2009); photosynthetic apparatus, for example pigment and carboxylating-enzyme content (Dale & Causton 1992; Kull & Niinemets 1998; Rothstein & Zak 2001; Niinemets & Valladares 2004; Poorter et al. 2006); lifespan (Williams et al. 1989; Bongers & Popma 1990; Valladares et al. 2000; Hikosaka 2005; Vincent 2006); and arrangement in the canopy (Poorter & Werger 1999). Spatial scales of adaptation range from global differences among biomes (Wright et al. 2005) to variation within the canopy of individuals (Niinemets and Valladares 2004; Sack et al. 2006).

In this study, I quantified plasticity in leaf traits of the temperate deciduous forest tree *Aesculus glabra* Willd. (Ohio buckeye, Hippocastanaceae; taxonomy follows USDA, NRCS 2014). *Aesculus glabra* is a medium-sized tree of mesic forests of eastern North America (Williams 1990). Its range extends from Maine and Minnesota to Texas and northern Georgia (USDA, NRCS 2014). It had importance values >10% in some pre-settlement mesic forests in the Midwest (Crankshaw et al. 1965; Williams 1990), and currently occurs as a frequent understory species with occasional representation in the canopy (Hicks & Michaelis 2009). *Aesculus glabra* is considered to be shade tolerant but occurs in a range of light environments from

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forest edges and gaps to heavily shaded understory (Williams 1990).

The unusual leafing phenology of *A. glabra* is noteworthy. It leafs out and drops its leaves several weeks earlier than co-occurring tree species such as *Acer saccharum* Marshall, *Carya cordiformis* (Wangenh.) Koch, *Fraxinus americana* L., *Tilia americana* L., and *Celtis occidentalis* L., which are common canopy dominants in the mesic forests where *A. glabra* occurs (Henderson et al. 1993; Augspurger & Bartlett 2003; Augspurger 2004; Augspurger & Reich 2008). It thus makes use of the period of high light availability in the spring in temperate deciduous forest understory (Hicks & Chabot 1985), a strategy similar to that of spring ephemeral herbs (DePamphilis & Neufeld 1989; Henderson et al. 1993; Augspurger & Reich 2008). Since leaves expand before the canopy develops, it was hypothesized that correlations of *A. glabra* leaf characteristics with the full-canopy light environment would be weak. The primary goal of this study was to test for such relationships.

Previous studies of leaf plasticity have typically compared leaf traits in seedlings grown in or mature plants maintained in contrasting environments. The current study examines phenotypic variation in relation to naturally-occurring light gradients in forest stands, a smaller spatial scale than has been typical of earlier studies.

METHODS

Study sites.—Research was conducted in two sites in Wabash County, Indiana, which is located in the Eastern Corn Belt Plains Ecoregion (EPA 2013). Both sites are second-growth, mesic forest fragments east of the town of Liberty Mills. Mean annual temperature at Wabash, about 25 km from the study area, is 9.4° C, and mean annual precipitation is 979 mm (Indiana State Climate Data Archive 2010). Soils in both study areas are Hapludalfs (Ockley and Fox series; Ruesch 1983).

Two sites with large populations of *A. glabra* were chosen. Previous measurements from these sites indicate that mean light levels decline from ca. 60% of values found in the open at the forest edge to ca. 10% of open values in the interior, and that light levels decrease by ca. 95% as the canopy leafs out (Hicks, unpublished; cf. Hicks & Taylor 2015).

The forest at the Taylor site (41°2′33.54″ N, 85°43′45.72″ W, elevation ca. 225-240 masl, 4.3 ha) is dominated by *Celtis occidentalis*, *Acer saccharum*, *Fraxinus americana*, *A. glabra*, *Carya cordiformis*, and *Fagus grandifolia* Ehrh. (Hicks & Taylor 2015). This site was never cleared completely for agricultural use, but was cut over for timber extraction in the 1930's (Taylor 1998). The largest canopy trees were approximately 65 cm in diameter at breast height (DBH). This site is bounded by open agricultural land to the south and the Eel River to the north.

The Flory-Gemmer site (41°2′23.60″ N, 85°41′9.78″ W, elevation ca. 230-240 masl, 9.0 ha) is about 3.5 km east-southeast of Taylor. This site was mostly deforested in the 1960's, but had been allowed to regrow for about 40 years at the time of the study. Canopy trees in the portion used in this study reached maximum sizes of about 30 cm DBH, with dominant species being *A. saccharum*, *C. occidentalis*, and *Prunus serotina* Ehrh. The site is bordered by open agricultural land to the north, east, and south, and by regenerating old field to the west.

Field and laboratory procedures.—*Aesculus glabra* saplings from 1.5 to 2.5 m tall were chosen by randomly locating two transects at each site. This size class was studied because *A. glabra* is a dominant understory species in this size category (Hicks & Taylor 2015), and for easy access to the canopy of study plants. To sample a wide range of light environments, one transect in each site was positioned near the boundary between the forest and adjacent open land, and one in the forest interior, ca. 15 m from the edge. Forty-one saplings were located along the transects, 26 at Taylor and 15 at Flory-Gemmer. However, since complete data were not available for all individuals, sample size varies from 38 to 41. Data from both sites were combined, as there were no significant differences between sites in soil type, light availability, or leaf traits.

Estimates of leaf life span were made for two branches on each sapling. One branch was near the bottom of the canopy and one in an exposed position at the top of the canopy. Branches were selected in the spring of 2006, prior to budbreak. I marked each branch with a loose wire tie, and counted leaves at intervals through the growing season. Leaf counts were made approximately weekly during leafing-out and leaf-fall and at approximately two-week intervals in the middle of the growing season. During leafing-out, leaves were counted as emerged if they had expanded to > 1 cm in total length (lamina plus petiole). Leaves were counted as missing if they were abscised or if all leaflets had turned yellow or brown. This method gives estimates that are aggregates for branches, rather than for individual leaves.

Leaf lifespan (LLS) was calculated on a per-branch basis. To measure LLS, I estimated the date at which 50% of the leaves on a tagged branch had emerged, and the date at which 50% of the leaves were absent. (This usually required a linear interpolation of the proportion of the leaves present on the sample dates before and after the 50% level was achieved.) LLS for each branch was the number of days between 50% leaf emergence and 50% absence.

The light environment for each lower-canopy branch was quantified with a hemispherical photo technique, which calculates light availability based on all forms of canopy openness, including gaps and stand edges (Rich 1989). Canopy photos were taken with a Nikon Coolpix 4500 camera equipped with a Nikon fisheye adaptor lens (Nikon Inc., Melville, New York, USA). Images were analyzed by the program Gap Light Analyzer (Frazer et al. 1999), calculated for the period 15 April—1 September. Light availability is expressed as the percent of light available at the photo point, relative to an area with no forest canopy or other overhead obstructions, integrated over the specified time period. The light environment for each lower-canopy branch was measured on 14 July 2006, a time when the canopy was fully leafed out. The camera was positioned on a tripod with the lens next to the marked branch. I collected the nearest adjacent branch for destructive sampling at the time when the canopy photos were taken. Samples were taken from two randomly chosen leaves on the sampled branch.

In the lab, disc samples were removed with a 1.27 cm^2 punch. Ten discs were massed, then dried (60° C, 24 hr) and remassed. Mass values were used to estimate leaf water content and leaf mass per area (LMA). Water content was calculated as (loss of mass on drying) / (dry mass). Ten more discs were used to determine total chlorophyll content using N,N-dimethylformamide extraction (Moran & Porath 1980; Inskeep & Bloom 1985). Finally, stomate density and length were determined on cuticle peels from the lower leaf surface. (No stomates occurred on the upper side of the blade.) Peels were made using Archer adhesive (Carolina Biological Supply, Burlington, North Carolina, USA). The peels were observed at 400 × with a Nikon Alphaphot-2 microscope (Nikon Inc., Melville, New York, USA) and the number of stomates in a field of known area was counted (three fields per leaflet, ca. 0.7 mm^2 per field). Lengths of guard cells were estimated with a calibrated eyepiece micrometer (three fields per leaflet, 10 stomates per field).

Statistics and calculations.—Statistical tests were performed by SPSS (SPSS 2013). Relationships of leaf characteristics with light were assessed by the Linear Regression procedure. Correlations of leaf traits with each other were determined by the Bivariate Correlation procedure using Pearson coefficients. Differences between upper and lower branches were evaluated with Paired-sample t-tests. A critical value of 0.05 was used to indicate significance in all cases. Use of nonparametric procedures (Kendall's tau and Wilcoxon) did not change the outcome of any statistical test.

Plasticity indices (PI) were calculated, following Valladares et al. (2000), as

$$100 \times [(\text{maximum value of trait}) - (\text{minimum value of trait})] / [\text{maximum value of trait}]$$

Maxima were taken as the trait values at 23% light and minima as values at 3% light levels in the regression analyses presented below; these were the maximum and minimum total light values found in the current study.

RESULTS

Light availability was significantly correlated with stomatal density ($r = 0.51$, $p < 0.001$), LMA ($r = 0.51$, $p < 0.001$), water content ($r = -0.58$, $p < 0.001$), chlorophyll on a mass basis ($r = -0.49$, $p = 0.002$), and leaf lifespan ($r = 0.37$, $p = 0.02$); data for all significant correlations are shown in Fig. 1. Chlorophyll on a per-area basis and stomatal length were not significantly correlated with light ($r = 0.08$, $p = 0.65$ and $r = 0.10$, $p = 0.53$, respectively). There were significant correlations between most pairs of leaf variables, other than those including chlorophyll on a per-area basis or stomatal length (Table 1).

PI values, indicating the response of leaf traits to the light gradient within the stand, were 0.43 for stomatal density, 0.39 for LMA, 0.11 for water content, 0.45 for chlorophyll per unit mass, and 0.21 for leaf lifespan.

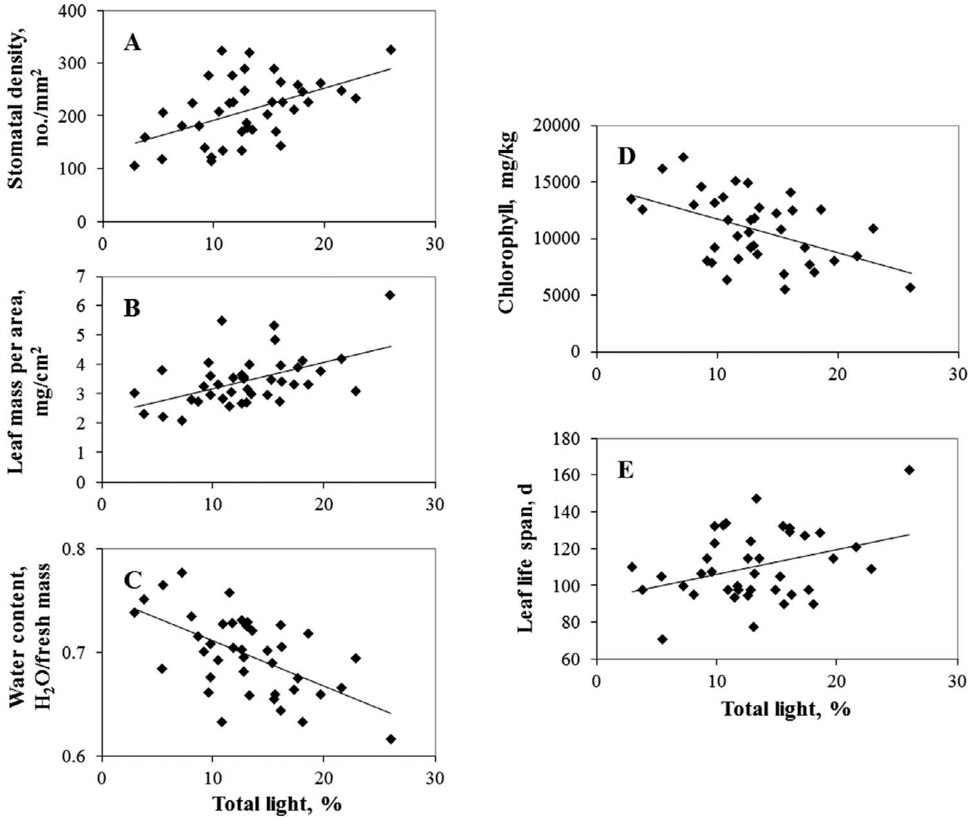

Figure 1.—Scatter plots of leaf characteristics in relation to light for *Aesculus glabra* from two deciduous forest sites near Liberty Mills, IN. N varies from 38 to 41. Regression equations are provided in the form Y = C + m × X, where Y = dependent variable, C = Y intercept, m = slope, and X = independent variable (total light in all cases). A. Stomatal density ($r = 0.51$, $p < 0.001$; Y = 132 + 6.11 × X). B. Leaf mass per area ($r = 0.51$, $p < 0.001$; Y = 2.31 + 0.090 × X). C. Water content ($r = -0.58$, $p < 0.001$, Y = 0.755 – 0.0040 × X). D. Chlorophyll ($r = -0.49$, $p = 0.002$, Y = 14760 – 300 × X). E. Leaf lifespan ($r = 0.37$, $p = 0.02$, Y = 93.0 + 1.35 × X).

Table 1.—Pearson correlation coefficients for relationships among leaf characteristics of *Aesculus glabra* leaves from two deciduous forest sites in Liberty Mills, IN. N varies from 38 to 41. Statistical significance is coded as: n = $p > 0.05$, * = $0.001 < p < 0.05$, ** = $p < 0.001$.

	Water content, gH_2O/gfw	Chlorophyll, mg/kg	Chlorophyll, mg/m^2	Leaf life span, d	Stomatal density, no./mm^2	Stomatal length, µm
Leaf mass per area, mg/cm^2	−0.88 **	−0.83 **	−0.15 n	0.52 *	−0.58 **	0.08 n
Water, gH_2O/gfw	–	0.86 **	0.26 n	0.52 *	0.56 **	0.01 n
Chlorophyll, mg/kg		–	0.61 **	−0.28 n	−0.51 *	−0.20 n
Chlorophyll, mg/m^2			–	0.24 n	−0.07 n	−0.28 n
Leaf life span, d				–	0.28 n	−0.28 n

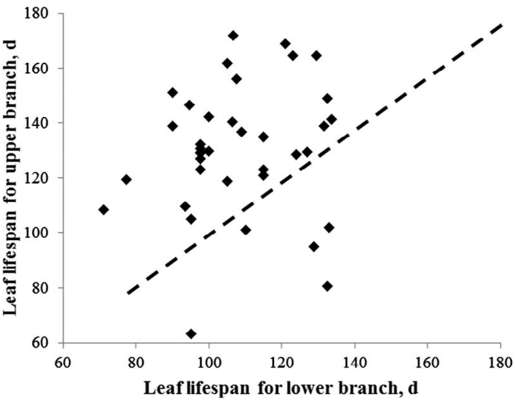

Figure 2.—Scatter plot of leaf lifespan of leaves on upper and lower branches of *Aesculus glabra* from two deciduous forest sites near Liberty Mills, IN. Dashed line indicates equal leaf lifespan in upper vs. lower branches. N = 39.

Leaf lifespan was significantly longer on upper branches than on lower branches of the same plant (Fig. 2; 108 ± 16 d for lower branches vs. 130 ± 24 d for upper branches, paired-samples t-test, t = −5.00, $p < 0.001$, n = 39).

DISCUSSION

Aesculus glabra displayed significant plasticity of leaf traits. Stomatal density, LMA, water content, chlorophyll per unit mass, and leaf lifespan were correlated with the light environment in a small-scale spatial gradient from the edge to the interior of a small forest stand. Also, leaf lifespan differed significantly within the canopy of individual plants.

Adjustment of leaf characteristics within a forest stand occurred on a scale of tens of meters, with many traits changing a correlated way. The relationships of light to stomatal density, LMA, water content, and chlorophyll per area found in this study are typical of those observed in a variety of species (cf. Boardman 1977; Gutschick 1999; Niinimets & Valladares 2004). PI values of stomatal density, LMA, chlorophyll on a mass basis, and leaf lifespan fell within the range found by other studies on temperate deciduous trees (Abrams & Kubiskey 1990; Ashton & Berlyn 1994; Lei et al. 1996; Sack et al. 2006; Baltzer & Thomas 2007; Seiwa & Kikuzawa 2011; Wyka et al. 2012; Legner et al. 2014). Data on plasticity of LLS do not appear to be available for temperate trees, but a study of four tropical species found PI values for LLS to range from 0.24 to 0.58 (recalculated from Vincent 2006). This suggests that *A. glabra* has relatively low plasticity for this trait. Stomate length and chlorophyll per area often do not change with changes in the light environment (Abrams & Kubiske 1990; Niinemets et al. 1998; Sack et al. 2006), as found in the current study. Consequently, although *A. glabra* initiates leaf expansion earlier than other woody species in the same environment, plastic variation in its leaf traits is generally of the same magnitude and in the same direction as documented for other temperate forest trees.

Plasticity of leaf traits in response to variation in the light environment, as documented in the current study, is regarded as adaptive. Plasticity increases the ability of leaves in a range of environments to take up carbon and to make a positive contribution to the carbon balance (photosynthetic fixation vs. respiratory loss of carbon) of the whole plant. Leaves in low-light environments typically have low LMA. Such leaves have lower content of structural materials relative to photosynthetic cells. This, combined with their maintenance of a greater leaf surface area per mass of leaf, increases their ability to capture light. High LMA in plants from high-light, open environments allows greater tolerance of stress such as physical damage (Niinemets et al. 1999; Valladares et al. 2002; Poorter et al. 2009; Catoni et al. 2015).

The increased leaf lifespan observed in *A. glabra* in higher light environments is unexpected. Theory predicts that leaves in environments with low resource levels, e.g., the low light levels of the forest understory, should be retained longer (Kikuzawa 1991). This prediction has been supported by observational and experimental data (Williams et al. 1989; Bongers & Popma 1990; Valladares et al. 2000; Hikosaka 2005; Vincent 2006). The reason that *A. glabra* does not fit this generalization may lie in its poor ability to maintain a positive carbon balance in the understory when the canopy has leafed out (Henderson et al. 1993; Augspurger & Reich 2008).

Plasticity in the narrow sense refers to variation in phenotypic characteristics of a particular genotype in relation to environmental variation (Schlichting & Pigliucci 1998). *Aesculus glabra* displays this form of plasticity, as indicated by the difference in LLS between upper- and lower-canopy branches of the same individual. Intracanopy variation has also been found in other temperate deciduous trees, indicating that this is a frequent strategy for plants whose canopies span a range of light environments (Niinemets et al. 1999; Niinemets & Valladares 2004; Sack et al. 2006; Wyka et al. 2012; Legner et al. 2014).

Gianoli & Valladares (2012) suggest that a broader view of plasticity, in which “related but not identical genotypes” are “exposed to different environments”, is useful in ecological studies. This concept of plasticity also fits the current study, although the individuals studied are of unknown genetic relatedness. The breeding system of *A. glabra* is unknown (Lim et al. 2002), although *A. pavia*, in the same section of the genus *Aesculus* as *A. glabra*, is capable of both outcrossing and selfing (Chanon 2005). The relatively high degree of intra-population relatedness found by Lim et al. (2002) suggests that *A. glabra* populations consist of similar genotypes. Consequently the broad concept of plasticity also applies to phenotypic variation in *A. glabra*’s response to light gradients.

Since *A. glabra* initiates leaf expansion early in the spring, before the forest canopy develops, how does it produce leaves whose characteristics are related to the summer light environment? At least two mechanisms might lead to the observed correlation, namely proximate light cues during *A. glabra* leaf growth, prior to canopy expansion, and carryover effects from previous years.

Proximate effects of light during leaf expansion are well known. Jurik et al. (1979) found that the light environment during leaf expansion affects subsequent development of leaf structure and function. *Aesculus glabra* may respond to shading by the trunks and branches of still leafless neighbors.

Carryover effects from the previous season also are known in deciduous forest trees. Kimura et al. (1998) and Uemura et al. (2000) used shading experiments to show that some shoot and leaf characteristics in Japanese *Fagus* species are affected by light availability during the previous growing season. The *A. glabra* populations studied here were in stands that had not experienced significant changes in structure in the previous year, so leaf characteristics may represent the effect of the previous year’s light microenvironment.

Augspurger & Reich (2008) showed that *A. glabra* leaf senescence occurred earlier in plants that were artificially shaded prior to canopy expansion. Effects were observed in the first year of shading, indicating importance of proximate cues. However, the effect was increased by several years of shading, consistent with a carryover effect.

Although *A. glabra* leafs out at a time when the light environment is rather uniform, it still possesses sufficient plasticity to respond appropriately to variation in the light environment at small spatial scales. The ability of *Aesculus glabra* to occupy a wide range of forest environments, from edges to gaps to understory in secondary forest in the current study, to the understory of older forests (Hicks & Michaelis 2009), is consistent with its well-developed plasticity. Currently, and in the near future, understory plants in temperate forests of northeastern North America face challenges from canopy opening due to the death of *Fraxinus* (Hoven et al. 2014). It is likely that *A. glabra* will be able to respond adaptively to gap formation and increased light; however, it is unknown whether this species has sufficient plasticity to outcompete shade-tolerant neighbors and shade-intolerant invaders in the race to the canopy.

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