Seasonal Variation of Leaf Morphology in *Hydrophyllum virginianum*

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ABSTRACT

The herbaceous layer of a deciduous forest varies throughout the growing season, with certain plants photosynthetically active in different months. *Hydrophyllum virginianum* (Virginia waterleaf) flowers in spring but also produces new leaves later in the season, remaining active long after tree leaf drop. In early spring, *H. virginianum* produces a set of leaves that are often highly variegated (white spots resembling drops of water). Later leaves do not show the same variegation. This pilot study was designed to characterize leaf morphology throughout the growing season, and determine whether chlorophyll a/b content and stomatal density likewise vary in spring, summer, and fall. Additionally, we calculated the proportion of each leaf that was variegated each month from May through October in a population of *H. virginianum* in a hardwood forest in northeast Iowa. Forty individual plants were monitored biweekly to track leaf emergence and senescence. Monthly, one proxy leaf for each plant was harvested from the surrounding population for lab analyses of chlorophyll a/b ratios, stomatal density, and quantification of proportion variegated. Data were analyzed with ANOVA and Tukey post hoc. Results indicate that as expected, variegation was higher in spring. Stomatal density was highest in early leaves, intermediate in mid-season leaves, and lowest in late season leaves ($p<0.0001$). Average chlorophyll a/b ratio did not differ between early and mid-season leaves ($p=0.1091$) but analyses by month revealed that chlorophyll a/b ratio is variable throughout the growing season ($p<0.0001$). Month-to-month fluctuation in leaf metrics did not follow the seasonal arc of tree canopy closure and senescence, which suggests that there may be a more complex interaction of environmental variables. As this was a pilot study, methods have been refined and monitoring and analyses are ongoing to further elucidate patterns.

INTRODUCTION

The herbaceous understory of a hardwood forest is critical for ecosystem functions such as community diversity and nutrient cycling.[1] The herbaceous layer is seasonally variable, so the dominant species in this community shift over the course of the year and most species senesce by the time trees lose their leaves in fall. However, *Hydrophyllum virginianum* (Virginia waterleaf), displays a complex life cycle with variable leaf morphology and photosynthetic activity in spring, summer, and fall.[2] *H. virginianum* has unique variegated morphology[3,4] that does not persist throughout the growing season in our region (based on field observations). We wanted to monitor seasonal change in this herb. We conducted this pilot study to quantify patterns in leaf characteristics such as stomatal density, chlorophyll a/b ratios, and proportion of leaf with variegation spots throughout the growing season.

METHODS

Field surveys

- Sampling occurred in a deciduous hardwood forest near Waverly, Iowa in 2017
- 40 plants were labeled and monitored biweekly from May through October
- Leaves were harvested from nearby plants monthly to assess morphology in the lab

Lab analyses

- Leaves collected in each variegation category (none, low, high) available each month
- Leaves traced on clear plastic to calculate total leaf area and proportion variegated area
- Stomatal casts made by applying and removing clear nail polish to leaf underside, casts viewed at 400x to estimate stomatal density (mean of 4 FOV per leaf)
- Leaves macerated and sample of tissue used for methanol extraction of chlorophyll
- Spectrophotometric absorbance of extraction samples was measured at 652nm and 665nm for chlorophyll a and b, respectively[5]
- Because serial dilutions were not consistently performed, we present only conservative, low-range chlorophyll concentrations
- Data analyzed with ANOVA and post hoc Tukey HSD
- Additional December sampling date added for stomatal density, protocol altered for chlorophyll measurements (not included in analyses)

Figure 1. Variegation typical of early season leaves of *Hydrophyllum virginianum*. Photo by H. Schmitz.

RESULTS

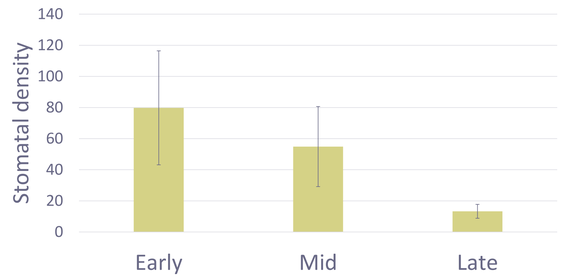

Figure 2: Mean stomatal density by season for *H. virginianum.* Error bars represent ±SD. Early (May-July), Mid (Aug-Oct), and Late (December); ($F=72.75$, $p<0.0001$).

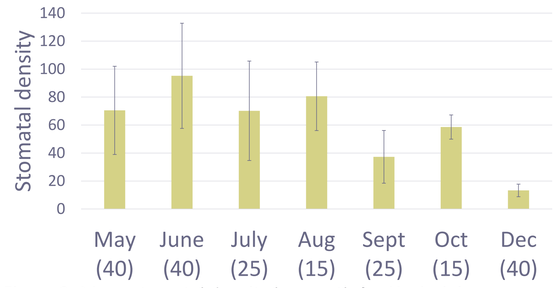

Figure 3: Mean stomatal density by month for *H. virginianum.* Number of samples listed below month. Error bars represent ±SD. ($F=36.66$, $p<0.0001$)

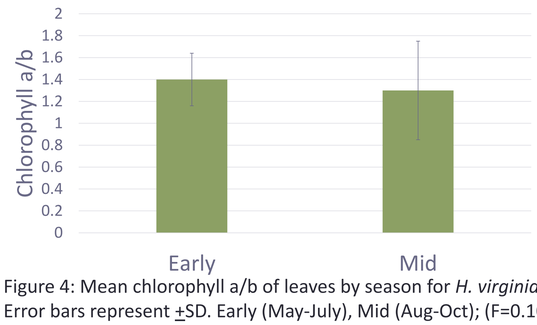

Figure 4: Mean chlorophyll a/b of leaves by season for *H. virginianum.* Error bars represent ±SD. Early (May-July), Mid (Aug-Oct); ($F=0.1091$, $p=0.7418$

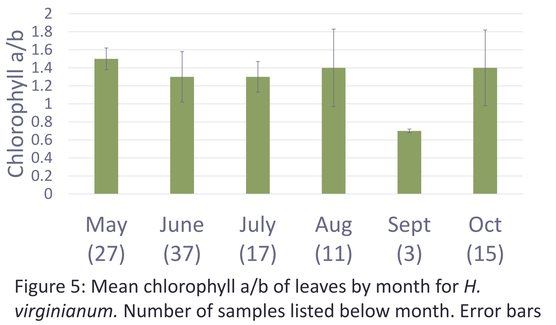

Figure 5: Mean chlorophyll a/b of leaves by month for *H. virginianum.* Number of samples listed below month. Error bars represent ±SD. ($F=5.72$, $p<0.0001$).

- Original (early) leaves senesced before August sampling, mid-season leaves senesced before December.
- Early leaves 0-50% variegated, but variegation was not seen in mid or late season leaves (Fig. 1).
- Stomatal density highest in early leaves, intermediate mid season, and lowest in late season (Fig. 2)
- Analyses by month indicated that June had highest and December lowest mean stomatal density (Fig.3).
- Average chlorophyll a/b ratio did not differ between early and mid-season leaves (Fig. 4).
- Analyses by month revealed that mean chlorophyll a/b ratio is variable throughout the season (Fig.5).
- December chlorophyll a/b ratio (not included in analyses, N=20 mean=3.02, ±2.22 SD) suggest that newly adapted protocol will allow us to detect a wider range of concentrations.

DISCUSSION

- Month-to-month fluctuation in leaf metrics did not follow the seasonal arc of tree canopy closure and senescence, suggesting complex interaction of environmental variables.
- Stomatal densities were lowest in September. The month of August 2017 was exceptionally dry and September leaves seem to reflect that (Fig. 3). Rainfall rebounded in September/October and similarly we see an uptick stomatal densities.
- Further monitoring has begun and will include additional data on ambient light and temperature levels, flower production, and new methods for chlorophyll extraction.

ACKNOWLEDGEMENTS

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