Bundle Sheath Extension Density Along a Light Gradient

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INTRODUCTION:

Leaves can be classified as either homobaric (uniform pressure) or heterobaric (varying pressure)[1]. The presence of bundle sheath extensions contributes to spatial variations in carbon dioxide pressure in heterobaric leaves. Bundle sheath extensions are formed by colorless cells that extend vertically from the top epidermal layer to the bottom epidermal layer and arise from a common vein.

Two heterobaric species were the main focus of this study: *Acer saccharum*, or sugar maple, and *Liquidambar styraciflua*, American sweet gum. Although it is unknown why bundle sheath extension density differs among plant species, lower density is observed in shaded leaves of an individual plant. Shaded leaves benefit from being less heterobaric than leaves in sunlight, since this increases their photosynthetic uptake.

Fig. 1: 0.75cm² section of *L. styraciflua*

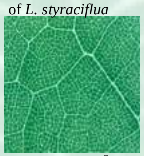


Fig. 2: 0.75cm² section of *A. saccharum*

METHODS & SAMPLING:

SAMPLING:

Leaves were sampled from two species, *Acer saccharum* and *Liquidambar styraciflua*, at the Chicago Botanic Garden in Glencoe, IL. Leaves in full sun, partial shade, and full shade were collected from each of the 8 cardinal directions of the tree. Luminosity was recorded at the exact position of each leaf sampled using a light meter.

EMBEDDING LEAF TISSUE & PREPARING SLIDES:

Small squares (approximately 1cm x 1cm each) of leaf tissue were fixed in FAA, dehydrated with an ethanol solution, and rehydrated with a chloroplast clearing agent, Citrisolv. The leaf tissue was then saturated with paraffin in a mold and cut into 12 micron thick sections using an 820 Spencer microtome at Chicago's Field Museum. Both paradermal and cross sections were mounted onto slides and stained using safranin and Astrablue.

Fig. 3: Spencer microtome

IMAGE-J ANALYSIS OF LIVE LEAF TISSUE:

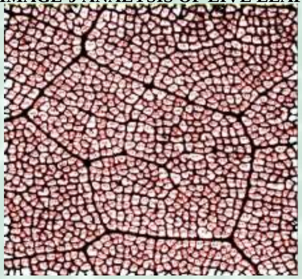


Fig. 4: Sample image obtained from ImageJ software, showing the automatically counted compartments which are formed by bundle sheath extensions.

Live leaf tissue from *Acer saccharaum* and *Liquidambar styraciflua* was photographed under a microscope using a Leica digital camera. The images were then loaded into ImageJ software to count the number of compartments formed by bundle sheath extensions in a 0.75 square centimeter section. The number of compartments formed was used as an estimate of bundle sheath extension density.

ABSTRACT:

The presence of bundle sheath extensions (or BSE) in the leaves of heterobaric plants limits lateral diffusion of CO_2[1]. The density of these bundle sheath extensions has been seen to vary among different leaves of an individual plant. This study investigated luminosity as a possible reason for variation in BSE density within an individual.

Leaves were collected from each of the 8 cardinal directions of a heterobaric species, *Acer saccharum*. Luminosity was recorded at the location of each leaf sampled, and ImageJ, a digital imaging program, was used to count the number of compartments formed by bundle sheath extensions in a 0.75cm² section of leaf. The number of compartments formed by bundle sheath extensions was used as an estimate for BSE density and was found to increase linearly as luminosity increased. The decreased BSE density observed in shaded leaves may allow them to maximize photosynthetic uptake of CO_2.

Fig. 5: *Acer saccharum* tree sampled at the Chicago Botanic Garden

HYPOTHESES:

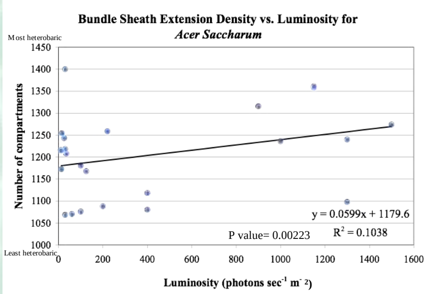
H_a: Within an individual, leaves receiving the greatest amount of sunlight will have the greatest density of bundle sheath extensions, while those receiving less sunlight will have a lower bundle sheath extension density.

H_o: No correlation observed between luminosity and bundle sheath extension density

RESULTS:

Fig. 6:

Linear regression plot of bundle sheath extension density plotted vs. luminosity. All data were recorded from a single Acer saccharum specimen, and each data point represents an average of the eight 0.75cm² sections analyzed per leaf.



A sun, partial shade, and shade leaf were sampled from each of the 8 cardinal directions, for a total of 24 leaves sampled. A total of 192 0.75cm² square sections (8 from each leaf) were photographed and analyzed using ImageJ.

The average number of compartments formed in a 0.75cm² square was plotted against the luminosity measurement for that leaf, and linear regression analysis was performed on Microsoft Excel. Shade leaves, indicated by low luminosity, were found to have decreased bundle sheath extension density on average.

Using the number of compartments formed by BSE's as an estimate for bundle sheath extension density, the BSE density was observed to increase linearly along with increased luminosity.

DISCUSSION:

The null hypothesis was rejected, and the average number of compartments formed by bundle sheath extensions was found to be positively linearly related to luminosity (p-value: 0.00223).

It may be advantageous for shaded leaves of an individual to have a decreased bundle sheath extension density, or be less heterobaric. Under "sun fleck" conditions, this would allow for increased lateral diffusion of CO_2 throughout the leaf, thus increasing photosynthetic productivity.

Observing and photographing live leaf tissue allowed for simple analysis of bundle sheath extension density and eliminated the need for time consuming preparation and staining of slides for microscopy.

PETM FIELD WORK IN THE BIGHORN BASIN, WYOMING:

***In conjunction with Scott Wing, Francesca Smith, Jon Bloch, Mary Kraus, and Rosemary Bush**

Background:

Following my research at the Chicago Botanic Garden, I spent four weeks in the Bighorn Basin doing paleoecological field work collecting samples from the Paleocene-Eocene Thermal Maximum (PETM). The PETM was a period of rapid global warming (5-10ºC) which occurred approximately 55.8 million years ago[2]. This global warming event may be analogous to future global warming caused by CO_2 output. During this time, a release of ^{13}C depleted carbon to the atmosphere, resulted in a global carbon isotope excursion[3]. The $\delta^{13}C$ and δD obtained through mass spectrometry provide insight into the carbon cycle and paleohydrology during the PETM.

Goals:

- Collect samples from a newly identified PETM section for $\delta^{13}C$ and δD analysis of fossil leaf waxes
- Characterize ecological and climatological effects of PETM warming period

Results:

55 samples of fossilized soils were collected from 20 trenches, which measured 66 meters in vertical distance.

The data collected will later be interpreted along with paleobotany, paleopedology, and vertebrate paleontology of the section for a more broad understanding of the PETM.

Fig. 7: Trenches spanning a PETM section of hillside in the Bighorn Basin

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