The Angiosperm Advantage: Evidence from carbon isotopes and the fossil record

Amanda Bender, Department of Geology, University of Maryland at College Park
Advisors: Nathan A. Jud, Dr. A. Jay Kaufman, Dr. Thomas R. Holtz, Jr.

I. Introduction

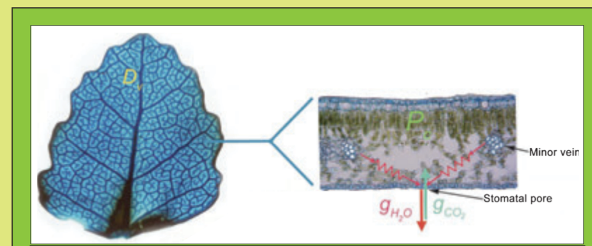


Fig. 1: Typical angiosperm leaf and cross-section of a leaf, showing the spatial relationship of leaf veins that bring water to the stomatal pores, which lose water (g_{H2O}) while they take in CO_2 from the atmosphere to photosynthesize. [Brodribb & Feild 2010]

Angiosperms (flowering plants) are the dominant terrestrial plant group today, due largely to their unrivalled photosynthetic rates, which is limited mainly by a plant's ability to supply water to the leaf (Brodribb & Feild 2010). During photosynthesis, a leaf opens its stomata to exchange carbon dioxide and oxygen with the atmosphere and loses water via transpiration. To combat the risk of dehydration, plants have evolved many innovative features to bring water closer to these sites of water loss (Sperry 2003), including the irrigation system of leaf veins (Roth-Nebelsick et al. 2001). Leaf veins are correlated as a measure of the maximum capacity for photosynthesis within a leaf (Sack & Frole 2006; Brodribb et al. 2007; Noblin et al. 2008; Boyce et al. 2009).

Modern angiosperms exhibit a larger density of leaf veins than other plant groups (e.g. conifers and ferns) (Brodribb et al. 2005), allowing for their increased rates of transpiration and higher rates of productivity. However, the evolution of this increased leaf vein density is not well-constrained. Recent phylogenetic and fossil evidence suggest that the key innovation(s) allowing for increased leaf vein density (>5 mm mm^{-2}) first appeared in leaves at ~100 Ma (Late Albian) (Brodribb & Feild 2010; Feild et al. 2011).

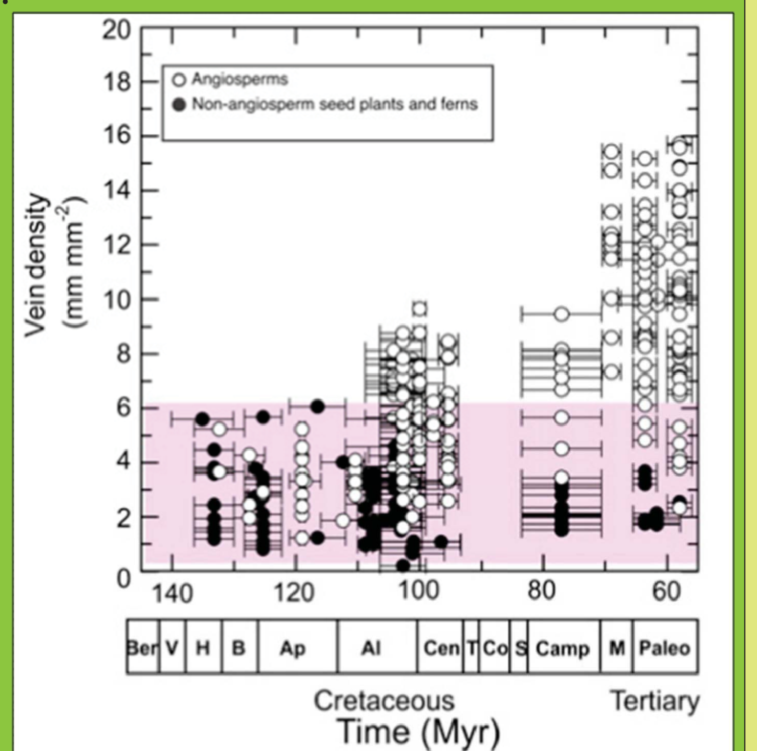


Fig. 2: Graph showing increase in leaf vein density of angiosperm fossils (open circles) from the Cretaceous through the Tertiary, and the steady vein density of non-angiosperm (closed circles) (Feild et al. 2011).

Of the two stable isotopes of carbon, ^{12}C and ^{13}C, the lighter ^{12}C comprises significantly more CO_2 in the modern atmosphere (98.89%). Carbon isotope signatures measured from modern and fossil leaves record how the leaf discriminates against the heavy ^{13}C isotope during photosynthesis, which occurs in C_3 plants mostly due to the enzyme RuBisCO. Farquhar et al. (1982) relate stomatal conductance to isotope discrimination, or fractionation. When the stomatal conductance is small (i.e. stomata are more closed), intercellular partial pressure is small, which nearly disables enzymatic fractionation. Consequently, the leaf fixates more ^{13}C in leaf tissue when its stomata are more closed (Farquhar & Sharkey 1982). Because leaf veins bring water closer to the stomatal sites of water loss, a greater density of leaf veins enable greater stomatal conductance, resulting in an increased intercellular partial pressure, and a greater discrimination against heavy ^{13}C. This results in a more negative $\delta^{13}C$ for leaves with high vein density.

$$\partial^{13}C = \left[\frac{\left(\frac{^{13}C}{^{12}C}\right)_{sample}}{\left(\frac{^{13}C}{^{12}C}\right)_{standard}} - 1 \right] * 1000$$

Fig. 3: Carbon isotope ratios are commonly expressed in δ-notation, compared to the standard Vee Pee Dee Belemnite.

II. Hypotheses

Rates of photosynthetic productivity increased during the evolution of modern angiosperms.

A. Angiosperm leaf vein density increased during angiosperm evolution, from the Early Cretaceous to modern time.

B. Increased rates of productivity in modern angiosperms results in more negative $\delta^{13}C$ values in comparison to both extinct angiosperms and living and extinct non-angiosperm plants.

C. $\delta^{13}C$ can be reliably measured from fossil plants, and can be constrained to reflect on isotope fractionation during photosynthesis.

III. Experimental design

Problem: To determine whether Early Cretaceous angiosperms from the well-defined lineage Proteales had high vein density and high productivity rates comparable to its modern relatives, or whether these characters were more comparable to contemporaneous ferns and conifers growing in the same habitat.

A. Compare leaf vein density between modern and Cretaceous angiosperms and ferns.
B. Measure average $\delta^{13}C$ from modern leaves from the same environment:
  (1) Trees of angiosperm *Platanus occidentalis* (American sycamore)
  (2) Ferns from Osmundaceae
  (3) Conifers from Cupressaceae
C. Measure $\delta^{13}C$ from carbonaceous compression fossils from the Early Cretaceous:
  (1) Early angiosperm protealeans, thought to belong to Platanaceae lineage
  (2) Ferns from Osmundaceae lineage
  (3) Conifers from Cupressaceae lineage

The modern-fossil pairs of ferns and conifers create control groups. The difference in $\delta^{13}C$ within these groups allows us to constrain change in $\delta^{13}C$ due to diagenetic effects and changes in pCO_2 and the abundance ratio of $^{13}C/^{12}C$ in the atmosphere between the Early Cretaceous and today.

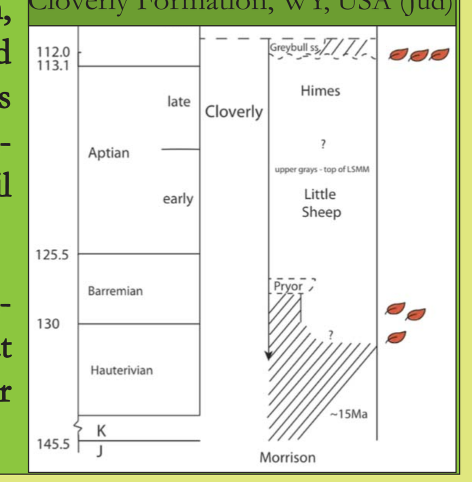
The difference in $\delta^{13}C$ between the modern sycamore and the Cretaceous platanoid is expected to be driven by these same processes, and the difference in carbon fractionation as driven by leaf vein evolution.

IV. Geologic setting

Fossilized leaves are to be collected from the Himes Member of the Cloverly Formation in the Bighorn Basin, WY, USA. Fossil angiosperms have been collected and identified from this site (Jud 2010), marked in Figure 4 as red leaves. Dated at 110-115 Ma, these represent the earliest angiosperm macrofossils known from the fossil record of the western UnitedStates.

The sediments in the Himes Member are generally identified as fluvial depositional environments, indicating that the leaves deposited would have had sufficient water supply.

Fig. 4: Stratigraphic column of Cloverly Formation, WY, USA (Jud)



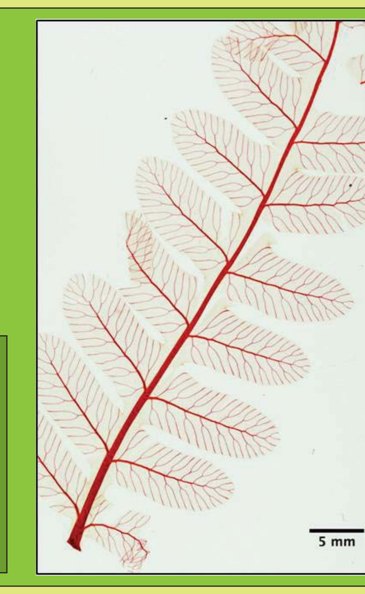
V. Methods

A. Leaf vein density measurements

Leaf vein density: length of veins (mm) divided by surface area of lamina (mm^2), measured using ImageJ (NIH Image, Bethesda, MD, USA)

Fig. 5 (left): Early Cretaceous platanoid, D_V=1.71 (±0.19 SD)

Fig. 6 (right): Modern *Osmunda claytoniana* from Cleared Leaf Collection, D_V=1.80 (±0.37 SD)



B. Carbon isotope abundance measurements

Step 1: Collect leaf specimens

Modern leaves from Seneca Creek State Park | Carbonaceous fossil leaves from Cloverly Formation

Fig. 7: *Osmundrastrum cinnamomeum*, fern fiddlehead

Fig. 8: Early Cretaceous platanoid

Step 2: Prepare specimens

Dry modern leaves
Crush modern leaves
Weigh 30-70 μg in tin cup

Scrape carbon from fossilized leaf
Weigh ~100 μg in tin cup

Step 3: Analyze for stable carbon isotopes

Load samples into loading carousel
↓
Combust samples in elemental analyzer
↓
Gas carried to mass spectrometer, ionized, and deflected into collector cups
↓
Ions measured by computer, outputs in Excel file

VI. Initial results

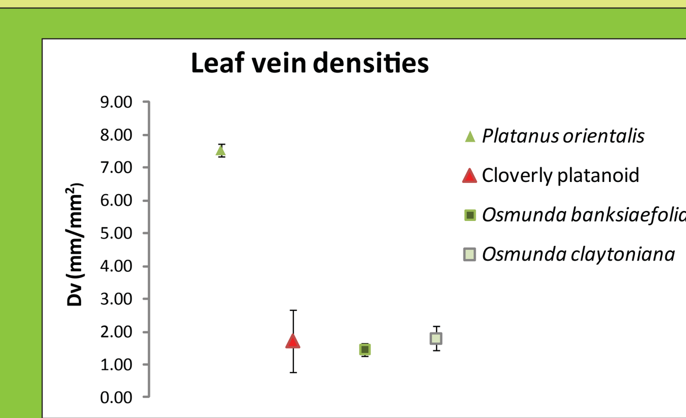


Fig. 9: Initial results of D_V measurements, with error bars showing one sigma.

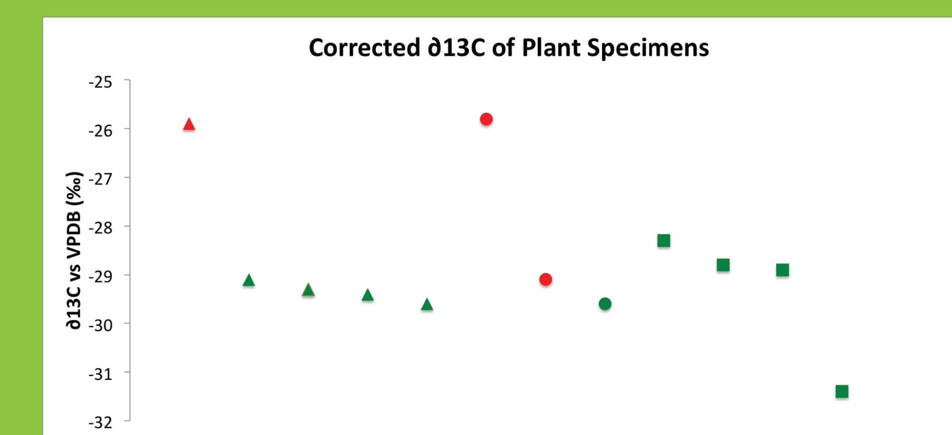


Fig. 10: Corrected $\delta^{13}C$ values for initial data. Initial data points include angiosperms (triangles), conifers (circles), and ferns (squares). Data were collected from both modern (green) and fossil (red) specimens.

VII. Discussion of initial results

The Cretaceous platanoid measured has a D_V much lower than modern Platanaceae, similar to D_V observed in modern ferns (Osmundaceae). This could be due to an actual difference in leaf vein density among this lineage between the Early Cretceous and the present day, or due to error from insufficient fossil preservation.

The modern sycamore (Platanaceae) measured in the initial carbon isotope study has a $\delta^{13}C$ that is nearly identical to $\delta^{13}C$ of the conifer. It is important to keep in mind that the sycamore material analyzed was merely the interior of a bud, as the plant had not fully begun to leaf at the collection time, and that the value does not reflect $\delta^{13}C$ of a fully photosynthesizing leaf.

Another interesting feature of the carbon isotope abundance measurements is that the $\delta^{13}C$ of two fossil taxa (between a fossil conifer and fossil angiosperm) are nearly identical. This may signal that there is a similar fractionation between the fossil conifer and fossil angiosperm.

VIII. Timeline of future work

Summer 2011: Collection of modern and fossil specimens

Fall 2011: Continue collection of modern specimens
Identify appropriate fossil specimens
Collect vein density and carbon isotope measurements
Interpret and analyze results
Speculate on further work to improve results/sampling method/etc.

IX. References

Boyce, C.K., T.J. Brodribb, T.S. Feild & M.A. Zwienecki. 2009. Angiosperm leaf vein evolution was physiologically and environmentally transformative. *Proceedings of the Royal Society B* 276: 1771-1776.
Brodribb, T.J., N.M. Holbrook, M.A. Zwienecki & B. Palma. 2005. Leaf hydraulic capacity in ferns, conifers and angiosperms: impacts of photosynthetic maxima. *New Phytologist* 165: 839-846.
Brodribb, T.J., T.S. Feild & G.J. Jordan. 2007. Leaf maximum photosynthetic rate and venation are linked by hydraulics. *Plant Physiology* 144: 1890-1898.
Brodribb, T.J. & T.S. Feild. 2010. Leaf hydraulic evolution led a surge in leaf photosynthetic capacity during early angiosperm diversification. *Ecology Letters* 13: 175-183.
Farquhar, G.D., & T.D. Sharkey. 1982. Stomatal conductance and photosynthesis. *Annual Review of Plant Physiology* 33: 317-345.

IX. References (continued)

Feild, T.S., T.J. Brodribb, A. Iglesias, D.S. Chatelet, A. Baresch, G.R. Upchurch, Jr., B. Gomez, B.A.R. Mohr, C. Coiffard, J. Kvacek & C. Jaramillo. 2011. Fossil evidence for Cretaceous escalation in angiosperm leaf vein evolution. *Proceedings of the National Academy of Sciences* Early Edition.
Jud, N.A. 2010. Angiosperms from the Early Cretaceous of Wyoming. *Geological Society of America Abstracts with Programs* 42: 482.
Noblin, X., L. Mahadevan, I.A. Coomaraswamy, D.A. Weitz, N.M. Holbrook & M.A. Zwienecki. 2008. Optimal vein density in artificial and real leaves. *Proceedings of the National Academy of Sciences* 104: 9140-9144.
Roth-Nebelsick, A., D. Uhl, V. Mosbrugger & H. Kerp. 2001. Evolution and function of leaf venation architecture: a review. *Annals of Botany* 87: 553-566.
Sack, L. & K. Frole. 2006. Leaf structural diversity is related to hydraulic capacity in tropical rain forest trees. *Ecology* 87: 483-491.
Sperry, J.S. 2003. Evolution of water transport and xylem structure. *International Journal of Plant Sciences* 164: S115-S127.

X. Acknowledgements

I am immeasurably grateful for Nathan Jud's guidance. His endless patience, infectious excitement, and passion for learning have been beyond invaluable for leading me to discover the world of paleobotany.
I would like to thank Jay Kaufman for his wisdom and leadership, especially with technical laboratory work and writing suggestions.
I owe many thanks to Thomas Holtz for his insight and encouragement, and especially for sparking my initial interest in geology and paleontology.
Thanks to Scott Wing for providing advice and consultation.
Thanks to Yongbo Peng, Natalie Sievers, and Sara Peek for assisting me in the geochemistry laboratory.
Thanks to my fellow seniors for supplying their good humor and various assistance.
Thanks to my family and friends for their unwavering support and encouragement.