Mechanical Diversity of Stomata and Water Use Efficiency in Michigan Plant Communities

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Introduction:

It has been known for a long time that different plant types have variations in stomatal structure (Franks and Farquhar 2007). Typically monocots, or grasses, have dumbbell stomatal shapes while many other angiosperms have a more kidney shape to their stomata (Franks and Farquhar 2007). In order for plants to survive and grow they need to be able to produce chemical energy through photosynthesis. In land plants photosynthesis is facilitated through their stomata located on the epidermis of leaf tissue. These stomata allow for the uptake of carbon dioxide but, water is always lost as transpiration when the pores open.

Water Use Efficiency (WUE) is a measurement of the amount of CO_2 fixed during photosynthesis per unit of water transpired. Plants' WUE is expected to increase as stomata close, because photosynthesis is reduced less than transpiration (Jones 1993). As leaf vapor pressure deficit (VpdL) increases, stomata will close and WUE should increase (Abbate 2004). Also, an increase in light should result in an increase in WUE because photosynthesis should increase (Fay 1993). Under soil water deficit stomata will close and there will be more light energy than there is CO_2 available to be converted into sugars. Excess light can cause damage to the photosynthetic machinery (Ort 2001). Water Use Efficiency may decrease if there is damage to the photosynthetic apparatus (Swarthout submitted).

Another important factor in understanding WUE is stomatal conductance. Stomata conductance is a measure of how open the stomata of a plant are. WUE is expected to remain fairly constant for C_4 grasses as stomatal conductance increases because the C_4 grass is better at maintaining proportional control of transpiration and photosynthesis levels in drought (Knapp, 1994). Stomata typically open and close in response to the direct effect of light on photoreceptors in the guard cells or indirectly in response to changes in internal CO_2 concentrations (C_i) (Huxman and Monson, 2003). The rates of stomatal opening and closure, or the conductance of the plant has also been shown to differ among C_3 and C_4 plants. The main difference between C_3 and C_4 plants is their variations in the photosynthetic fixation pathway. According to Huxman (2003) stomata of C_3 plants open and close more directly to light while C_4 plants' stomata respond more directly to internal CO_2 conditions.

Recently it has been suggested by Franks and Farquhar (2007) that ratio of stomatal pore to total stomatal area was critical in determining rates of stomatal opening and closure in *Triticum aestivum*. It was proposed that the smaller the pore area to total stomatal area ratio means that there can be a greater conductance over a smaller area of the leaf's epidermis allowing for a greater control over the conductance of the plant. This is important because conductance is what allows transpiration to occur. Thus if there is a higher control over the transpiration of the plant the Water Use Efficiency of the plant is expected to be higher.

We performed the study to investigate whether internal CO_2 and light levels impact stomatal conductance differently in C_4 and C_3 plants. Also, investigated using the model species of *Elymus repens* (Quack Grass), *Solidago altissima* (Tall Goldenrod), and *Andropogon scoparis* (Big Bluestem) was if WUE correlated with the ratio of pore area to total stomatal area. The working hypotheses were that a C_4 plant, *Andropogen scoparis*, will have a higher water use efficiency as conductance increases due it's different method for fixation of carbon into sugars. Also it was hypothesized, based upon work done by Frank and Farquhar (2007) that the species with the lowest stomatal pore to total stomata ration will have the highest water use efficiency.

Materials and Methods:

Study Site:

First three species were selected from a possible list of 25 plant species at Pierce Cedar Creek Institute (PCCI) near Hastings, Michigan. The list was narrowed down the ten species dependent upon availability and accessibility in the field. The next selection was done through a series of stomatal imprint collections from these ten species on June 7^{th} and 8^{th}. 2007. These imprints were done using clear fingernail polish that was painted on the leaf and then removed using tape and placed upon microscope slides. We then based our final selection on stomatal size and density, which were viewed using a light microscope at 400x magnification. The area of the view was taken using a micrometer and the average number of stomata per mm^2 was determined per species. The plants examined were *Elymus repens* (Quack Grass), *Solidago altissima*, (Tall Goldenrod), and *Andropogon scoparis* (Big Bluestem).

Estimation of Stomatal Pore Size:

Four plants per species were transported back to the Hope College growth chamber on July 14^{th} to perform further controlled experiments involving light responses. On July 16^{th}, 17^{th}, and 18^{th}, 2007, epidermal peels were taken from the transplanted Tall Goldenrod, Big Bluestem, and Quack Grass in order to view the stomatal sizes and densities. The slides were exposed to varying natural light conditions and submerged in a buffer solution comprised of 25mM MES buffer (pH.6.5) with 1mM KCl and 0.1mM $CaCl_2$ to stimulate stomatal opening. Using a compound microscope equipped with a Nikon camera, pictures were taken of the stomata. These pictures were then analyzed using ImageJ software (NIH, version 1.37) and a drawing tablet (Wacom, Vancouver, WA). The pixel to mm ratio was determined using a stage micrometer. Using this equipment, total stomatal area, the area of the guard cells, including the pore, and the area of the pore were obtained.

G-C_i and G-I Curve:

Light and internal carbon dioxide data was obtained, on July 24^{th}, 25^{th}, and 26^{th}, 2007, using the Li-Cor system at PCCI. For the G-I curve three plants were sampled for the Big Bluestem and Quack Grass, while only two were sampled of the Tall Goldenrod due to unfavorable weather conditions; during this period, only one plant of each species was sampled per day. For the G-C_i data three plants were sampled for the Quack grass and only two plants were sampled for Tall Goldenrod and Big Bluestem. One leaf was evaluated per plant for both the light and internal CO_2 curves. For the light curve, settings were controlled on the Li-Cor consol to vary the light levels that the leaf was exposed to. Starting with a light level of 2000, the machine systematically dropped the light levels every five minutes for one hour until the leaf was exposed to no light. Afterward, the Li-Cor system was programmed to a light level of 1400 for fifteen minutes to reactivate the leaf's photosynthetic machinery. The internal CO_2 curve was run in a similar fashion; the consol was programmed to first decrease and then increase the CO_2 levels that were exposed to the plant. During these curves, the leaf temperature and humidity levels remained constant; for the light curve, the internal carbon dioxide levels remained constant at 400 umol, while for the CO_2 curve, the light level remained constant at 1400 units. Data was analyzed using Sigma Plot on July 30th-August 10th , 2007.

Statistical analysis:

Data analysis will be done using a curve of the mean conductance plotted against the mean internal CO_2 levels and a curve showing the mean conductance as mean light intensity increases for *Elymus repens, Solidago altissima* and *Andropogon scoparis*. A conductance (g) versus internal CO_2 (C_i) curve is a variation of the more typical ACI curve. This method allows for a determination of the initial Rubisco limited portion of the curve (Warren 2006) and a G-C_i curve is a modified A-I curve that indicates the responsifity of the biochemical machinery, to the CO2 supply, and its role in determining the conductance of the plant (Barradas 1996)

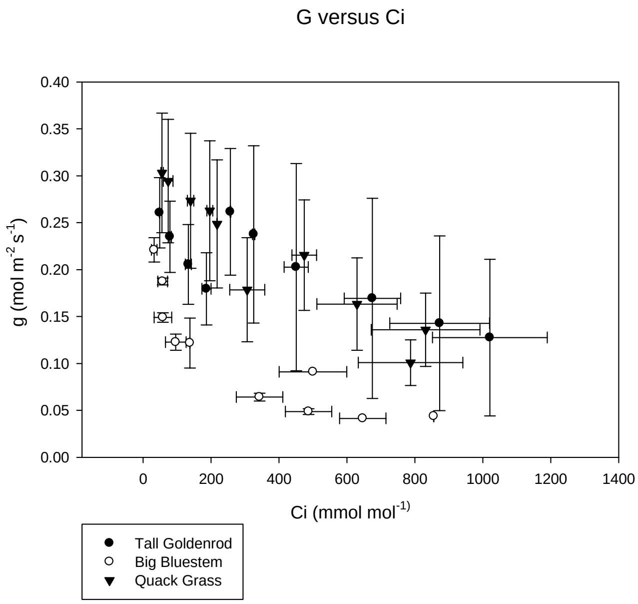
Results:

G-C_i and G-I Curve:

A G-C_i curve was created using the data obtained on July 24th, 25th and 26th 2007. This data showed a much steeper decrease in conductance as the internal carbon dioxide increased for *Andropogon scoparis*. This trend was also observed for *Elymus repens* and *Solidago altissima* but it happened more gradually (Figure 1). In fact *Andropogon scoparis* had a steeper slope then did *Elymus repens* and *Solidago altissima* as C_i increases ($F_{2,6}$ = 8.5788, $p<0.05$) During this time period a G-PARI curve was run and there was no species difference was observed between the light level and the conductance level (Figure 2).

Stomatal Pore Size:

When the stomata were observed under the microscope there was a definite difference in their shape. The *Solidago altissima* had a kidney shaped stoma while *Andropogon scoparis* and *Elymus repens* had dumbbell shaped stomata (Figure 3,4,5). Also, *Elymus repens* was the only species to have plentiful stomata on both the top and bottom of the leaf. *Solidago altissima* and *Andropogon scoparis* had no or very few stomata on the top of their leaves. The mean numbers of stomata counted on each species are found in Table 1. The average stomata area was found for the total stomata, the subsidiary cells, if present, the guard cell and the pore. The mean pore area was then divided by the mean total area for each species. It was found that species with the smallest pore area to stomatal area ratio was *Elymus repens* followed by *Andropogon scoparis* (Table 2). However, it was not found that the species with the smallest ratio had the highest Water Use Efficiency. Instead *Andropogon scoparis* had the highest WUE while having the medial total pore to total stomata ratio (Figure 6).



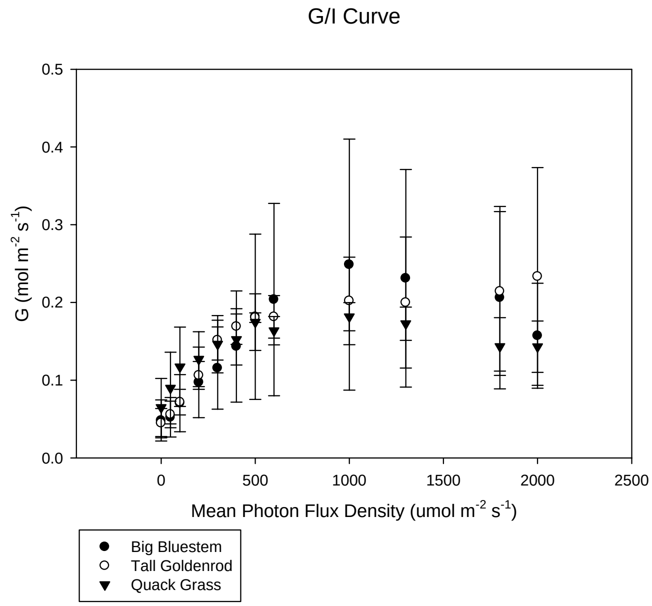


Figure 1: G-C_i curve for *Solidago altissima*, (y = -0.000575622x + 0.284486763) *Elymus repens* (y = -0.000284882x + 0.316455874), and *Andropogon scoparis* (y = -0.001509189x + 0.262198843). All regressions are for C_i <200 ppm which is common operating point.

Figure 2: G-I curve for *Solidago altissima*, (y=) *Elymus repens* (y=), and *Andropogon scoparis* (y=). All regressions are for I<200 ppm which is the common operating point.

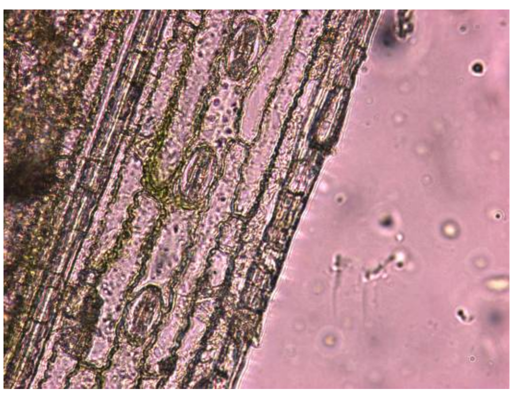


Figure 3: Big Bluestem epidermal peel taken after a half-hour exposure to light at 400x magnification.

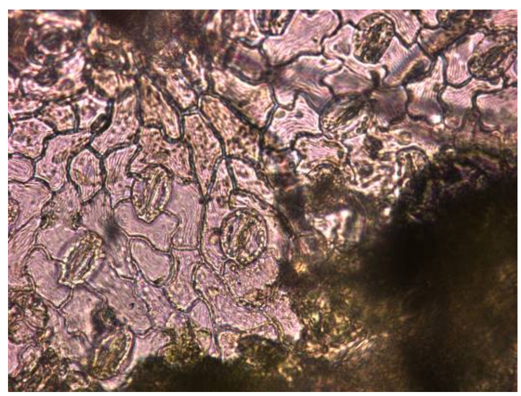


Figure 5: Tall Goldenrod epidermal peel taken a after a half-hour exposure to light at 400x magnification.

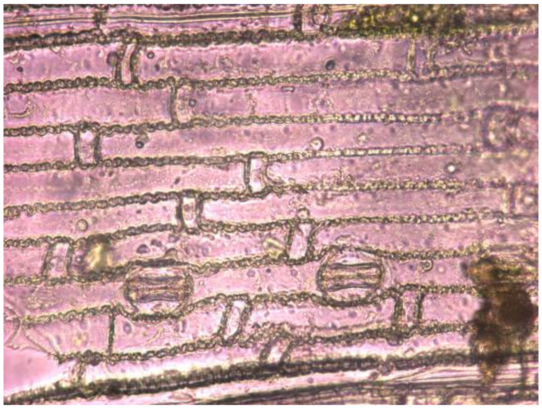


Figure 4: Wild Rye epidermal peel taken after a half-hour exposure to light at 400x magnification.

Species		Stomatal Density (stomata/mm^2)
	Top	Bottom
Elymus repens	91.70	88.07
Solidago altissima	0.70	132.81
Andropogon scoparis	0	124.42

Table 1: The mean number of stomata found on the top and bottom of the different plant species.

Species	Mean Total Area	Mean Subsidiary Cell Area	Mean Guard Cell Area	Mean Pore Area	Pore Area / Total Area
Andropogon scoparis	523.39 +/- (213.67)	326.98 +/- (32.443)	151.40 +/- (16.33)	61.45 +/- (2.56)	0.117
Solidago altissima	711.734+/- (78.78)	0 +/- (0)	582.88 +/- (33.39)	128.85 +/- (14.26)	0.181
Elymus repens	746.00 +/- (18.65)	390.75 +/- (17.03)	311.41 +/- (15.65)	44.05 +/- (3.33)	0.059

Table 2: A comparison of the areas of different structures in the stomata for the three species.

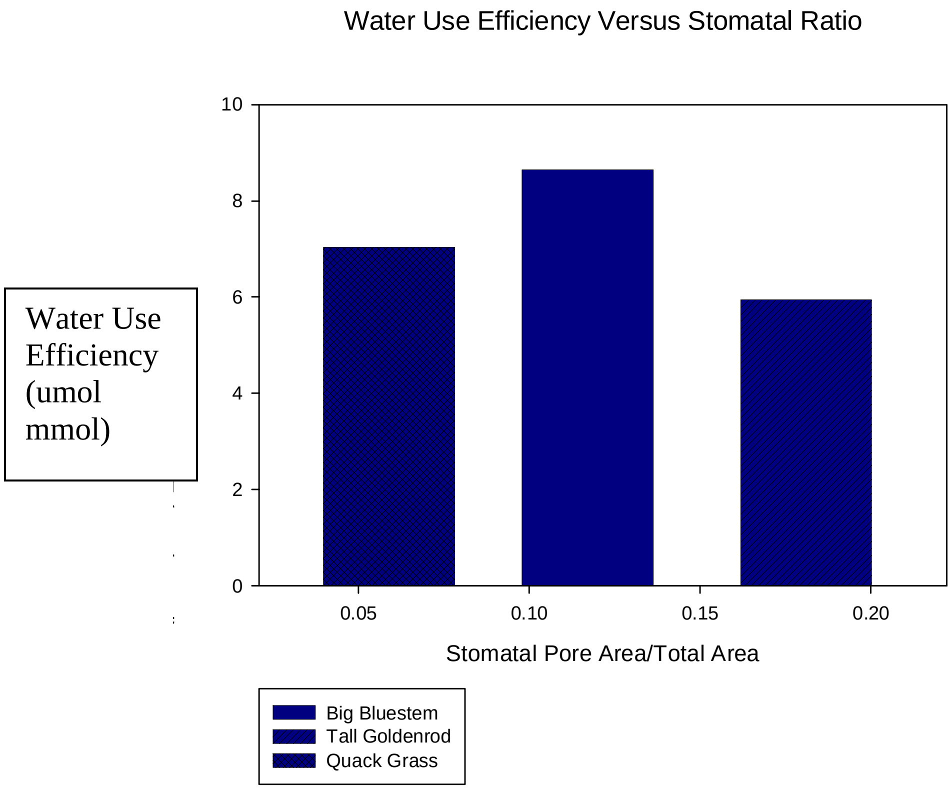


Figure 6: A comparison of the stomatal pore area to total area ratio to Water Use Efficiency for *Andropogon scoparis*, *Elymus repens*, and *Solidago altissima*.

Discussion:

The results of this experiment did not agree with the conclusions drawn by Frank and Farquhar (2007). Instead of the plant with the smallest pore area to total stomatal area ratio having the largest Water Use Efficiency it had the medial WUE. However, it did have a higher WUE then the other C_3 plant. Perhaps if the only plants studied had been C_3 this trend would have held true however, *Andropogon scoparis*, a C_4 grass, over wrote this trend perhaps because of its different method for fixing carbon. This would indicate that our data supports the conclusions made by Huxman and Monson (2003) that C_4 plants have an on average higher WUE then C_3 plants. In addition this higher WUE is probably due to their method of elimination of photorespiration.

This increased mean WUE in C_4 is probably due to their increased sensitivity to their internal CO_2 levels which was seen in our data. Our data suggests that because the C_4 grass maintained a higher WUE, due to its decrease in conductance as internal carbon dioxide increased, C_4 grasses may have a greater tolerance of drought. The reason this might be true is that WUE is the photosynthesis of the plant divided by its conductance, thus if conductance is decreasing faster then photosynthesis then WUE is maintained or increased.

An interesting thing found in this research is that the C_3 grass had a higher WUE and smaller ratio then the C_3 herbacious shrub, *Solidago altissima*. This may indicate that C_3 plants that have a smaller pore area to total area ratio do maintain a higher WUE then those plants without this ratio. If this were the case then it would support Frank and

Farquhar's (2007) research. In their study they did not look at a C_4 plant and it may be that C_4 grasses are just even better suited to maintain WUE then C_3 plants with this low ratio. By increasing the number of C_3 and C_4 plants studied this trend may become more apparent.

If it is true that WUE is an accurate measurement of a plant's ability to manage drought conditions then our data indicates that C_4 plants should tolerate drought conditions better then C_3 plants. Further research should include testing this trend in more C_3 and C_4 plants to see if C_4 plants do maintain a higher WUE then C_3 plants with a low stomatal pore to total stomatal area ratio and those without this ratio. Another thing that could be tested to see if C_4 grasses should continue to be investigated for biofuel production is to measure the biomass accumulation of different C_3 and C_4 plants under stressed and unstressed conditions.

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