# Screening for Drought Tolerance in Michigan Plant Communities

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*Abstract:*

The purpose of this experiment was to investigate the differences in water use efficiency for $C_3$ and $C_4$ plants and to see if water use efficiency varies with leaf vapor pressure deficit, light; and stomatal conductance; and to see if high water use efficiency promotes drought tolerance. Three species were studied: Big Bluestem, a $C_4$ grass; Tall Goldenrod, a $C_3$ shrub; and Quack Grass, a $C_3$ grass. Data was collected twice, once in June and once in July, to maximize possible environmental effects due to the differences in light and dryness. The data was analyzed using SYSTAT and it was found that water use efficiency varied significantly with time and species, and that conductance had a significant effect on water use efficiency. The C4 species had the lowest stomatal conductance and highest water use efficiency at all sampling times. These results indicate that $C_4$ plants have a different internal mechanism for dealing with drought stress than $C_3$ plants.

*Introduction:*

On the leaves of all plants are small epidermal pores that allow for gas exchange and transpiration: these are stomata. Stomata are able to regulate the amount of carbon dioxide that enters a plant and the amount of water leaves a plant depending on the rates of stomatal opening and closure and the size of the stomata. This activity is directly correlated to the water use efficiency (WUE) of the plant. WUE corresponds to the ratio of carbon dioxide fixed during photosynthesis per unit of water transpired, and plants can control their WUE through adjusting their stomatal behaviors (Jones, 1993). Stomata change their conductance levels when impacted by internal and external conditions, such as carbon dioxide levels, leaf temperature, light conditions, and other gas pressures.

However, the stomata are not the only factors when considering optimal water use efficiencies. $C_3$ and $C_4$ plants do not fix carbon in the same way due to their physiological differences. In $C_3$ plants, the enzyme Rubisco (found in the mesophyll cells) fixes oxygen and carbon dioxide, which enter immediately through the stomata, and converts them into carbon dioxide and sugars, respectively. Rubisco also fixes oxygen via the respiratory pathway and at ambient CO2, the amount of $CO_2$ that can be used in photosynthesis is directly impacted by stomatal conductance. This mechanism is different in $C_4$ plants, however. In $C_4$ plants, carbon dioxide enters through the stomata, and is immediately converted to $HCO_3$. Then that is converted into a four-carbon acid by the enzyme PEP Carboxylase in the mesophyll cells. This four-carbon acid is then converted back into $CO_2$ in the bundle sheath cells, and fixed there by Rubisco into sugars. Therefore, in $C_4$ plants, PEP-carboxylase is very efficient at capturing low CO2 concentrations and photorespiration is eliminated. (Knapp, 1993). Therefore, WUE is expected to be higher for $C_4$ plants as stomatal conductance changes because the $C_4$ plant has a way of concentrating $CO_2$ around Rubisco. This means that their change in WUE is fairly independent of stomatal conductance (Knapp, 1993). So, in this experiment, the follow questions were investigated:

1. Are there any differences in WUE in $C_3$ versus $C_4$ plants?
2. If so, does WUE vary with leaf vapor pressure deficit (VpdL), light (PARi), or stomatal conductance?
3. Does a higher WUE infer higher drought tolerance?

*Methods*

First three species were selected from a possible list of 25 plant species at Pierce Cedar Creek Institute (PCCI). 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 7th and 8th. 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. The plants chosen were Elymus repens (quack grass), Solidago altissima. (Tall goldenrod), and Andropogon scoparis (big bluestem). Quack grass and Big Bluestem are monocots while Tall Goldenrod is a dicot; Quack Grass also had stomata on both the top and bottom of its leaves, while Big Bluestem and Tall Goldenrod only had stomata on the bottom.

A total of twelve plants were chosen as the experimental plants at the Pierce Cedar Creek Institute on June 14th 2007. There were four replicates of each of the three species (goldenrod, wild rye and big bluestem). Using an Infrared Gas Analyzer (IRGA, Li-Cor 6400, Lincoln, NE), we measured the rates of photosynthesis, transpiration, stomatal conductance and internal leaf CO2 concentrations on three leaves per plant. Environmental conditions in the leaf chamber were set to temperature and light at various times during the day, based upon readings from a weather station (Li-Cor 1400) which provided data about the temperature, humidity and light levels. For the grasses, leaf areas in the chamber were estimated by measuring the average width of the leaf, in the grasses and multiplying that by the known length of the chamber, which was 3 cm. The area of the goldenrod leaf was taken differently because the shape of the leaf was more oval than rectangular, and could not be measured by a simple area calculation. The leaves were marked at the edges of the chamber before removing the entire leaf from the chamber and the plant. The area that occupied the chamber was measured with a leaf area meter. Data was collected and stored over two 24 hour periods, with two collections a day between 9:00 am and 12:00pm and 2:00 and 5:00pm, in the field at PCCI on June 14$^{th}$ and 15$^{th}$.

Also in the field, predawn plant water status was measured twice between 6:30 and 7:00 am on June 13$^{th}$ and 14$^{th}$ by the BOMB. This provided water potential measurements before any stomata opened. The water potential data gives information about how much water is available in the soil which is important in understanding the transpiration of the plant and whether the plants are optimizing their water supply. In order to achieve this, black plastic bags were placed over the plants were placed over the plants the evening before to prevent light from causing stomatal opening in the morning and preventing transpiration from influencing the predawn water status of the plant. One leaf per plant was sampled with the Scholander Pressure Bomb.

*Statistical Analyses*

Two Way Analysis of Variance was used to see if species and time impacted predawn water potentials, WUE, light, VpdL, and stomatal conductance at time of sampling using SYSTAT.

The Scholander Pressure Bomb was once again used to obtain pre-dawn water potentials of the replicates in order to recognize the amount of water stress they were experiencing. The analysis involving SYSTAT also remained the same for newly acquired data.

*Results*

Figure 1 shows the relationship between VpdL and WUE for each species and each collection period. No significant relationships were found between VpdL and species or between VpdL and time when gas measurements were made. Figure 2 shows the relationship between PARi (light) and WUE. There was not found to be a significant difference in light across the sampling times nor between species when gas exchange measurements were taken.  Figure 3 shows the relationship between G (stomatal conductance) and WUE.  There was a significant difference found between conductance and time ($F_{1, 92}$=25.2, $p<0.05$) and between conductance and species ($F_{2, 92}$=5.69, $p<0.05$).  Figure 4 shows the relationship between WUE and sampling time.  There was a significant relationship found between time and WUE (F=26.98, df=1.92, $p<0.05$) and between species and WUE (F=18.8, df=2.92,$p<0.05$).So overall, conductance was found to be significantly lower in July than in June, and Big Bluestem was found to have the lowest conductance.  Also, Big Bluestem was found to have the highest WUE and in general, WUE for all species was found to be higher in July than in June.

Table 1 shows the predawn water potential readings for each species in both June and July.  These readings show how much water stress the plants are under during different environmental conditions, and correlate with the WUE of the plants.  All of the water potentials were clearly lower in June than July, which was unexpected considering July generally has drier conditions than June. Also, tall goldenrod had higher water potentials in both June and July than the other two species.  This could explain lower stomatal conductance in this species.

| | **JUNE** Mean | **JUNE** St. Error | **JULY** Mean | **JULY** St. Error |
|---|---|---|---|---|
| Big Bluestem | -1.00 | 0.19 | -3.38 | 0.75 |
| Tall Goldenrod | -2.56 | 0.54 | -4.44 | 0.71 |
| Quack Grass | -2.13 | 0.38 | -2.25 | 0.27 |

**Table 1: Mean Water Potentials in June and July**

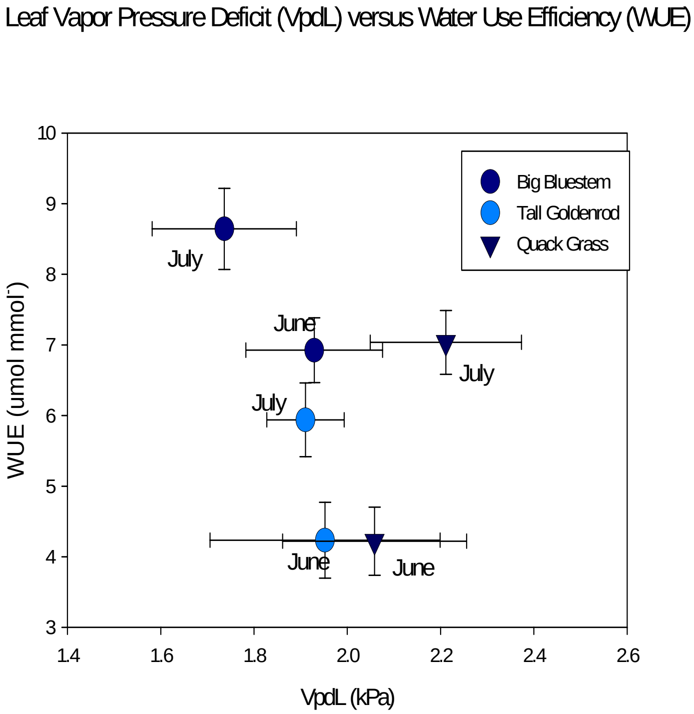


**Figure 1: VpdL versus WUE**

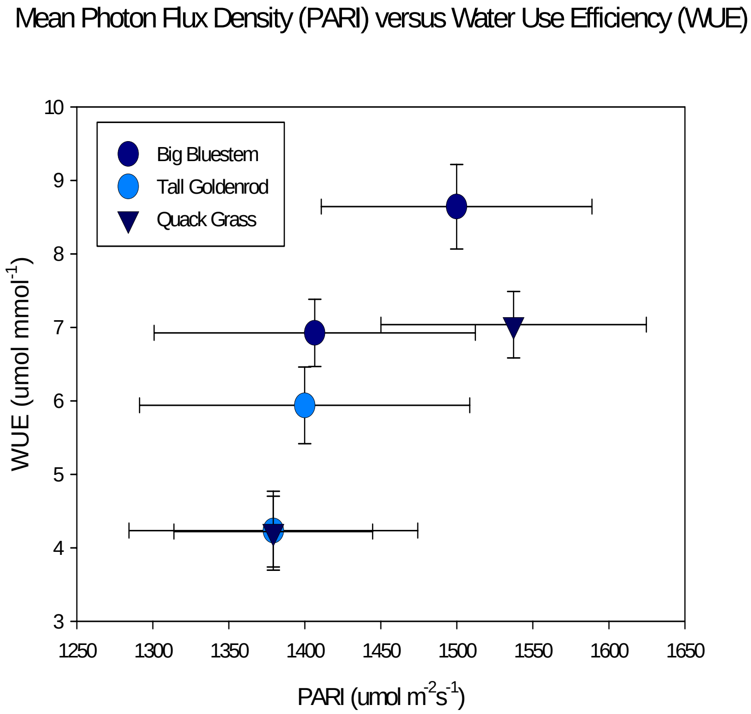


**Figure 2: PARi versus WUE**

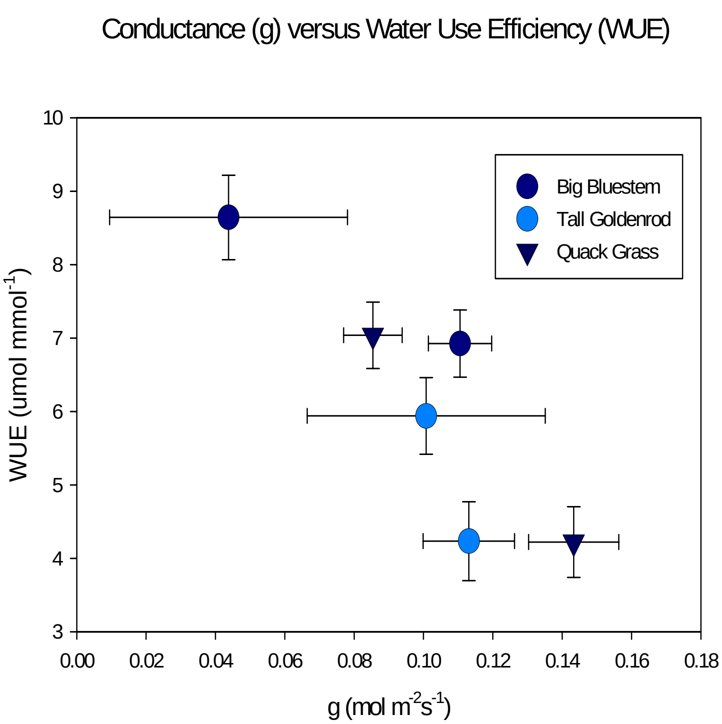


**Figure 3: G versus WUE**

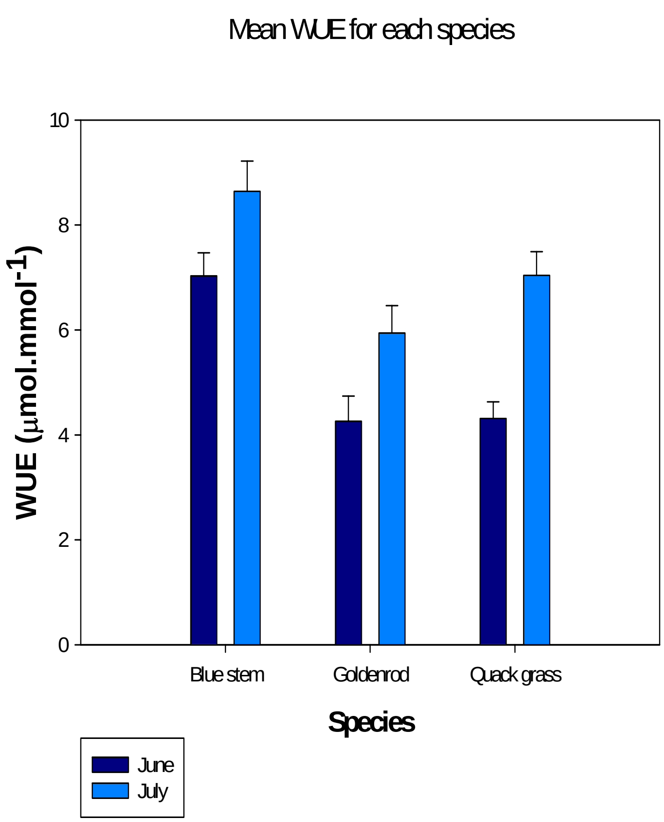


**Figure 4: Species versus Mean WUE**

*Discussion:*

From this experiment, it can be concluded that genetic differences had a larger impact on WUE than environmental conditions. This relates to the differences between the $C_4$ and $C_3$ carbon fixation pathways as well as general differences in stomatal morphology. As expected, Big Bluestem, the $C_4$ plant, maintained a higher WUE for both collection periods despite the changes in VpdL and light. Once again, this relates back to the PEP-Carboxylase carbon dioxide concentration mechanism that allows WUE to remain relatively high and stable.

WUE was affected by stomatal conductance and not environmental conditions. So, as the stomata close, less water is lost and WUE increases. This was especially noticeable in the Big Bluestem because it not only had the highest WUE for each collection period, but it also had the lowest conductance per collection period. It was also found that time had a significant effect on stomatal conductance, but environmental conditions did not affect this. Therefore, the variations in stomatal conductance may be due to leaf tissue water content and soil water content; the stomata would react to these variables by opening/closing the stomata in order to maximize water use efficiency.

The relevance of this experiment to the science is not only to gain a better understanding of the mechanisms by which plants deal with drought stress, but also to aid the scientific community on its search for plants that can be used as biofuels. Scientists are currently looking for plants that can quickly accumulate large biomasses that can withstand drought stress to be used as biofuels. Since plants gain biomass when more CO2 enters the plant as less water leaves, stomatal conductance and WUE are very important in this search. This study could provide very basal information about plants that optimize their water use efficiency which may be good candidates for biofuel production. The data suggests that C4 perennials would be more drought tolerant and different C4 species should be compared to see if genetic differences among them gives rise to more biomass accumulation under varying soil moisture availability.

*References*

Jones, H.G. (1993) Drought tolerance and water-use efficiency. JAC Smith and H. Griffillis, Plant responses from cell to community. Bios Scientific Publishers: 193-202.

Knapp, A.K. (1993) Gas exchange dynamics in C3 and C4 grasses: consequences of differences in stomatal conductance. *Ecology* **74**, 113-123.