Stomatal kinetics in response to CO_2 and its relation to stomatal size and density

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1 Introduction

Water use efficiency (WUE) is very often topic lately. The level of greenhouse gas CO_2 is rising. That means, inorganic C is more available for plants to assimilate, but for photosynthesis is important water also. So on the other hand there is problem with water sufficiency, because of greenhouse gases, the average temperature of the planet is rising and dry areas are expanding. Plants are under a constant stress, because of drought.

2 Materials and methods

2.1 Measuring stomatal conductance and photosynthesis

Materials:

- Two plants Platanus orientalis (normal and stressed)
- Fern *Microsorum diversifolium*
- Two instruments GFS-3000 Portable Photosynthesis System

Procedure:

There were used two plants *Platanus orientalis* and one fern *Microsorum diversifolium*. One *P. orientalis* was not watered for seven days and the second *P. orientalis* was well watered. Plants were kept under artificial conditions. Plane trees were kept under the light intensity of 1000 µmol/m²s, 22 °C and 65% humidity. The *M. diversifolium* was kept under artificial light at 600 µmol/m²s, 22 °C and 65% humidity. Experiments were done in the plant physiology laboratory.

The stomatal conductance and the photosynthesis were measured with two instruments GFS-3000 Portable Photosynthesis System. At first chambers of the machines were adjusted to these settings: temperature 25 °C and humidity 65%. The light settings differed: For plane

trees it was set to 1000 µmol/m²s and 600 µmol/m²s for *M. diversifolium*. The CO_2 levels in chambers were changed during measurements. At first the experiment begun with 400 ppm of CO_2, then 100 ppm and at the end 800 ppm. Changes of CO_2 levels were made after a stabilization of assimilation and stomatal conductance. There was also measured the time of a stabilization.

Figure 1: GFS-3000 Portable Photosynthesis System

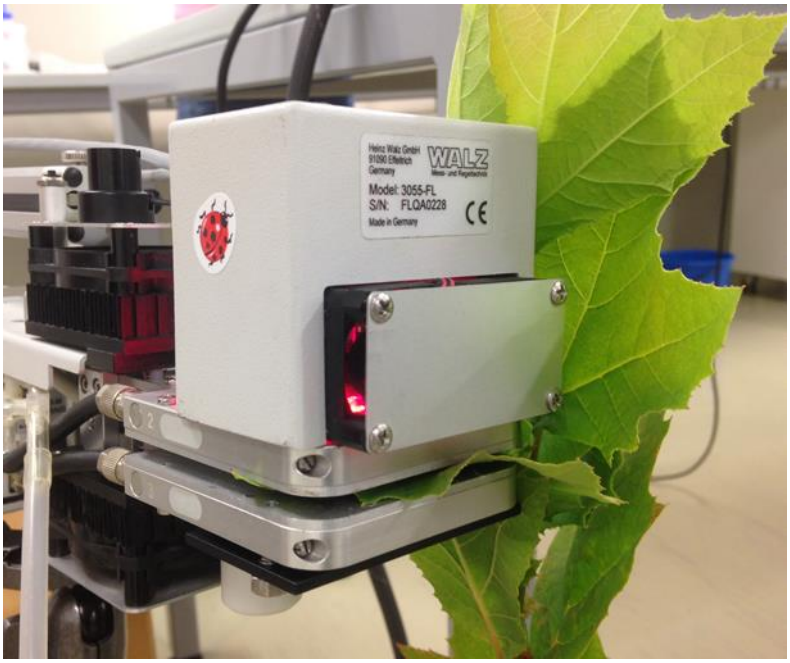

Figure 2: *P. orientalis* leaf in measurement chamber

2.2 Stomatal size and density measurement

Materials:

- Transparent nail polish, transparent tape, slides
- Light microscope, PC, Image-J software

Procedure:

The “varnish”-method was used. The leaf which was previously used for measuring, was coated with clear nail polish on the bottom of the leaf. Leaf-veins were avoided. After the nail polish was dry, transparent tape was sticked on it and pulled off. The tape with the dry varnish was sticked on the slides and analysed under the light microscope. Photos of the leaf bottom imprint were taken in a magnification of 100 and 200. To calculate the stomatal size the length and width of 10 stomata for each plant were measured with the computer program ImageJ. The stomatal size was calculated by multiplying the length with the width. To obtain the stomatal density the amount of stomata on three different parts of the leaf bottom were counted. To acquire average density the formula: number of stomata / area.

Figure 3: Leaf bottom surface imprint on slides

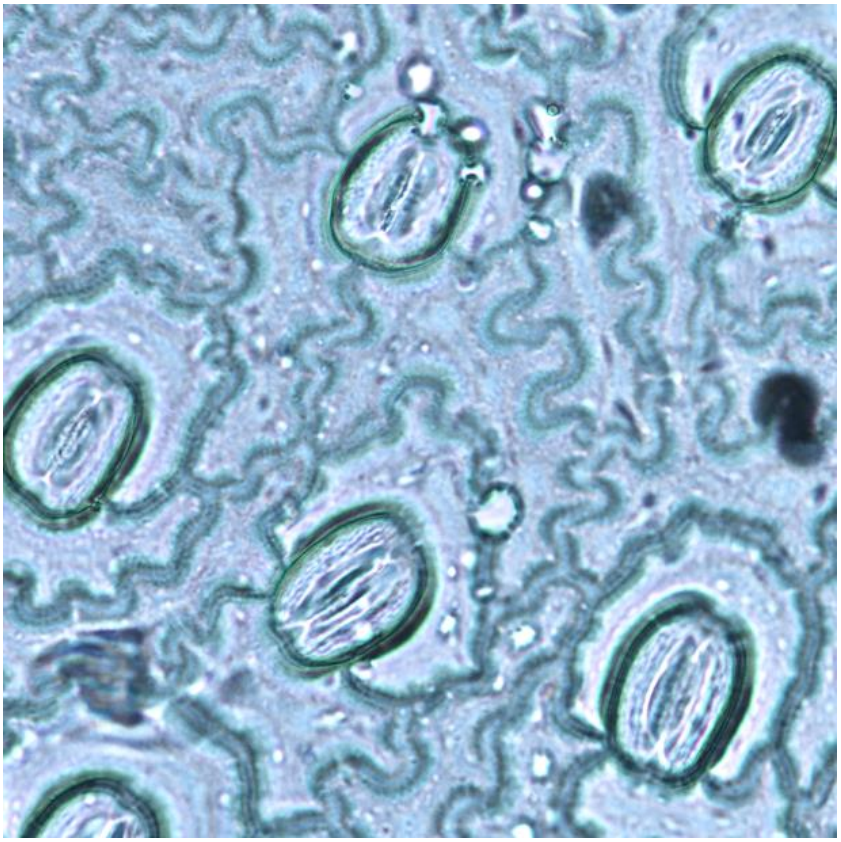

Figure 4: Stomata of *M. diversifolium* at 200 magnification

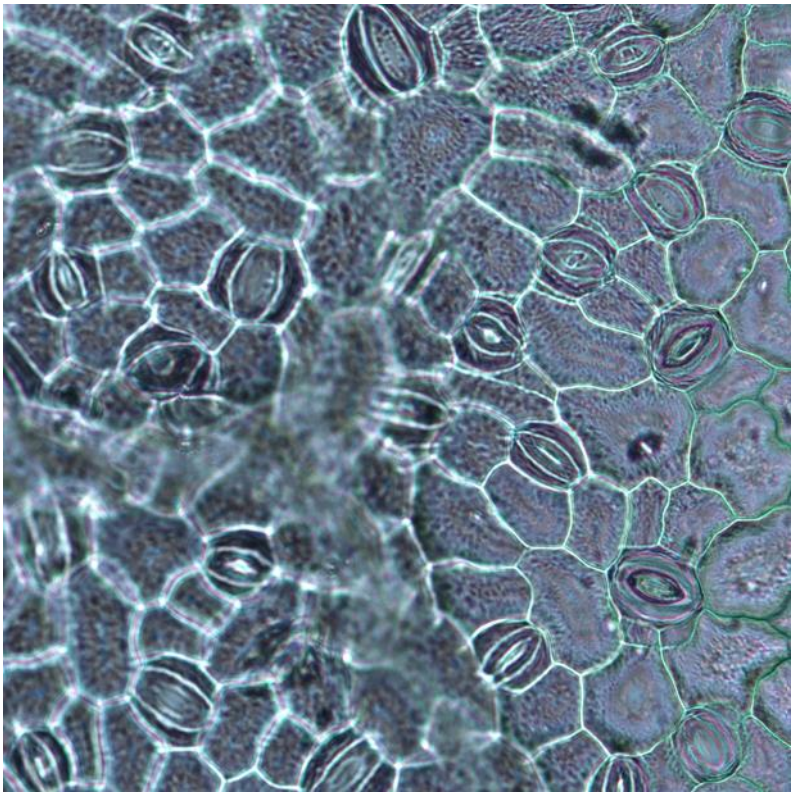

Figure 5: Stomata of *P. orientalis* at 200 magnification

3 Results

During the first experiment gained data about the stomatal conductance and the photosynthesis are in tables 1 and 2. Graphs in figure 6 and 7 were constructed from these data and from CO_2 levels. We calculated values of WUE according to this formula:

$$WUE_{photosintesis} = \frac{Asimilation}{stomata\ conductance}$$

Table 1: CO_2 Assimilation of the three test plants

CO2 [ppm]	*P. orientalis* (normal)	*P. orientalis* (drought)	*M. diversifolium*
100	1,07	0,02	0,51
400	6,86	0,97	3,5
800	12,26	2,37	6,84

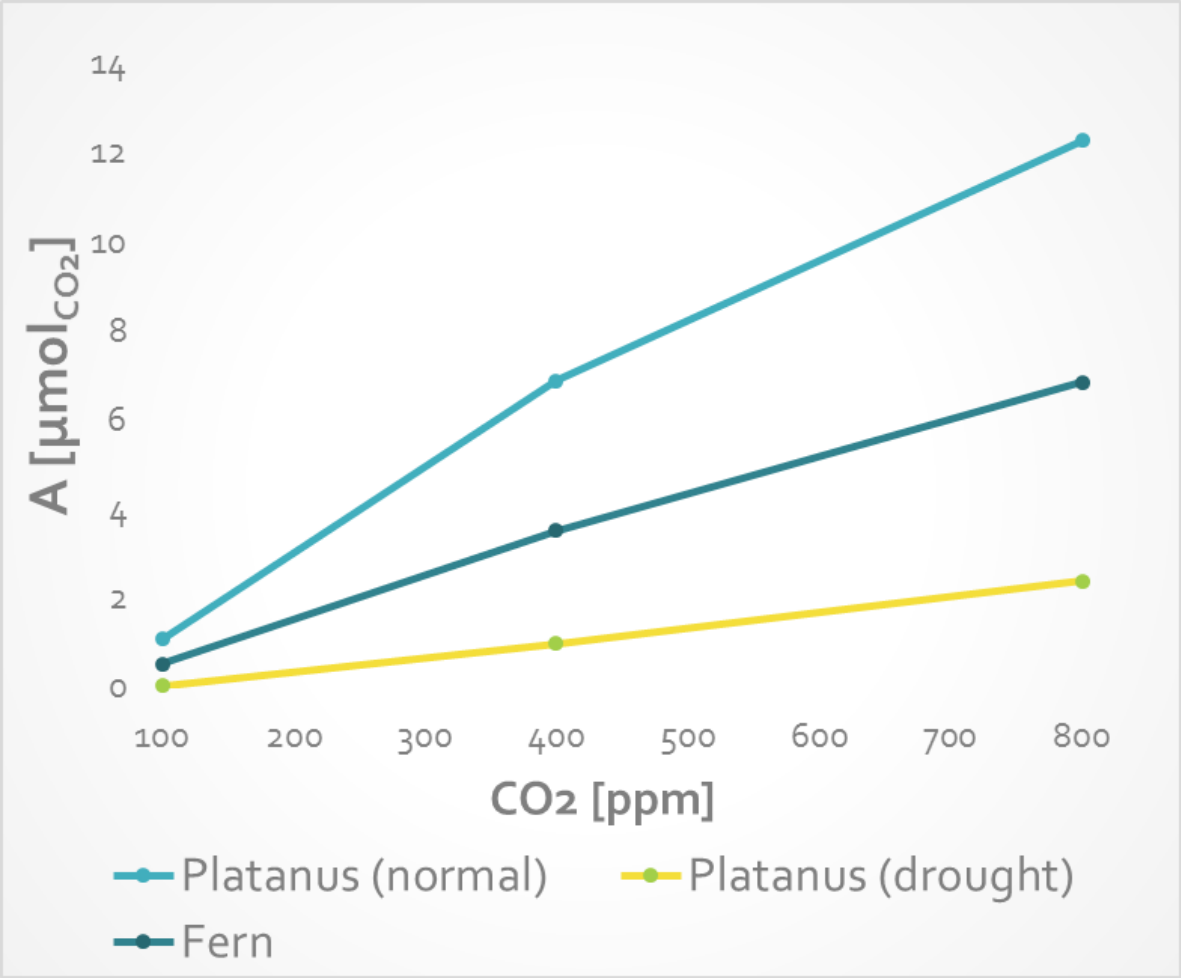

Figure 6: CO_2 Assimilation of the three test plants

Table 2: Stomata conductance of the three test plants

CO2 [ppm]	*P. orientalis* (normal)	*P. orientalis* (drought)	*M. diversifolium*
100	103,4	9,1	52,2
400	58,6	9,9	33,7
800	42,2	9,6	33,8

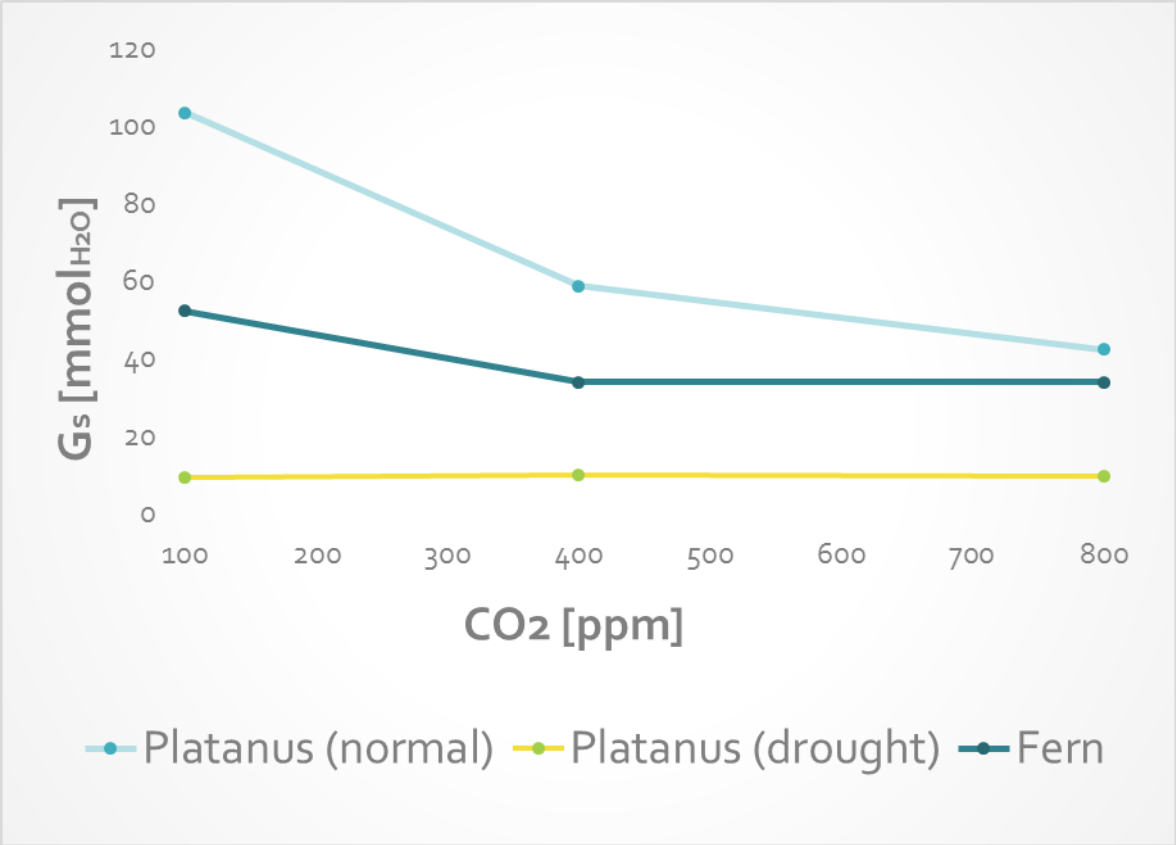

Figure 7: Stomata conductance of the three test plants

WUE values were transferred into the table 3 and figure 8. Stabilization periods of plants was inserted into the table 4.

Table 3: WUE of the three test plants

CO2 [ppm]	*P. orientalis* (normal)	*P. orientalis* (drought)	*M. diversifolium*
100	10	2	9
400	117	97	103
800	290	246	202

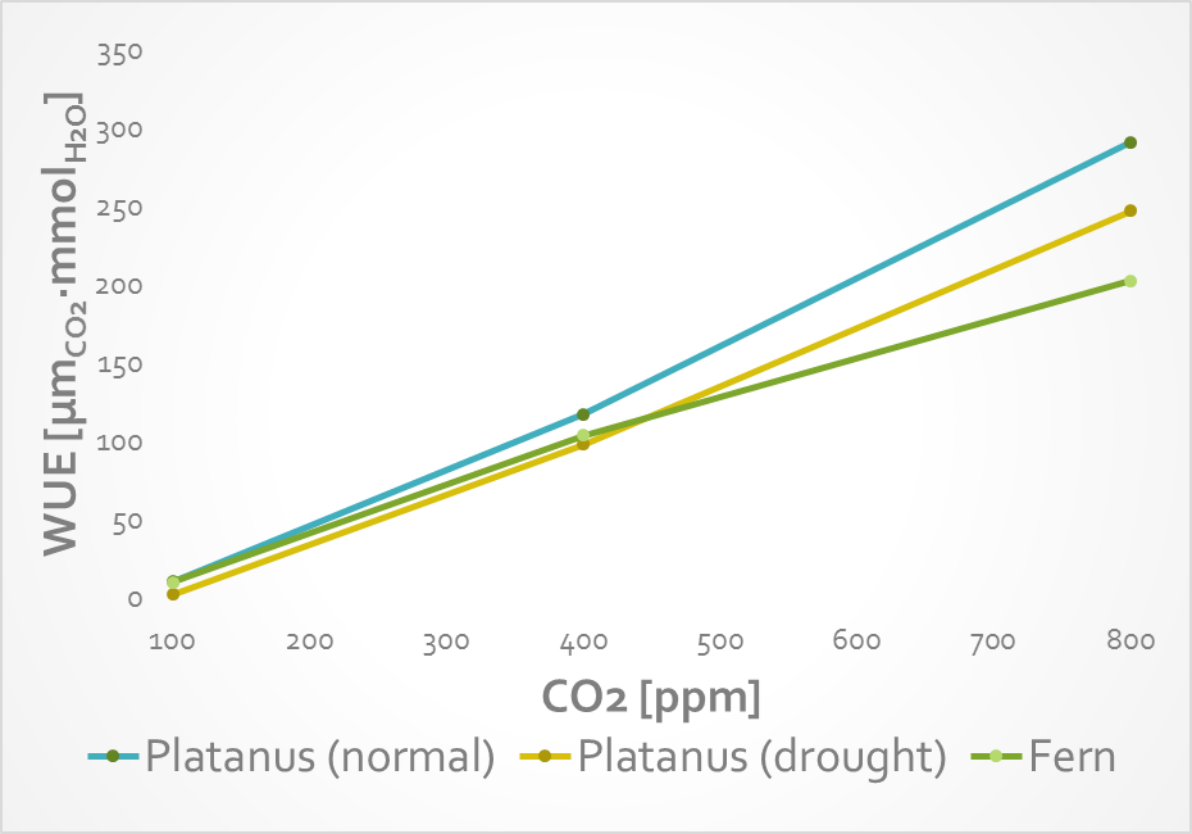

Figure 8: WUE of the three test plants

Table 4: Stabilization period of the three test plants

Measurement	*P. orientalis* (normal)	*P. orientalis* (drought)	*M. diversifolium*
1.	40 min	8 min	62 min
2.	13 min	4 min	13 min
3.	22 min	5 min	17 min
Summary	75 min	17 min	92 min

The stomatal size and density are shown in figure 9 and 10.

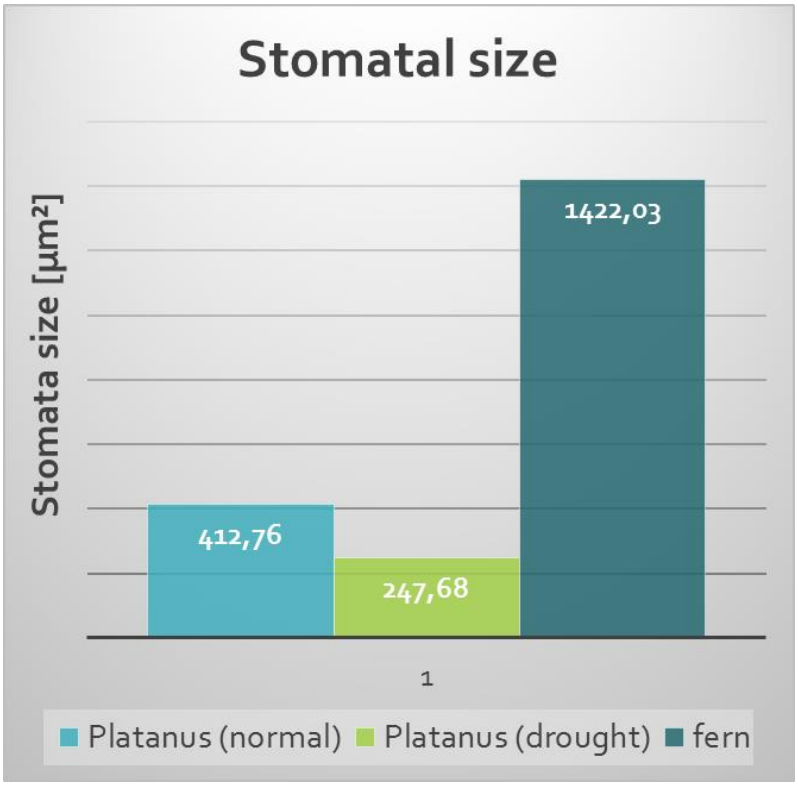

Figure 9: Stomatal size of the three test plants

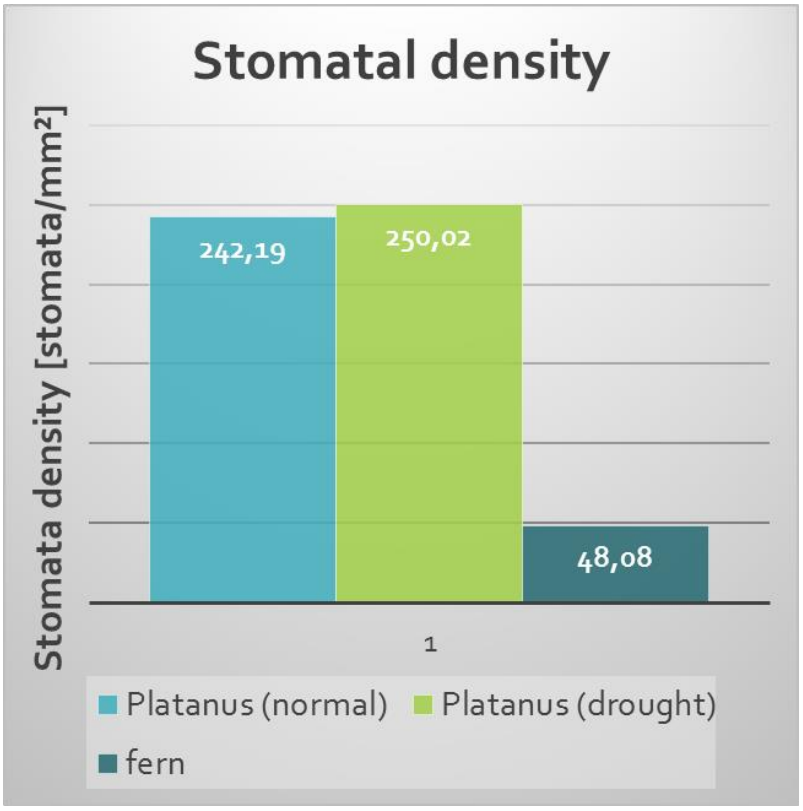

Figure 10: Stomatal density of the three test plants

4 Discussion

We observed reactions of stomata to different levels of CO_2. The plant which was stressed by drought had lower stomata conductance than other plants. It could be related to the physiological mechanism which tend to protect the plant from complete dryness. The plant was stressed even more when there was low CO_2 level. At the level 100 ppm well-watered plants managed to open the stomata more than the plant which suffered by drought. At the level 800 ppm plants had enough of CO_2 in the chamber so they could close stomata. Even though the difference between plane trees is very visible, for better significance and good strength of the experiment, it would be good to do the experiment with more plants and different species.

The varnish method showed us how simple it is to observe stomata structures and compare them between different plant families as gymnosperm and angiosperm. According to the evolution, we can suggest that angiosperm plants have smaller stomata structure and higher density compared to ancient gymnosperm family. On the other hand the habitat of the fern is more humid so it can manage to have bigger stomata than the plane tree. We realized that the fern needs more time to open stomata and have slower reaction to the changes of CO_2 levels. For better resolution, there would be needed to have wide range of gymnosperm and angiosperm species to compare.

5 Conclusion

The plane tree which suffered by drought had low stomata conductance during the whole experiment. The measuring took for 17 minutes in total and g_s values were 9,1; 9,9 and 9,6 $mmol_{H2O}$. WUE values were 2; 97 and 248 $\mu mol_{CO2} \cdot mmol_{H2O}^{-1}$. Well-watered plane tree had higher values. Stomatal conductance values were 103,4; 58,6 and 42,2 $mmol_{H2O}$. WUE values for well-watered plane tree were 10; 117 and 290 $\mu mol_{CO2} \cdot mmol_{H2O}^{-1}$. The measuring took for 75 minutes. The fern had slightly lower values in comparison to well-watered plane tree. Values of stomatal conductance were 52,2; 33,7 and 33,8 $mmol_{H2O}$. WUE values were 9; 103 and 202 $\mu mol_{CO2} \cdot mmol_{H2O}^{-1}$. The measuring of the fern took for 92 minutes.