

Drought and plants

Participants:

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Introduction

In a context of climate change the frequency, the intensity and the lasting of summer drought is expected to increase in the second half of the 21st century in Europe (Meehl and Tebaldi, 2004). As a consequence of this, the soil water availability will decrease and the transpiration of plants will increase. This lack of water impacts different steps of the water flowing through the plant. It impacts the soil-root and leaf-atmosphere interfaces and the water column present from the soil to the leaves. Furthermore, the heat will increase the transpiration, the driving force for water transport. It maintains the gradient of water potential in the plant. With low soil moisture and a high transpiration the water potential of the tree will decrease all along the pathway (Bréda et al., 2006). As a result of this, the tree will close its stomata. The stomata are situated on the epidermis of plant leaves. They control the influx of CO₂ which will be used for photosynthesis (CO₂ assimilation), as well as the water loss in the atmosphere due to transpiration (Schroeder et al., 2001). How do the plants find the best compromise between CO₂ uptake and water loss?

The flow of gases is related to stomatal conductance. It is the inverse of the physical resistance for gas movement between the atmosphere and the inside of the leaf. It is related to the density of the stomata, its size and its aperture. Therefore that photosynthesis in light conditions and transpiration increase and decrease in accordance with stomatal conductance.

The study was conducted on three tree species growing in European temperate forest: Hazelnut tree (*Corylus avellana*), Sycamore maple (*Acer pseudoplatanus*) and Common hornbeam (*Carpinus betulus*). The study objectives were (i) to investigate the relationship between stomatal conductance (gs) and photosynthesis, (ii) to determine the correlation between stomatal density and size, (iii) to compare mean vessel area between the tree species and (iv) determine the correlation between the vessel size and their density. We also measured the water potential to see how well watered the plant was which has an effect on the water content in the system of the plant.

Materials and methods

Measuring stomatal conductance and photosynthesis

Our practical work took place in the greenhouses of the Botanical garden of the University of Ulm. The plant material consisted of three different tree species, hazelnut (*Coryllus avellana*), maple (*Acer pseudoplatanus*) and common hornbeam (*Carpinus betulus*) and were cultivated in pots. Every species was provided twice, one with a normal water supply and the other one having been kept ten days under drought conditions (no watering). The first step was to measure CO₂ assimilation and stomatal conductance (gs) with the gas-exchange system (figure 1a). Li-6400 XT, Li-Cor gas analyzer system. Around a clamped leaf, gas composition was independently controlled and monitored (figure 1b).

The gas analyzer measured the CO₂ concentration before and after the air passes the leaf, and then calculates stomatal conductance and photosynthetic rate. At first at 400 ppm of

CO₂ until there was no significant change of the anymore in photosynthesis rate and stomatal conductance. After that, we reduced the concentration to 100 ppm CO₂. For light we chose 1000 quantum per m², for temperature 20 °C for both CO₂ concentrations. Every minute within ten minutes, we noted the measured value of the stomatal conductance and the CO₂ assimilation.

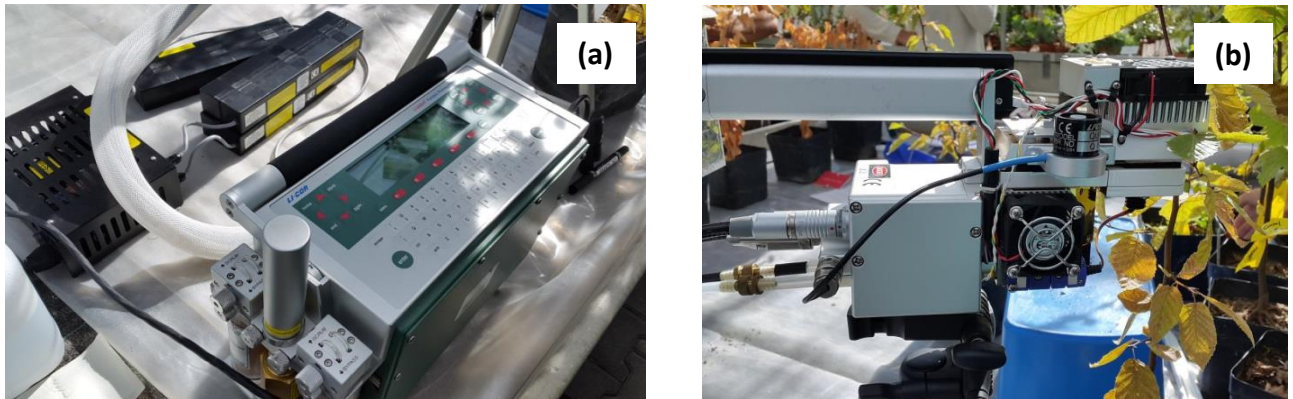


Figure 1: Console of Li-Cor gas analyser (a) and the sensor head with the chamber for clamping leaves (b).

Measuring stomatal size and density

To be able to find out the density and size of stomata we used the „Varnish“ method. We painted a thick patch of transparent nail polish on the lower (abaxial) side of the leaf surface being studied and let it dry completely. Then we taped a piece of clear tape to the nail polish patch and gently peeled the patch from the leaf. We got a leaf impression which we fixed on a microscope slide which we observed under a light-microscope. We used 100x magnification for counting the stomata and 400x magnification for measuring size of the stomata and taking pictures.

We also measured the length and width of stomata, using freeware Image-J and calculated the average size of stomata. For counting the stomata, we measured the area, counted the stomata and then calculated stomata per projected surface area (mm²).

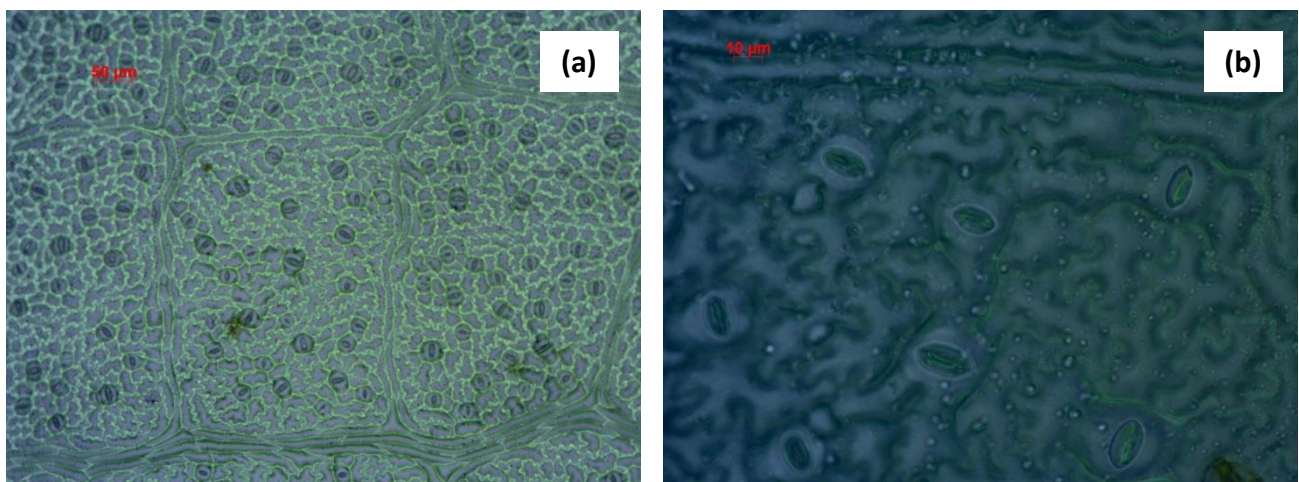


Figure 2: Picture of hazelnut tree stomata 10x magnification (a) and 40x magnification(b).

Measuring vessel size and density

For analyzing the wood anatomy, we prepared wood sections by using a hand microtome. The sections were transferred to a petri dish of water and treated with a Safranin solution (1% Safranin in 50% ethanol) and Alcian blue solution (1% Alcian blue in 50% aqueous with a little added phenol) for a few minutes until the section changed color. The sections were transferred to petri dishes with ethanol (50%, 70% and 90%) and prepared for the microscope by mounting them with glycerol (temporary slides).

By using a microscope, we took pictures of the samples of all three species. Those pictures were used to analyze the vessel density and area of the three species. With the program freeware Image-J, ten vessel area of each species were measured. For analyzing the density of the vessels, a square was drawn on the picture and the vessels in the square were counted. For being able to compare the density of the different species, the counted number of the vessels was divided by 100 and multiplied by the area of the square:

$$(\text{counted vessels} / 100) \times \text{square area}$$

Pictures of wood sections, in which the areas of the vessels were measured and the density analyzed, can be seen on pictures 1, 2 and 3 in the appendix.

Results

Stomatal conductance and photosynthesis

The first part of our practical work dealt with the stomatal conductance (gs), the CO₂ assimilation and the link between these processes for three different tree individuals, maple, hornbeam (both of the control treatment) and hornbeam (of the ten day drought treatment), which differs to the well-watered trees in case of appearance. It had wilted leaves, also brown colored and dry. This is confirmed by our data of the water potential. The control plant (hornbeam) showed a water potential of 18 bar whereas the drought treated hornbeam had a water potential > 21 bar.

In figure 3 one can see the conductance-photosynthesis relation at 400 ppm, which are relatively ambient conditions. Hornbeam was ten days under drought conditions, while the other hornbeam and maple were well watered. Well-watered hornbeam has a lower conductance (0.02 mol H₂O m⁻² s⁻¹) and in our case a lower photosynthesis rate (1.5 mol H₂O m⁻² s⁻¹) than maple (0.03 mol H₂O m⁻² s⁻¹ and 3.5 mol H₂O m⁻² s⁻¹). For hornbeam under dry conditions we measured a negative photosynthesis rate (-0.8 mol H₂O m⁻² s⁻¹) and almost no conductance (0.004 mol H₂O m⁻² s⁻¹). This is redundant to the figure. The drought treated plant had lower conductance and CO₂ assimilation than the plants of the control treatment, corresponding to the well documented closure of the stomata under dry conditions. The relationship between stomatal conductance and CO₂ assimilations is linear because of Rubisco.

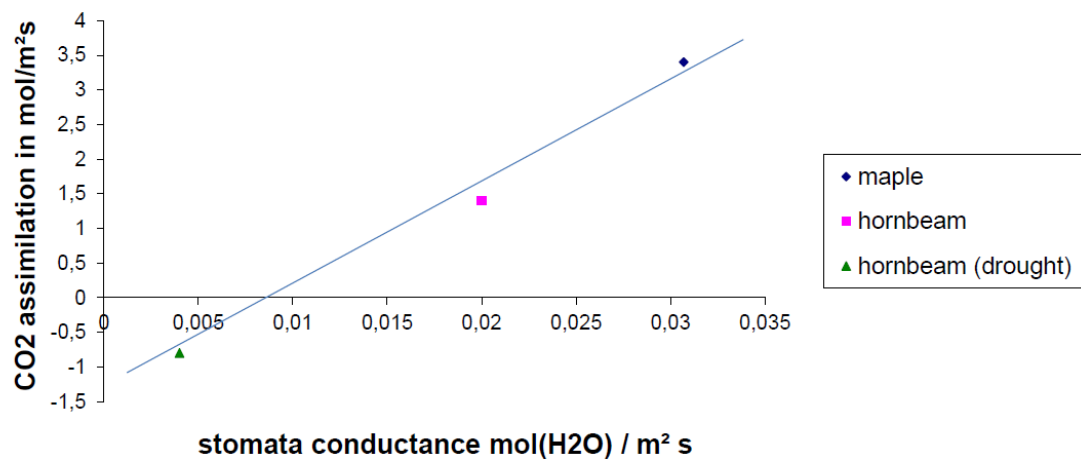


Figure 3: Stomata conductance and CO₂ assimilation for well-watered maple and hornbeam as well as hornbeam under drought at 400ppm.

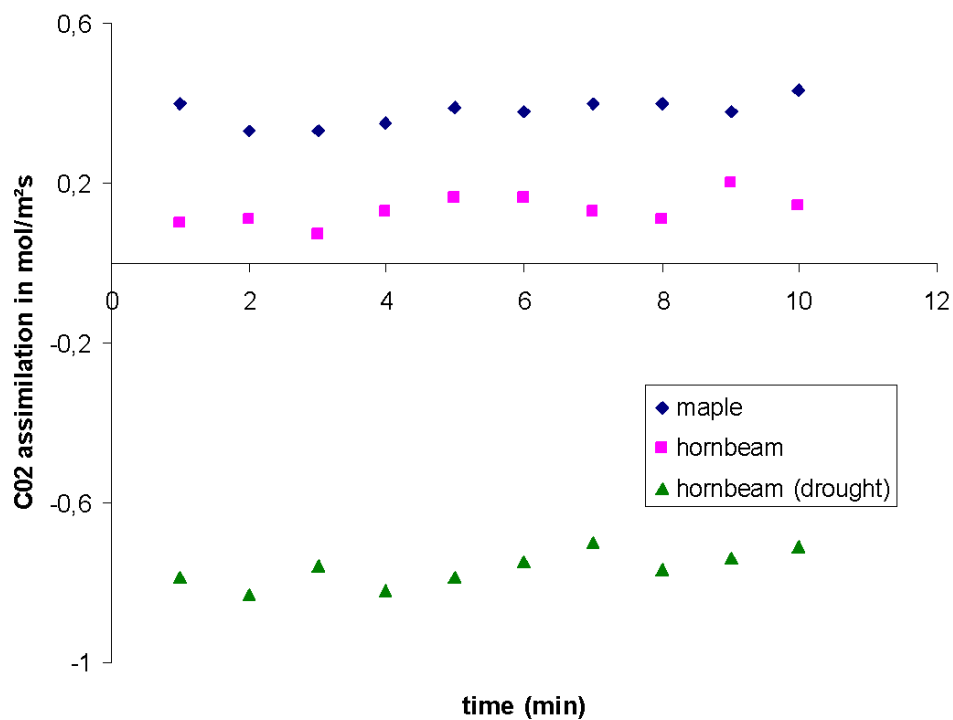


Figure 4: CO₂ assimilation over time at 100ppm of CO₂ in the chamber for well-watered maple and hornbeam as well as hornbeam under drought.

In the first figure 4 you can see the different CO₂ assimilation over time for the three tree species (maple, hornbeam, and hornbeam (drought)). It is obvious to see, that the CO₂ assimilation of the hornbeam under drought is negative. The values of the two others species, maple and hornbeam, aren't high either, but there is a big difference to notice and the values are above zero. Another point is that all values of the various species are fluctuated.

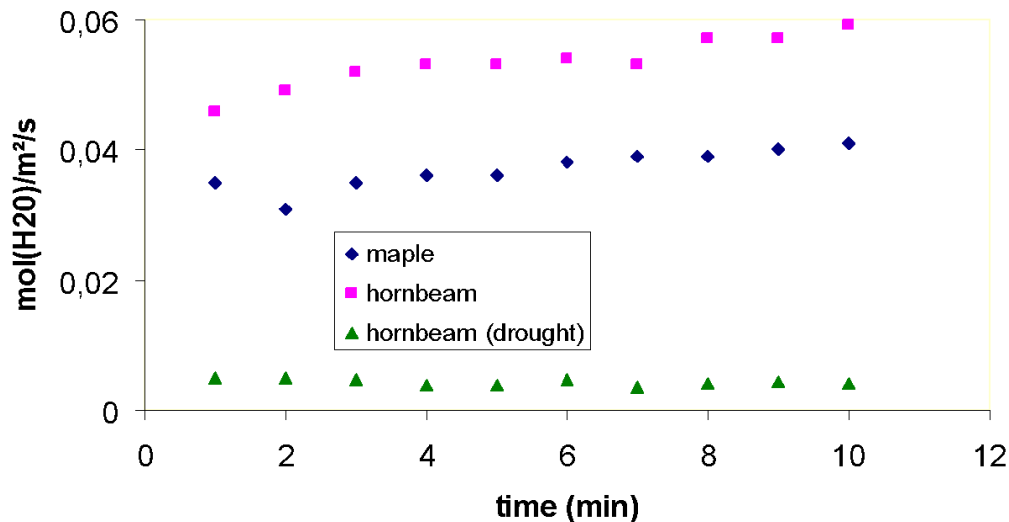


Figure 5: Stomatal conductance over time at 100ppm of CO₂ in the chamber.

The stomatal conductance (gs) in mol (H₂O)/m²s over time is shown in figure 5. There is a clear increase in gs in maple and hornbeam of the control treatment. In addition the latter has the highest stomatal conductance as well. It's discernible, that the hornbeam under drought has again the lowest values after the second measurement.

The size and density of stomata

Stomatal size and density relationships between the analyzed three species are illustrated in Figure 6.

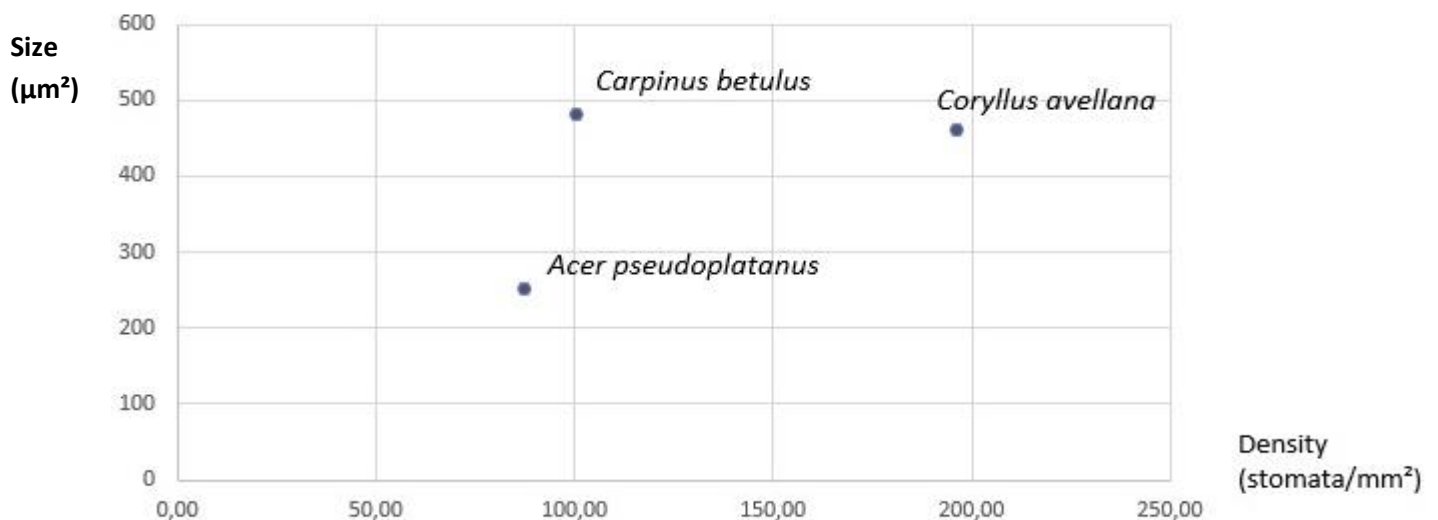


Figure 6: Stomatal size and density relationship.

We see on this figure that Common hornbeam has the biggest and Maple has the smallest stomata size. Hazelnut tree has the highest and Maple has the lowest density of stomata. And we can see that there's no correlation between stomatal size and density.

Vessel size and density relation

The differences of vessel areas between the analyzed three species are illustrated in Figure 7. To be able to recognize the differences easier, the measured vessel areas are classified due to their circle area in μm^2 .

Results show, that the vessel areas of *A. pseudoplatanus* are between 132 μm and 446 μm , as can be seen in Table 1. Therefore, *A. pseudoplatanus* shows a range between 100 μm^2 up to 500 μm^2 shown in Figure 7. The average of that species is about 284 μm .

Measured areas for *C. avellana* are between 123 μm and 291 μm and show an average about 198 μm . It has vessel areas in the first two classes, 100 μm^2 to 200 μm^2 and 200 μm^2 to 300 μm^2 . For *C. betulus* areas between 457 μm and 1582 μm are measured and an average of 1015 μm calculated. Data can get from Table 1 and Figure 7 shows that it has the biggest range and the largest vessel areas with vessel areas between 500 μm^2 and 1600 μm^2 .

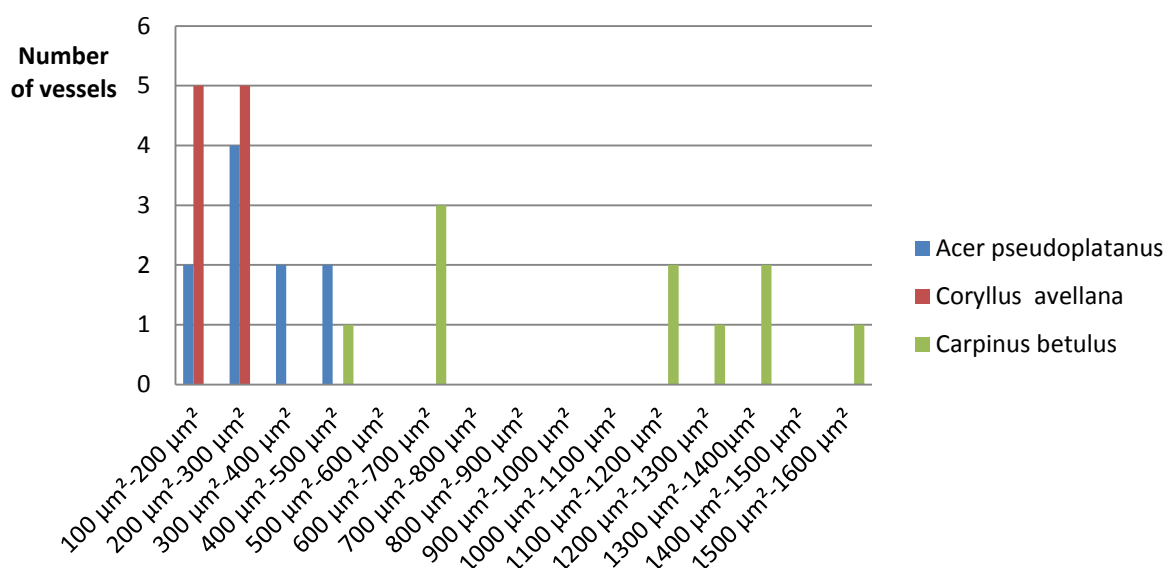


Figure 7: The three species vessel density, classified according to their area.

Table 1: Maximal and minimal measured vessel area, the area of the square, counted vessels in the square

	<i>Acer pseudoplatanus</i>	<i>Coryllus avellana</i>	<i>Carpinus betulus</i>
max. vessel area [μm^2]	446	291	1581,925
min. vessel area [μm^2]	132	123	457
mean vessel area [μm^2]	284	198	1015
area of square [μm^2]	120353	37630	271455
counted vessels	54	29	42
density	0,04	0,08	0,02

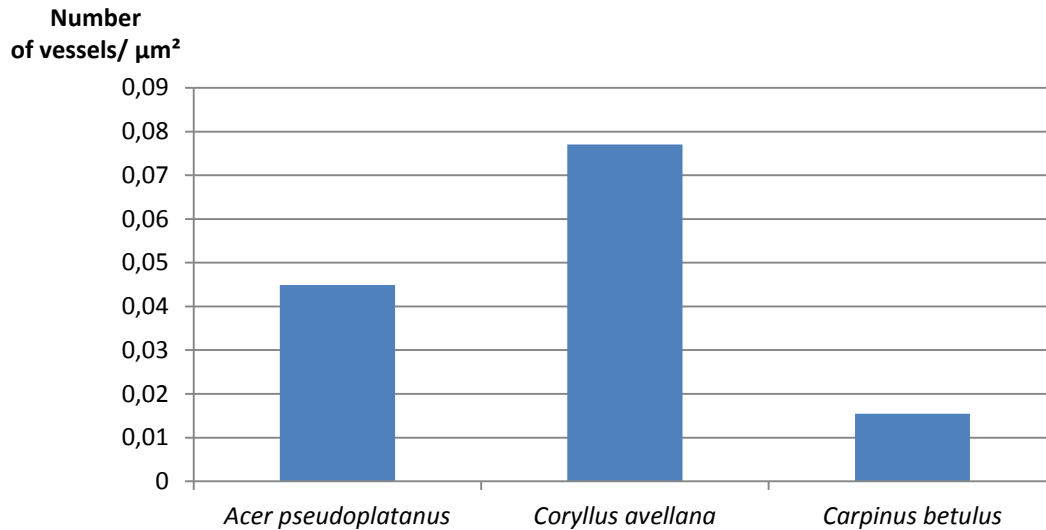


Figure 8: Vessel density of the three species.

The density between the vessels varies between the three species, as can be seen in Table 1. For *A. pseudoplatanus*, 54 vessels are counted in a square of 120353 μm², whereas in a square of 37630 μm², for *C. avellana* 29 vessels are counted. *C. betulus* has 42 vessels in a square of 271455 μm², also can be taken from Table 1. Vessel density of the three species is shown in Figure 8.

The relationship between vessel area and density is shown in Figure 9. *C. avellana* with the highest vessel density shows the smallest vessel areas, whereas *C. betulus* with the lowest vessel density shows the biggest vessel areas in average.

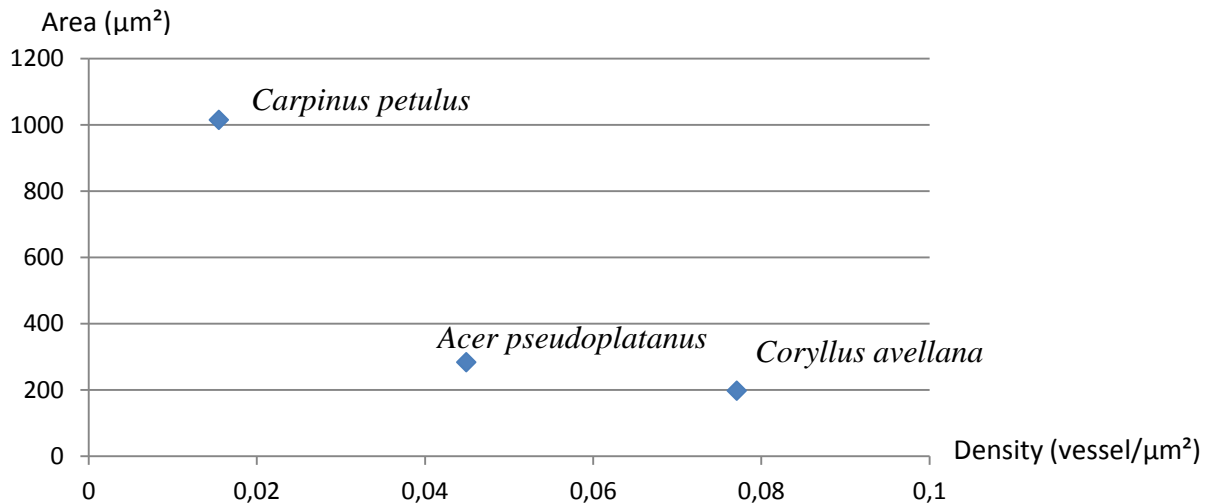


Figure 9: Correlation between vessel density and vessel area of the three species

Discussion

We couldn't measure the CO₂ Assimilation and the stomatal conductance of each tree in our first part of our practical work. The drought treatment had a severe impact on the plants, which showed wilted leaves and in general dry appearance. Hazelnut and maple under drought belonged to these trees, which were excluded from gas exchange measurements, water potential measurements and determination of the varnish experiment for stomatal density.

As one can see in the first diagram maple opens its stomata very wide and has a higher photosynthesis rate than hornbeam under normal conditions. The negative photosynthesis rate measured for the hornbeam under drought shows that the plant respire more than doing photosynthesis. This negative CO₂ assimilation can be observed in figure 2. Low amount of CO₂ even further decreases CO₂ assimilation. Due to drought, the tree is too water-stressed, therefore reduces its transpiration by closing stomata, thereby decreasing photosynthetic activity. The other two plants have a low assimilation as well. For our measurements we expected at least 2 mol/m²s. Reasons for these results could be the hot summer this year, preparation for winter (senescence) or the disadvantages of a pot plant, like high temperature in the soil affecting the root system.

A typical response to water availability is a reduction of photosynthesis as water becomes scarce. Eventually photosynthesis reaches zero or become negative. There are two reason of decreasing photosynthesis during a water deficit: stomatal and non-stomatal effects. Stomatal effect refers to stomatal closure. The non-stomatal effect refers to internal problems because of lack of water and down-regulates the photosynthesis apparatus. A debate has been running since the first paper on the photosynthesis-water relation. Which one between stomatal closure and metabolic impairment mainly limits photosynthetic activity?

Stomatal closure depends also on CO₂ concentration. In the figure 3, you can see that the hypothesis, decrease of CO₂ implicates an opening of the stomata in the leaves is confirmed by our practical work for plants of the control treatment.

The *g_s* of the hornbeam under drought is very low and doesn't change because the plant is too stressed. This results in a decrease of C assimilation leading to a reduced photosynthetic activity.

The low photosynthesis rate can also be explained by a metabolic impairment. Drought reduces biochemical capacity for C assimilation and utilization. The photosynthesis rate is linked to the activity of RubisCO (Ramachandra Reddy, 1996; Tezara et al., 1999; Chaitanya et al., 2002a; Parry et al., 2002). In fact RubisCO activity is limited with CO₂ concentration; at a low CO₂ concentration it is not efficient. Rubisco catalyses two reactions, (carboxylation and oxygenation) those are directly competitive for CO₂ assimilation. The relationship between these two is driven by the ratio of the partial pressure of CO₂ and O₂ at the enzyme. As O₂ partial pressure stays rather constant, lowering the CO₂ partial pressure reduces net CO₂ assimilation. Therefore RubisCO makes the link between stomatal conductance and photosynthesis efficiency.

All this is based on a permanent gas movement between the leaf and the atmosphere. Through transpiration the leaf loose more water than it can take from the soil through the roots, because of low soil moisture. So the water potential in the leaves gets more negative, because the pressure in the xylem is increasing due to the transpiration suction. This phenomenon can lead to embolism. It has been shown that a tight control of water-loss through stomatal closure protects from embolism (Jones and Shuterland, 1991; Tyree and Sperry, 1988). Usually embolism starts when stomatal conductance drops below 10% of initial values (Bréda et al., 2006). In figure 8 (size-density relationship of stomata), we can see that there's no correlation between stomatal size and density. Because of literature, we expected a negative correlation between the size and the density of stomata as a response to water availability and CO₂

concentration (Gindel, 1969; Upreti et al., 2002). A study from Hetherington and Woodward (2003) proposes that small stomata have shorter response times to environmental parameters and combined with their high density, this should allow the leaf to obtain a high stomatal conductance quickly under favorable conditions and a low conductance under less favorable conditions.

The plants have many possibilities to react to drought in an appropriate way. We already mentioned processes with short term adaptations, but there are long term adaptations as well. Trees can develop deep root systems to enhance the water transport or increase hydraulic conductivity by producing a smaller diameter in vessels with a lower cavitation risk.

The differences of vessel areas between those three species are illustrated in Figure 7. Referring to literature, it is an advantage for *C. avellana* having smaller vessel. In fact, having wider vessel increase the susceptibility of drought-induced cavitation (Hacke, 2001; Ladjal et al., 2005). The density between the vessels varies between the three species, as can see in Table 2. Concerning the correlation between those two parameters we can say that there is a negative correlation between the vessel size and the density. This means that the tree has to find a trade-off between hydraulic conductivity and xylem safety (Sellin et al., 2008).

To sum up, we realized that plants have short- as well as long-term adaptations to cope with drought. Stomata size and density, vessel size and density, water potential and stomata conductance, as well as CO₂ assimilation are all parameters which differ in plant species. There will be several strategies to keep the essential processes in plants running also if droughts appear more often and might last for longer periods. For sure, some plants will adapt by modulating one or more of the mentioned parameter, we dealt with in this work. There will be lots of different strategies and it is not possible to give a clear outlook about the impact of drought and plants, because of the high number of parameters needed to be noticed. In the following years, we will find out, which plants can adapt to drought and deal the best with climate change.

REFERENCES:

- Bréda, N., Huc, R., Granier, A., & Dreyer, E. (2006). Temperate forest trees and stands under severe drought: a review of ecophysiological responses, adaptation processes and long-term consequences. *Annals of Forest Science*, 63(6), 625-644.
- Chaitanya, K. V., Masilamani, S., Jutur, P. P., & Reddy, A. R. (2002). Variation in photosynthetic rates and biomass productivity among four mulberry cultivars. *Photosynthetica*, 40(2), 305-308.
- El-Sharkawy, M., & Hesketh, J. (1965). Photosynthesis among species in relation to characteristics of leaf anatomy and CO₂ diffusion resistances. *Crop Sci*, 5(1), 517-521.
- El-Sharkawy, M., Hesketh, J., & Muramoto, H. (1965). Leaf photosynthetic rates and other growth characteristics among 26 species of *Gossypium*. *Crop Sci*, 5, 173-175.
- Franks, P. J., & Beerling, D. J. (2009). Maximum leaf conductance driven by CO₂ effects on stomatal size and density over geologic time. *Proceedings of the National Academy of Sciences*, 106(25), 10343-10347.
- Gindel, I. (1969). Stomatal number and size as related to soil moisture in tree xerophytes in Israel. *Ecology*, 263-267.
- Hacke, U. G., & Sperry, J. S. (2001). Functional and ecological xylem anatomy. *Perspectives in plant ecology, evolution and systematics*, 4(2), 97-115.
- Hetherington, A. M., & Woodward, F. I. (2003). The role of stomata in sensing and driving environmental change. *Nature*, 424(6951), 901-908.
- Neales, T., & Incoll, L. D. (1968). The control of leaf photosynthesis rate by the level of assimilate concentration in the leaf: a review of the hypothesis. *The botanical review*, 34(2), 107-125.
- Upreti, D. C., Dwivedi, N., Jain, V., & Mohan, R. (2002). Effect of elevated carbon dioxide concentration on the stomatal parameters of rice cultivars. *Photosynthetica*, 40(2), 315-319.
- Jones, H. G., & Sutherland, R. A. (1991). Stomatal control of xylem embolism. *Plant, Cell & Environment*, 14(6), 607-612.
- Ladjal, M., Huc, R., & Ducrey, M. (2005). Drought effects on hydraulic conductivity and xylem vulnerability to embolism in diverse species and provenances of Mediterranean cedars. *Tree Physiology*, 25(9), 1109-1117.
- Muchow, R. C., & Sinclair, T. R. (1989). Epidermal conductance, stomatal density and stomatal size among genotypes of *Sorghum bicolor* (L.) Moench. *Plant, Cell & Environment*, 12(4), 425-431.
- Parry, M. A., Andralojc, P. J., Khan, S., LEA, P. J., & Keys, A. J. (2002). Rubisco activity: effects of drought stress. *Annals of Botany*, 89(7), 833-839.
- Reddy, A. R. (1996). Fructose 2, 6-bisphosphate-modulated photosynthesis in sorghum leaves grown under low water regimes. *Phytochemistry*, 43(2), 319-322.
- Sellin, A., Rohejårv, A., & Rahi, M. (2008). Distribution of vessel size, vessel density and xylem conducting efficiency within a crown of silver birch (*Betula pendula*). *Trees*, 22(2), 205-216.
- Tezara, W., Mitchell, V. J., Driscoll, S. D., & Lawlor, D. W. (1999). Water stress inhibits plant photosynthesis by decreasing coupling factor and ATP. *Nature*, 401(6756), 914-917.
- Tyree, M. T., & Sperry, J. S. (1988). Do woody plants operate near the point of catastrophic xylem dysfunction caused by dynamic water stress? Answers from a model. *Plant physiology*, 88(3), 574-580.

Appendix

Table 1: Measured vessel area (μm^2) and mean vessel area for *Acer pseudoplatanus* (a), *Corylus avellana* (b) and *Carpinus betulus* (c).

(a)	vessel size (μm^2)	(b)	vessel size (μm^2)	(c)	vessel size (μm^2)
1	445,728	1	201,666	1	1357,027
2	322,83	2	188,534	2	655,116
3	290,493	3	267,178	3	1228,105
4	401,575	4	143,099	4	643,657
5	238,488	5	175,703	5	1581,925
6	132,148	6	291,028	6	1351,775
7	171,637	7	242,422	7	1111,597
8	250,848	8	209,516	8	624,557
9	274,557	9	142,797	9	496,59
10	311,248	10	122,57	10	1102,047
Mean	283,9552	Mean	198,4513	Mean	1015,2396



Figure 1: Picture of a wood section of *A. pseudoplatanus* (sycamore maple)

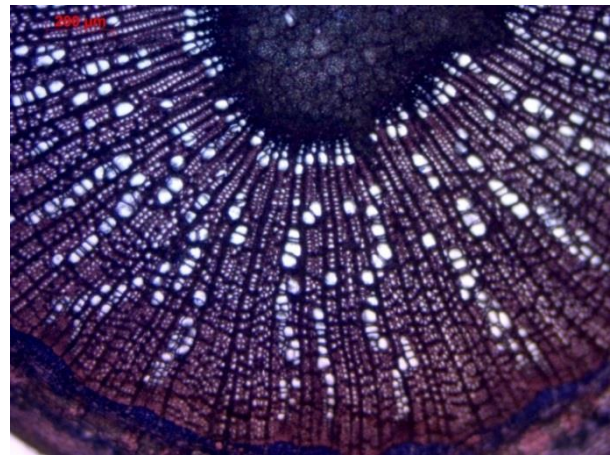


Figure 2: Picture of a wood section of *C. betulus* (common hornbeam)

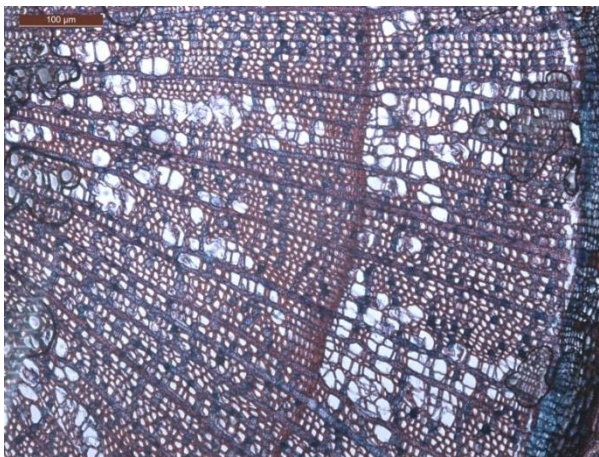


Figure 3: Picture of a wood section of *C. avellana* (hazelnut tree)

