THE POPLAR CLONE (*POPULUS MAXIMOWICZII* A. HENRY × *P. NIGRA* L.) GROWTH UNDER THE CONTROLLED ENVIRONMENT OF GROWTH CHAMBERS

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The objective of this study is to test the clone J-105 (Populus maximowiczii A. Henry × Populus nigra L.) responses to high temperatures under different water supply, CO_2 and VPD levels and. The experiment was established in May, 2014 by 144 cuttings planted into the pots. Sixty pots were placed into 5 growth chambers in July with the number of 12 pots per 1 chamber. The pots were split into 2 variants per 1 chamber – wet and dry treatment. The measurements, consisted in measurements of the plant heights, soil moisture (using ThetaProbe Soil Moisture Sensor) and CO_2 response curves (using LI-6400 Portable Photosynthesis System), were carried out weekly. There were 4 parameters set up within each chamber under 2 protocols: (1) daily temperature course, (2) relative humidity (Rh) that together with temperature enable to control VPD values, (3) CO_2 values and (4) PAR. The protocol for the control group was also established. The randomization between chambers was carried out weekly. The initial hypothesis was based on the assumption that elevated CO_2 concentration (EC) will improve the water use efficiency and thus reduce the negative impact of drought, while increased air temperature and higher VPD will, on the contrary, amplify the negative effects of drought.

Keywords: short-rotation coppice, poplar clone J-105, growth chamber

INTRODUCTION

Carbon dioxide is an essential substrate for photosynthesis leading to increased carbon uptake and assimilation, thereby increasing plant growth. Plant growth is stimulated by elevation of CO_2. The application of more CO_2 increases plant water use efficiency (WUE) and results in less water use (Prior et al., 2011). The elevated CO_2 may reduce transpiration (e.g. Allen et al., 1994; Jones et al., 1984) that together with increased photosynthesis contributes to WUE increase (e.g. Baker et al., 1990; Morison, 1985). The transpiration reduction caused by elevated CO_2 leads to the effects of drought raising (Bazzas, 1990) and allows plants to maintain enhanced photosynthesis (e.g. Acock and Allen, 1985). By the study of Prior et al. (2010) the elevated CO_2 results also in water infiltration increase and sediment loss (through runoff) decrease. The vapour pressure deficit (VPD) can negatively affect plant growth as plants reduce stomatal conductance to water vapour in response to increasing VPD, limiting the ability of plants to assimilate carbon (Ocheltree et al., 2013). Plants reduce stomatal conductance in response to large VPD between the leaf and atmosphere and it leads to global reductions in ecosystem productivity (Zhao and Running, 2010). By reducing stomatal conductance to water vapour, plants minimize water loss and maintain the hydration of plant cells as VPD increases (Zhao and Running, 2010). When CO_2 is elevated, the most limiting resource becomes water or nutrients (Prior et al., 2011). Poplar (*Populus* spp.) species belongs to the most sensitive woody plants to water stress (Marron et al., 2003). Due to the mutual relationships among the mentioned parameters, it is necessary to study the responses of plants in relation to these factors. A study of the higher number of factors (such as CO_2, drought, VPD and temperature) is possible only through growth chambers where all factors of environment are completely controlled.

MATERIALS AND METHODS

The poplar clone J-105 (*Populus maximowiczii* A. Henry × *Populus nigra* L.) hardwood cuttings were planted into the 12-liters pots with pot diameter approximately 30 cm at the research company ZEMSERVIS – Test Station Domanínek, Ltd. (situated in Bystřice nad Pernštejnem within Bohemian-Moravian Highlands in the Czech Republic – altitude 530 m a.s.l.) in the beginning of May, 2014. The pots were placed on a field into the dug hole to avoid pots drying out and overheating. The plants were regularly watered and weeded. The pots were transported to the Global Change Research Centre, Academy of Sciences of the Czech Republic, v. v. i. at the end of July, 2014. The 60 plants were put into the 5 growth chambers there. The five growth chambers of FytoScope FS-SI 3400 model (Photon Systems Instruments LLC, www.psi.cz) were used for the experiment. These chambers enable to set up the cultivation conditions both manually and through various protocols simulating diurnal changes of light intensity, spectral composition, temperature, relative humidity and CO_2. Vapour pressure deficit (VPD) was controlled via actual temperature and relative humidity. There were 4 parameters set up within each chamber under 2 protocols: (1) daily mean temperature course (the same for both protocols, it was established as a mean hottest day in Domanínek according to the long-term climatic data), (2) relative humidity (Rh) that together with temperature enable to control VPD values (less than 1 kPa and higher than 1 kPa protocols), (3) CO_2 values (400 and 700 ppm protocols) and (4) photosynthetically active radiation – PAR (a daily maximal value 1,500 μmol.m^{-2}.s^{-1} in all chambers). The protocol for the control group was established thus: CO_2 at the level 400 ppm, daily temperature curve was based on a mean July day in Domaninek and so the Rh values. The 12 pots were subsequently split into 2 variants within 1 chamber: (1) wet treatment – it was assessed as a weakly rainfall from Domanínek [mm] recalculated into adequate water volume in liters per each pot (500 ml watering), (2) dry treatment – it was watered only if the soil moisture decreased below 12 % (measurements were carried out using ThetaProbe Soil Moisture Sensor). The protocols are presented within the Table 1. The measurements, consisted in measurements of the plant heights, soil moisture, chlorophyll content and CO_2 response curves, were carried out weekly.

Table 1: The particular protocols within the growth chambers.

		Hot day, high VPD			Hot day, low VPD		
Time	PAR [μmol m^{-2} s^{-1}]	T [°C]	Rh [%]	VPD [kPa]	T [°C]	Rh [%]	VPD [kPa]
0:00	0	15	90	0,09	15	90	0,09
4:30	0						
5:30		15	90	0,09	15	90	0,09
8:00	1200	24	60	1,19	24	80	0,90
11:30	1500	30	45	2,33	30	80	1,06
15:00	1500	32	35	3,09	32	80	0,95
18:00		30	45	2,33	30	80	1,06
20:00	0						
21:00		20	80	0,47	20	80	0,47
		Control, no stress					
Time	PAR [μmol m^{-2} s^{-1}]	T [°C]	Rh [%]	VPD [kPa]	**Protocols**	**Signs**	**CO_2 [ppm]**
0:00	0	12	90	0,14	Hot day,		
4:30	0				high VPD	H_AC	400
5:30		14	90	0,08		H_EC	700
8:00	1200	21	60	0,99	Hot day,		
11:30	1500	24	50	1,49	low VPD	L_AC	400
15:00	1500	25	50	1,58		H_AC	700
18:00		23	50	1,40	Control,		
20:00	0	15	90	0,17	no stress	C_AC	400
21:00		12	90	0,07			

* The empty fields within the Table 1 means that the temperature changed gradually up to the value following in the table.

The actual soil moisture was measured using ThetaProbe Soil Moisture Sensor (Delta-T Devices Ltd, http://www.delta-t.co.uk) as procentual values. The chlorophyll content was measured using DUALEX SCIENTIFIC+™ (FORCE-A, http://www.force-a.eu) for the chlorophyll index evaluation. The CO_2 response curves were measured through LI-6400 Portable Photosynthesis System (LI-COR, Inc., http://www.licor.com/env/) by the module A/Ci curves – i.e. net CO_2 assimilation rate, *A*, versus calculated substomatal CO_2 concentration, Ci (Manter and Kerrigan, 2004). The data sets of light saturated CO_2 assimilation rate at growth CO_2 concentration (A_{max}) were evaluated from the LI-6400 Portable Photosynthesis System instrument.

RESULTS

The main objective of this study was to evaluate the response of the hybrid poplar to drought stress in the combination with other environmental factors that influence the CO_2 assimilation and transpiration rate by the regulation of stomatal closure. Simultaneously, these are factors whose importance is increasing in the context of global change: higher air temperature, elevated CO_2 concentration (EC) and higher vapour pressure deficit (VPD). The basic hypothesis was based on the assumption that EC will improve the water use efficiency and thus reduce the negative impact of drought, while increased air temperature and higher VPD will, on the contrary, amplify the negative effects of drought.

As it is evident from Table 2, the soil moisture decreased to around 10 % within a 14 days of the experiment duration. The rate of decline in soil moisture was relatively little affected by the air temperature and VPD. However, there is evident a trend showing that the higher temperature and higher VPD accelerate soil drying. Relatively significant downward effect on soil moisture, however, has the EC. Here it was observed that the decrease in soil moisture is lower in both measurements under EC, indicating improved water use efficiency in EC due to the effect on stomatal closure.

Higher air temperature generally led to a small increase in chlorophyll content per area unit, in both well-watered and drought-stressed plants (Fig. 1). This increase was the lowest under high VPD combined with EC. Drought stress generally caused a decrease in chlorophyll content, and this decline was lower in low VPD. It is obvious, that the effect of EC on the chlorophyll content is different under low VPD where increase of chlorophyll content was observed and under high VPD, where chlorophyll content decreased. This interaction is probably due to a complex action of most factors on the leaf thickness, which under moderate drought stress, higher temperature and EC conditions increases, and thus indirectly increases the chlorophyll content per area unit. In contrast, under conditions of severe drought stress the chlorophyll content decreases as a result of accelerated leaf senescence.

Table 2: Mean soil moisture and standard deviation (n=6) measured at 7th and 14th day of the experiment under individual drought, VPD, CO_2 concentration and temperature treatments.

Day		Treatment variant					
		C_AC		L_AC		L_EC	
		WET	DRY	WET	DRY	WET	DRY
7th day	Mean soil moisture [%]	25,33	16,26	23,41	16,33	27,19	17,73
	Standard deviation	±2,20	±2,10	±2,29	±1,67	±2,94	±1,96
14th day	Mean soil moisture [%]	21,42	11,43	20,13	10,88	24,56	12,25
	Standard deviation	±2,08	±1,60	±1,97	±1,16	±2,15	±1,41
		H_AC		H_EC			
		WET	DRY	WET	DRY		
7th day	Mean soil moisture [%]	24,20	16,01	23,89	17,23		
	Standard deviation	±1,93	±2,11	±1,79	±2,41		
14th day	Mean soil moisture [%]	17,73	10,43	17,94	12,00		
	Standard deviation	±1,46	±0,86	±1,19	±1,49		

One of the most important parameter monitored during experiment was light saturated CO_2 assimilation rate at growth CO_2 concentration (A_{max}; Fig. 2). In the first stage (after 7 days) of experiment, there were no apparent differences in A_{max} if the plants were sufficiently supplied with water regardless of temperature, VPD and EC effects. Conversely, the impact of drought on A_{max} was pronounced already seven days from the beginning of the experiment and also showed a clearly mitigating effect of EC on the negative drought effect. In both low and high VPD was the A_{max} decrease caused by drought, reduced almost by half under EC. A similar, but less pronounced alleviating effect of EC was also observed 14 days from the beginning of the experiment. In contrast, the stimulation effect of EC was highlighted after 14 days in treatments sufficiently supplied with water, particularly under low VPD. These results suggest that the EC contributes to drought stress alleviation in its initial stages, whereas longer exposure to EC has a stimulation effect on A_{max} particularly in combination with higher temperature and sufficient water supply.

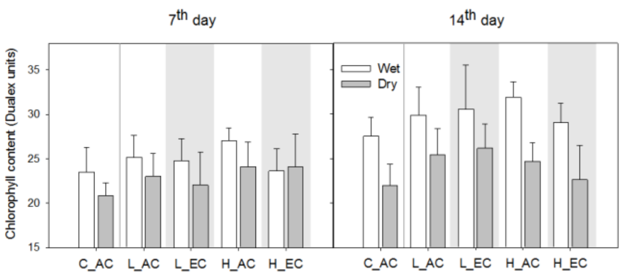

Figure 1. Changes in chlorophyll content per area unit in poplar leaves in response to drought stress, VPD, CO_2 concentration and air temperature. The means (vertical bars) and standard deviations (error bars) are presented (n=6).

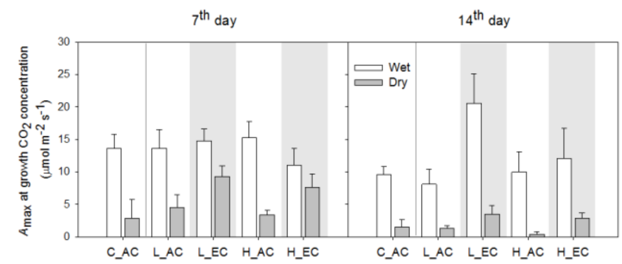

Figure 2. Changes in light saturated CO_2 assimilation rate measured at growth CO_2 concentration in response to drought stress, VPD, CO_2 concentration and air temperature. The means (vertical bars) and standard deviations (error bars) are presented (n=6).

Different interaction effect of EC and drought on A_{max} can be also seen from the relationship between soil moisture and A_{max} and it is evident that the impact of EC changes over time (Fig. 3). From the change of slope of this relationship it is evident that EC initially acts positively under drought stress, while later the stimulatory effect of EC is more pronounced under sufficient water supply. This changing response is probably related to the effect of drought on growth and limitation of sink for assimilates. After prolonged exposure to drought the sink for carbohydrates is reduced and this results in feedback regulation of photosynthesis under EC. On the contrary, under sufficient water supply the EC and higher temperatures boost plant growth, thus creating a higher sink for assimilates and photosynthesis is then stimulated.

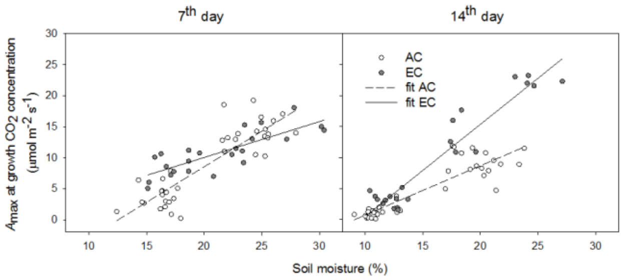

Figure 3. Changes in relationships between soil moisture and light saturated CO_2 assimilation rate measured at growth CO_2 concentration (A_{max}) determined separately for ambient (AC- white symbols) and elevated CO_2 concentration (EC- gray symbols).

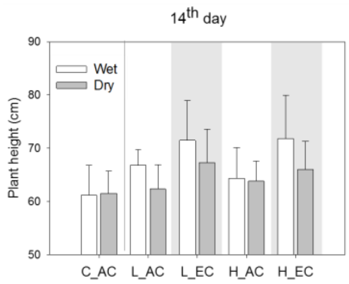

Figure 4. Plant height at the end of experiment as affected by drought stress, VPD, CO_2 concentration and air temperature. The means (vertical bars) and standard deviations (error bars) are presented (n=6).

From the evaluation of plant height after 14 days of the experiment it is evident that the higher air temperature stimulates the growth particularly in combination with EC (Fig. 4). Conversely, drought stress reduced height increments. VPD impact on the growth of poplars was relatively small. These findings suggest that A_{max} stimulation by EC is determined by rapid plant growth and thus by a high sink capacity. Sufficient height growth in the case of this experiment, was ensured by a higher temperature in combination with a sufficient water supply.

CONCLUSION

The results showed that the EC mitigates the drought stress but only in the beginning, the mitigation is insufficient later. Contrarily, later, EC stimulates photosynthesis and growth only under the conditions with water sufficiency and this is because of sufficient sink for photosynthesis products is created.

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