Impact of short-term heat wave single and in combination with drought on gas exchange parameters of barley

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Abstract. In this study there were examined the short-term (3-day long) impact of heat wave (31 ºC *vs.* 21 ºC) single and in combination with drought (i.e. fully and not watered during the heat wave period) on gas exchange parameters of barley (*Hordeum vulgare* L.) plants grown in growth chambers at control environment, as well as the recovery following stress. Short-term heat wave by itself has had minor or no effect on soil water content, photosynthetic rate, stomatal conductance and intercellular CO_2 concentration during the heat wave period, although increased transpiration rate and decreased water use efficiency, but all these parameters fully recovered after one-day regeneration period. In contrast, the combination of heat wave and drought brought much more pronounced negative effect on soil water content and gas exchange parameters, whereof not all could return to the control value after one-day regeneration period. Hence, the obtained results showed that at the early development stage barley plants may by capable to cope with short-term heat wave event under future climate, if they would be well watered, but heat wave in conjunction with drought could bring irreversible changes in their leaf physiology.

Keywords: Heat wave, drought, gas exchange, barley

1. Introduction

The occurrence of extreme weather events such as heat waves defined as prolonged periods of excessively hot temperatures over a region are predicted to become more intense, longer lasting, and/or more frequent, as a consequence of the increased inter-annual variability and increased average summer temperatures (IPCC 2014; Steffen *et al*., 2014). Linkages between extreme heat waves and drought conditions are also reported with drought conditions increasing the intensity of heat waves (Ameye *et al*., 2012; Carmo-Silva *et al*., 2012; Lobell *et al*., 2015; Ruehr *et al*., 2016).

The physiology of plants' response to drought at the whole plant level is highly complex and involves deleterious and/or adaptive changes. Early responses of plants to drought stress usually help the plant to survive for some time. One of the primary responses of plants to water deficit is a rapid closure of stomata to avoid further loss of water through transpiration that enables to maintain hydraulic function, but restricts diffusion of carbon dioxide from the environment into the leaf that lowers the intercellular CO_2 concentration (C_i), resulting in a limitation of photosynthesis (Flexas *et al*., 2006; Woodruff *et al*., 2015). While the effect of heat wave on stomata behavior can be the opposite – plants can increase transpiration rate with increasing air temperature for leaf cooling in order to avoid overheat and prevent deleterious damages induced by heat stress (Ameye *et al*., 2012). On the other hand, temperature increases can be a major factor in driving drought stress because vapor pressure deficit increases nonlinearly with higher air temperatures and the consequent stomatal closure and subsequent reduced transpiration rate decreases the capacity for evaporative cooling, increasing leaf temperature and, consequently, the damages of heat stress on photosynthesis (Duursma *et al*., 2014), leading to growth inhibition and reproductive failure (Nguyen *et al*., 2013).

In this study there were examined the leaf gas exchange response of barley (*Hordeum vulgare* L. cv. 'Aura DS') plants at their early development stage to short-term effect of heat wave single and in combination with drought as well as the recovery following stress. Two key research questions were addressed: (1) to what extent the combined effect of heat wave and drought on gas exchange parameters of barley would be stronger than the effect of single heat wave treatment; and (2) if these parameters would recover completely to the control level after one-day regeneration period following stress.

2. Materials and methods

2.1. *Plants' growing conditions*

Experiments were conducted in two closed control environment plant growth chambers, located at Vytautas Magnus University, in 2017 m, with each chamber volume of 10 m^3 (2 x 2 x 2.5 m). Barley plants (*Hordeum vulgare* L. cv. 'Aura DS') were sown on 11th January and grown in 3-liter (21 cm in height and 10.6 cm in diameter) plastic pots (15 plants per pot), filled with a mixture of field soil, perlite and fine sand (volume ratio 5:3:2). A nutrient supply corresponding to 90 kg ha^{-1} of nitrogen was used during the sowing. Additional fertilization with the complex nutrient (NPK 12-11-18 + microelements) solution, increasing N level until 150 kg ha^{-1}, was applied one week before the treatment on the 26th day of January. A photosynthetically active radiation (PAR) of ~270 μmol

m^{-2} s^{-1} photon flux density in each of the chamber was provided by a combination of ten natural day-light luminescent lamps (Philips, Waterproof OPK Natural Daylight LF80 Wattage 2×58 W/TL-D 58 W) and one high-pressure sodium lamp (Philips MASTER GreenPower CG T 600 W). The pre-set values were also identical in both of the chambers for the duration of a 14 h (8:00 a.m. to 10:00 p.m.) photoperiod and an atmospheric carbon dioxide concentration of 400 μmol mol^{-1}. RH in the heated growth chamber was 25±3% during the period of 6.5 h per day of heat wave treatment that gradually increased to 45±4% after that till 10:00 p.m. and maintained overnight. While in the control chamber there was 50±5% during the day and 70±5% at night. In order to minimize the effects of differences in growing conditions on plant performance within the same growth chamber, each pot was rotated under the same growing condition every day.

2.2. Treatments and experimental design

Initially, all plants were grown in the control chamber under the conditions of the ambient air temperature of 21/14 °C day/night until full expansion of the third leaf (growth stage 14, Zadoks). Then, the heat wave (HW)-exposed plants were transferred into another growth chamber, where the heat wave (i.e. day temperature of 31 ºC for 6.5 h per day and 21 ºC night temperature) was imposed for 3 days, while the other one was maintained at ambient air temperature conditions. The temperature in the heated growth chamber was increased gradually from 21 to 31 °C between 9:00-11:00 a.m., holding the temperature of 31 °C until 5.30 p.m., and then was gradually decreased from 5:30-7:30 p.m. to 25 °C that was maintained till 10:00 p.m. until the period of night began, when it decreased to 21 °C and was maintained overnight. In this case, plants were subjected to a 3-day, 6.5 h per day, of +10 °C HW treatment. Before the HW treatment, the volumetric soil water content (SWC) (vol. %) for all plants was set at ca. 30% and every pot was weighed in the morning (between 11:30-12:00 h) each day in order to determine gravitation water loss and to maintain the target SWC level. Then, the HW-exposed plants were divided into two groups, where half of the HW-exposed plants were watered normally to the target SWC level of 30%, while other one was left without additional watering during the 3-day long HW period. At the 4th day of the treatment, after nocturnal HW, when the SWC and the leaf gas exchange measurements were made, ~3:00 p.m. HWN plants were also re-watered to the control SWC level of 30% and all pots from the HW chamber were moved back to the ambient temperature conditions for one-day regeneration period. So, there were three treatments of all in this experiment: (1) ambient temperature, fully watered (ATW); (2) heat wave, fully watered (HWW); (3) heat wave, not watered (HWN). All treatments were run in three replicates.

2.3. Leaf gas exchange measurements

The measurements of gas exchange were performed using a portable closed infrared gas analyser LI-COR 6400 (LI-COR, Inc., Lincoln, NE, USA) with randomly selected youngest fully expanded leaves during the course of experiment between 11:00 a.m. and 3:00 p.m. The photosynthetic rate determined at the corresponding growth conditions (A_{growth}, μmol CO_2 m^{-2} s^{-1}), stomatal conductance (g_s, mmol H_2O m^{-2} s^{-1}), transpiration rate (E, mmol H_2O m^{-2} s^{-1}) and intracellular CO_2 concentration (C_i, μmol CO_2 mol air^{-1}) were recorded automatically for 15 min. at 10 sec. interval. Water use efficiency (WUE, μmol CO_2 mmol H_2O^{-1}) was calculated according to the manufacturer instructions as A divided by E. The measurements were carried out at least in three replicates.

2.4. Statistical analysis

The Student's t-tests were used to estimate the difference between reference and treatment values in all parameters. In all tests, treatments and sampling date were fixed effects and a p-value < 0.05 was the threshold for significance. All analyses were performed by *STATISTICA 8* and the results were expressed as the mean values and their standard error (p<0.05) (±SE).

3. Results and discussion

3.1. Soil water conditions

The volumetric soil water content (SWC) in HWW was not lower than that in ATW treatment, indicating that soil water conditions did not differ between temperature treatments (Fig. 1A). By contrast, it declined sharply in HWN treatment during the heat wave period with the reduction of SWC nearly three times from the control level of 30 % at the beginning to about 11 % (p < 0.05) on the day 3 (the last day, 6.5 h per day, of +10 °C heat stress treatment) and continually decreased y*et al*most two times from that level to 6 % (p < 0.05) on the 4th day of the treatment, i.e. after nocturnal heat wave (treatment with temperature of +7 °C), before HWN-treatment plants were rehydrated to the target SWC level of 30 %.

3.2. Leaf gas exchange response

The imposed heat wave impact (6.5 h per day of +10 °C heat stress treatment) on fully watered (HWW) barley plants did not have any considerable effect on photosynthetic rate (A_{growth}) during the first two day of the treatment, but A_{growth} in HWW declined significantly by 14 % (p < 0.05) on the 3th day of the treatment compared to the ATW-treatment plants (Fig. 1B), indicating that the progressed heat wave of temperature +10 °C has had a significant negative effect on photosynthesis. By contrast, A_{growth} in HWN treatment declined sharply and significantly, as did SWC, during the 3-day long heat wave period with substantially reduction of A_{growth} by 56 % (p < 0.05) compared to the ATW treatment and by 49 % (p < 0.05) compared to the HWW treatment on the 3th day of the treatment (Fig. 1B), indicating that combined impact of heat wave and drought brought much more pronounced negative effect on photosynthesis than the single heat wave treatment. This negative effect on photosynthesis of the combination of heat and drought was even more pronounced on the day 4, i.e. after the nocturnal HW, before HWN-treatment plants were rehydrated to the control level, when A_{growth} in HWN treatment significantly declined almost 40 % ($p < 0.05$) more and was by 74 % ($p < 0.05$) lower than that in ATW treatment, while A_{growth} in HWW treatment returned to the control level and did not differ significantly from ATW treatment (Fig. 1B). These results reflect that drought-impacted plants suffered substantially more from heat wave than fully watered ones, which is consistent with previous studies (*e.g.* Ruehr *et al*., 2016; Duan *et al*., 2017), and that the crucial negative factor for photosynthetic rate of barley plants in this combined treatment was drought, but not heat. Although A_{growth} in HWN treatment was recovered to large extent after one-day regeneration period, it could not fully return to the control value and was 4 % ($p < 0.05$) lower than that in ATW treatment.

During the course of experiment, stomatal conductance (g_s) in HWW treatment did not differ significantly from ATW treatment, while it was signally and significantly decreased in HWN treatment on the 2th and the 3th days of heat wave treatment compared to the ATW treatment with the most pronounced reduction of g_s by 84 % ($p < 0.05$) on day 4, after the nocturnal HW, before the HWN-treatment plants were rehydrated (Fig.1C), as it was case with SWC and A_{growth} (Fig. 1A and B). As stomata closure is the well-known first responsive event of plants to water deficiency (Lisar *et al*., 2012), the obtained results show that, in this combined impact of heat wave and drought, with sharply decreasing g_s from the beginning in HWN treatment, barley plants responded more to drought than heat induced stress. It is known that stomatal closures are more closely related to soil moisture content than leaf water status, and it is mainly controlled by chemical signals such as abscisic acid (ABA) produced in dehydrating roots (Lisar *et al*., 2012). At the end of experiment, after one-day regeneration period, full recovery of g_s in HWN treatment was not observed − g_s in HWN treatment was only 79 % of g_s in ATW treatment (*t*-test: $p < 0.05$).

Despite the fact that there was no significant increase of g_s in HWW on the 1th day of treatment compared to the ambient temperature treatment, transpiration rate (E) in HWW treatment increased significantly by 82 % ($p < 0.05$) compared to the ATW treatment and was maintained to a similar significant ($p < 0.05$) higher level during the second two days of heat wave treatment, until it sharply decreased and returned to the control level after the release of heat wave on day 4 and did not differ from ATW treatment after one-day regeneration period on day 5 (Fig.1D). By contrast, from initially increased by 80 % ($p > 0.05$) on the 1th day of HW treatment, while the SWC in HWW and HWN treatments was the same (Fig.1A), E in HWN treatment decline sharply as combined impact of heat wave and drought prolonged with the most significant reduction of E by 77 % ($p < 0.05$) compared to the ATW treatment on day 4, after the nocturnal HW, before the HWN-treatment plants were rehydrated. The same tendency was found with the SWC and other leaf gas exchange parameters discussed above, once more implying that, in the combination of heat wave and drought, drought had considerably more negative effect on leaf physiology of barley plants than did it heat wave. So, alongside current evidence (Carmo-Silva *et al*., 2012; Ruehr *et al*., 2016; Duan *et al*., 2017), the results of this study confirmed that water availability has a dominant role in determining plant physiological responses. However, contrary than A_{growth} and g_s, after one-day regeneration period, E in HWN treatment fully recovered to the control value (Fig. 1D).

During the 3-day long heat wave period, water use efficiency (WUE) in HWW treatment was substantially reduced by 47 % ($p < 0.05$) on average compared to the ambient temperature treatment (*i.e*. ATW), suggesting that heat wave had large negative impact on WUE. However, it more than returned to the control level after the release of HW on day 4 and, the same as E, did not differ from ATW treatment after one-day regeneration period on day 5 (Fig.1E). On the 2th and the 3th days of heat wave treatment, WUE in HWN treatment was enhanced by 41 % ($p < 0.05$) and 49 % ($p > 0.05$), respectively, compared to fully watered ones at the same temperature (*i.e.* HWW treatment), but reduced by 25 % ($p < 0.05$) and 24 % ($p > 0.05$), respectively, compared to the ATW treatment. These results indicate that under combined impact of heat wave and drought, drought-induced water-saving effect due to reduced g_s and subsequently reduced E, was offset of water loss due to enhanced transpiration rate and declined A_{growth}, leading to the reduction in WUE, under high air temperature conditions. According to Allen and Prasad (2004), even a small increase in air temperature would more than offset the water-saving effect via reduced stomatal conductance. Nevertheless, on day 4, i.e. after the release of heat wave, WUE in HWN treatment was even higher by 13 % ($p < 0.05$) compared to the ATW treatment, although on day 5, i.e. after one-day regeneration period, it already declined and, the same as A_{growth}, was 4 % ($p < 0.05$) lower than that in ATW treatment (Fig.1E).

For the entire experimental period, the intercellular carbon dioxide concentration (C_i) in HWW treatment did not differ significant from ATW treatment. In contrast, it was considerably reduced by 20 % ($p < 0.05$) on average during the 2th and the 3th days of heat wave treatment, as well as on day 4, after the nocturnal HW, before the HWN-treatment

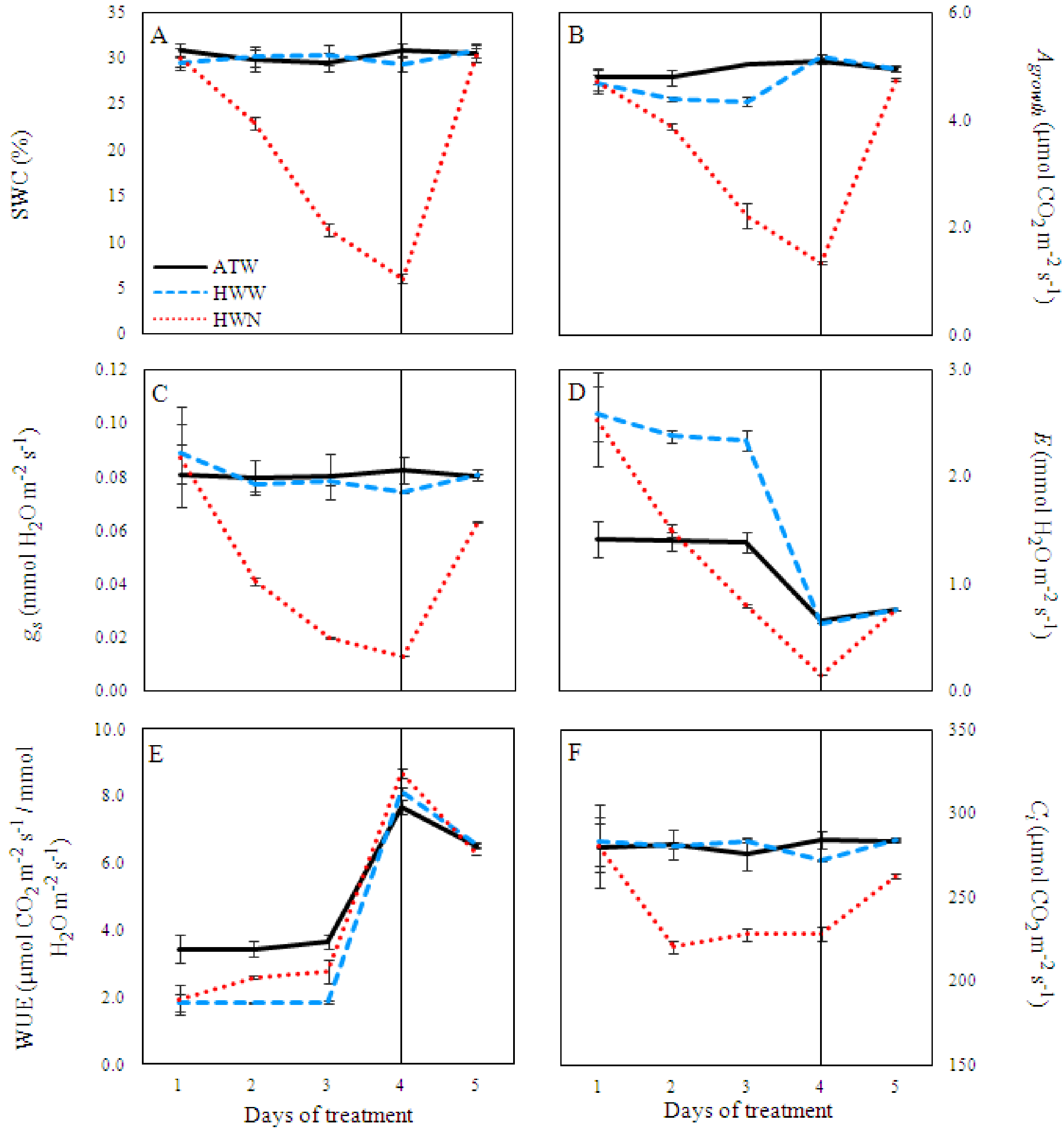

Figure 1. The changes of (A) soil water content (SWC), (B) growth photosynthetic rate (A_{growth}), (C) stomatal conductance (g_s), (D) transpiration rate (E), (E) water use efficiency (WUE), (F) and intercellular CO_2 concentration (C_i) in barley (*Hordeum vulgare* L.) plants grown under different temperature and soil water conditions during the course of experiment: ATW-ambient temperature, fully watered; HWW-heat wave, fully watered; HWN-heat wave, not watered. The vertical line represents the day when the regeneration period was started. Error bars are SE ± of the mean.

plants were rehydrated (Fig. 1F). These results indicate that considerably reduction in photosynthesis in HWN treatment to large extent could be attributed to stomatal closure and consequent reduced intercellular CO_2 concentration. The same assumption was made by Duan *et al*. (2017), who found that under high soil water availability, despite the initial sharp rise in leaf stomatal conductance and transpiration at the onset of the heat wave, photosynthesis declined gradually in parallel with stomatal conductance as heat wave progressed, maintaining a relatively low leaf level water use efficiency.

Moreover, similarly as in the case with A_{growth}, after one-day regeneration period, C_i in HWN treatment was 7 % ($p < 0.05$) lower than that in ATW treatment, what means that full recovery of C_i in HWN plants also was not achieved.

Conclusion

This study demonstrated apparent importance of soil water availability even during the short-term heat wave period, as combined impact of heat wave and drought brought much more pronounced negative effect on leaf physiology of barley plants than the single heat wave treatment. When the heat wave was imposed alone, leaf gas exchange parameters of barley recovered completely after one-day regeneration period, while, in the combination of heat wave with drought, full recover of photosynthetic rate, stomatal conductance and intercellular CO_2 concentration was not observed.

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