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Research paper

Water availability as dominant control of heat stress responses in two contrasting tree species

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Heat waves that trigger severe droughts are predicted to increase globally; however, we lack an understanding of how trees respond to the combined change of extreme temperatures and water availability. Here, we studied the impacts of two consecutive heat waves as well as post-stress recovery in young *Pseudotsuga menziesii* (Mirb.) Franco (Douglas-fir) and *Robinia pseudoacacia* L. (black locust) growing under controlled conditions. Responses were compared under water supply close to the long-term average and under reduced irrigation to represent drought. Exposure to high temperatures (+10 °C above ambient) and vapour pressure deficit strongly affected the trees in terms of water relations, photosynthesis and growth. Douglas-fir used water resources conservatively, and transpiration decreased in response to mild soil water limitation. In black locust, heat stress led to pronounced tree water deficits (stem diameter shrinkage), accompanied by leaf shedding to alleviate stress on the hydraulic system. The importance of water availability during the heat waves became further apparent by a concurrent decline in photosynthesis and stomatal conductance with increasing leaf temperatures in both species, reaching the lowest rates in the heat–drought treatments. Stress severity determined both the speed and the amount of recovery. Upon release of stress, photosynthesis recovered rapidly in drought-treated black locust, while it remained below control rates in heat ($t = -2.4$, $P < 0.05$) and heat–drought stressed trees ($t = 2.96$, $P < 0.05$). In Douglas-fir, photosynthesis recovered quickly, while water-use efficiency increased in heat–drought trees because stomatal conductance remained reduced ($t = -2.92$, $P < 0.05$). Moreover, Douglas-fir was able to compensate for stem-growth reductions following heat (–40%) and heat–drought stress (–68%), but most likely at the expense of storage and other growth processes. Our results highlight the importance of studying heat waves alongside changes in water availability. They further suggest that we should look beyond the actual stress event to identify lagged effects and acclimation processes that may determine tree resilience in the long term.

Keywords: drought stress, evaporative demand, growth, heat wave, photosynthesis, *Pseudotsuga menziesii* (Douglas-fir), recovery, *Robinia pseudoacacia* (black locust), transpiration, water deficit.

Introduction

Weather extremes triggering severe heat waves and droughts are increasing globally, with fundamental implications for the terrestrial carbon (C) and water cycles (Easterling et al. 2000, Reichstein et al. 2013). The net effects range from a decline in forest productivity (Ciais et al. 2005, Schwalm et al. 2012) to stress-induced tree mortality (Williams et al. 2013, Mitchell et al. 2014). The intensity of the stress response is likely affected by

antecedent conditions, the length of the drought period and related increases in temperature. Drought events typically co-occur with warm temperatures and increases in evaporative demand (e.g., Chaves et al. 2003, De Boeck and Verbeeck 2011). The latter further amplifies stress, because plants have to face the dilemma of water shortage when evaporative demand is largest (Williams et al. 2013, Ruehr et al. 2014). However, while periods of heat and drought naturally co-occur,

there are few experimental studies that account for the coupled effects of heat and water deficit (e.g., De Boeck et al. 2011, Bauweraerts et al. 2013).

Both drought and heat greatly affect plant C and water relations. Plants regulate transpiration rates through stomata within the limits of the hydraulic system to avoid the build-up of large embolisms (Meinzer and McCulloh 2013). Any given increase in leaf transpiration in response to heat (including large evaporative demand) is therefore finely tuned by stomatal conductance. If water availability decreases, then transpiration declines, due to stomata closure. The price plants pay to maintain hydraulic function is a reduction in evaporative cooling and photosynthetic C uptake. Apart from stomatal limitations, C assimilation can also be affected by direct metabolic limitations, such as downregulation of leaf photochemistry, which often occurs under high temperatures (e.g., Chaves et al. 2003, Wahid et al. 2007, Ameje et al. 2012). The consequence of reduced assimilation is that less C is then available for storage, maintenance and growth processes. Alongside this indirect effect, growth can also be impaired directly if water deficit results in cell turgor too low for cell expansion (Hsiao 1973, Muller et al. 2011).

Heat stress experiments show that plant responses depend on both temperature and water availability (e.g., De Boeck et al. 2011, Zhao et al. 2013, Bauweraerts et al. 2014), but the overall effects and underlying processes are less than clear. For example, comparing heat responses of well-watered versus chronically reduced-watered oaks showed that photosynthesis in water-deficient trees was surprisingly higher during mornings, but assimilation strongly decreased below the levels of the well-watered trees throughout the afternoons (Bauweraerts et al. 2013). The changes in photosynthesis were related to stomatal aperture, while a similar study on well-watered heat-stressed oaks and pines found no change in stomatal conductance but instead a down regulation of the photosynthetic apparatus (Ameje et al. 2012). Because thermotolerance of photochemistry is generally found to increase with drying (e.g., Chaves et al. 2002), lower water availability may protect the photosynthetic apparatus. Our understanding of plant growth in response to heat waves is also not clear and may depend on species and experimental design. After being exposed to a single 10-day heat wave, no response or even a stimulation of growth could be found in well-watered grassland communities (De Boeck et al. 2011, Dreesen et al. 2012), while trees exposed to multiple heat waves showed a slight reduction in growth and biomass (Bauweraerts et al. 2014). A combination of heat stress and low water availability, however, markedly reduced productivity in grasses as well as in trees. This further indicates that reliable predictions of plant responses to heat waves can only be made if they are studied alongside changes in water availability.

In addition, and to allow a holistic view of stress impacts, we need to consider plant recovery responses (Flexas et al. 2006,

Niinemets 2010). There is some information indicating that the larger the water deficit is, the longer the recovery time of photosynthesis will be (Miyashita et al. 2005, Gallé and Feller 2007). Following heat stress, recovery of photosynthesis can be species-dependent (Ameje et al. 2012), as well as being affected by the maximum temperatures during preceding stress (Haldimann and Feller 2004), and such recovery can therefore take from minutes up to weeks (Hamerlynck and Knapp 1996, Haldimann and Feller 2004). Grassland communities were massively affected by combined heat and drought, with recovery of net photosynthesis beginning weeks after the stress (De Boeck et al. 2011). For trees, existing data on recovery following heat–drought stress are sparse, but longer-term effects are indicated by a decline in growth, with the underlying processes as yet unknown. Douglas-fir growing in France, for example, showed reduced growth over several years after an extreme summer heat wave and drought in 2003 (Sergent et al. 2014). However, detailed information on processes related to tree recovery after heat–drought stress is largely absent.

Here, we examine the effects of tree water relations, photosynthesis and growth under heat and combined heat–drought conditions in two contrasting tree species: Douglas-fir (*Pseudotsuga menziesii* var. *menziesii* (Mirb.) Franco) and black locust (*Robinia pseudoacacia* L.). The two species differ in their life strategies: while Douglas-fir is an evergreen coniferous tree with bud development in autumn and terminated shoot and needle growth, black locust is a nitrogen-fixing deciduous tree with continued growth of leaves and shoots throughout the growing season. Both tree species are economically important for timber as well as biomass production and are grown extensively outside their native range. Under continued climate warming, these relatively drought-tolerant tree species (DeGomez and Wagner 2001, Lévesque et al. 2014) may be planted more intensively in Europe. In this context, we investigated the individual species' responses to heat waves under two levels of irrigation, and addressed the following research questions: (i) what are the impacts of high temperatures on tree water relations, growth and photosynthesis, and does water availability matter? (ii) How do photosynthesis and water-use efficiency (WUE) change with increasing temperature and atmospheric demand? (iii) Are post-stress rates of photosynthesis, transpiration and growth similar to pre-stress and controlled conditions?

Materials and methods

Experiment

Trees were grown in a controlled greenhouse facility in Garmisch-Partenkirchen, Germany (708 m above sea level, 47°28'32.9"N, 11°3'44.2"E). The greenhouse is equipped with special UV-transmissive glass. The environmental conditions in the greenhouse, such as light, temperature and air humidity are computer-regulated (CC600, RAM Regel- und Messtechnische

Apparate GmbH, Herrsching, Germany). Air temperature is regulated by combining ventilation and air conditioning, thus allowing well-mixed conditions. The air conditioning units are equally distributed in each greenhouse compartment. Relative humidity is maintained through fogging nozzles spraying distilled water, or by ventilation with the outside air.

Three-year-old *P. menziesii* var. *menziesii* (85 cm height) and 3-year-old *R. pseudoacacia* (80 cm height) were planted in individual pots (120 l, ~55 cm in diameter, 70 cm in height; Brute, Rubbermaid, Atlanta, GA, USA) in a mixture of humus with sand (2 : 3, pH = 7.5, N-content = $0.2 \pm 0.02\%$ and C : N ratio of $11 \pm 1\%$) in September 2012. To mimic soil temperature profiles, the pots were insulated with Styrofoam and bubble-sheeting and, to allow cooling, a coiled water pipe (made from stainless-steel) was installed at the bottom of the pots. By regulating the cooling, we were able to mimic soil temperature profiles to match pre-defined temperatures at 50 cm soil depth (air temperature averaged over previous 20 days).

Trees were placed into two adjacent, individually controllable compartments (each 36 m², 6 m height) of the greenhouse facility in May 2013 (see Figure S1 available as Supplementary Data at [Tree Physiology](#) Online). The trees from the heat and heat-drought treatment were placed in a compartment next to that of the control and drought treatment. In total, 24 trees were grown per compartment, with each of the four treatments containing six trees per species. Outside the stress periods, the trees were grown under ambient air temperature, as measured by a meteorological station in front of the greenhouse (average annual air temperature during 2013 was 7.3 °C). Relative humidity in the greenhouse was set to mimic the diurnal of long-term (20-year) monthly averages from a meteorological station in Garmisch-Partenkirchen. Pots were watered using automated drip irrigation (Rain Bird, Azusa, CA, USA). To assure homogenized wetting of the soil, six pressure-regulated drippers were distributed equally on the topsoil. Trees were watered every second day (except during recovery) with the amount of water close to the long-term (20-year) monthly sum of precipitation. During the course of the experiment, irrigation of the Douglas-fir trees was halved (as compared with black locust trees), whilst water content remained close to field capacity. In the drought and heat-drought treatments, irrigation was reduced for ~6 weeks (25 June to 10 August). During this period, irrigation in drought-treated trees was 65% lower in black locust ($0.6 \text{ l tree}^{-1} \text{ day}^{-1}$ on average) and 80% lower in Douglas-firs ($0.3 \text{ l tree}^{-1} \text{ day}^{-1}$ on average) than in control and heat-treated trees (black locust: $1.6 \text{ l tree}^{-1} \text{ day}^{-1}$ and Douglas-fir: $1.2 \text{ l tree}^{-1} \text{ day}^{-1}$ on average). Constant irrigation, rather than adapting to pre-defined soil water content, creates depletion in soil water, which is often found during natural heat waves (De Boeck et al. 2010).

Two heat waves were simulated during summer 2013: the first took place between 6 and 21 July (15 days), and the second between 28 July and 10 August (14 days, see Figure 1). Air

temperature was increased by ~10 °C above ambient to a maximum of 46 °C, and relative humidity was decreased by 17% so that water concentrations in the air were similar between ambient and extreme temperature conditions. This resulted in a sharp increase in vapour pressure deficit (VPD). The two heat phases were separated by a 7-day recovery period with ambient temperature, in which the trees were watered daily (drought treatment trees received half of the water of the control and heat treatment). Following the second heat stress event, trees were watered daily until the differences in soil water content between treatments subsided.

Measurements

Abiotic drivers Air temperature, relative humidity and photosynthetically active radiation (PAR) were measured automatically in each of the four treatments (see Figure S1 available as Supplementary Data at [Tree Physiology](#) Online). The PAR sensors (PQS 1, Kipp & Zonen, Delft, The Netherlands) were placed above canopy height, and the temperature and relative humidity sensors (CS215, Campbell Scientific Inc., Logan, UT, USA) at ~1 m vegetation height (above the soil surface), enclosed in aspirated radiation shields (43502, Young, Traverse City, MI, USA). Carbon dioxide concentrations in each greenhouse cabin were monitored with a CO₂ probe (GMT 222, Vaisala, Helsinki, Finland). To quantify soil conditions, each pot was equipped with soil moisture (10HS, Decagon Devices, Inc., Pullman, WA, USA) and temperature sensors (T107, Campbell Scientific Inc.) at 10 cm soil depth. To assess soil profiles, additional sensors were installed at 30 and 50 cm soil depth, in one pot per treatment and species. All sensors (including soil moisture and temperature probes) were measured half-hourly using data loggers (CR1000, Campbell Scientific Inc.), either directly connected to a logger or via a multiplexer (AM16/32B multiplexer, Campbell Scientific Inc.).

Relative extractable soil water (RSW) was calculated from volumetric soil moisture content (SWC), as

$$\text{RSW (\%)} = 100 \times \frac{\text{SWC}_{\text{actual}} - \text{SWC}_{\text{min}}}{\text{SWC}_{\text{max}} - \text{SWC}_{\text{min}}} \quad (1)$$

where SWC_{min} was set at $0.04 \text{ m}^3 \text{ m}^{-3}$, the minimum of daily soil water content at 30 cm depth in the drought-treated black locust trees, and SWC_{max} the maximum of mean daily SWC per sensor ($0.23\text{--}0.26 \text{ m}^3 \text{ m}^{-3}$). To integrate soil water availability over the entire rooting depth per treatment and species, RSW from shallow (10 cm) and deep soil layers (30 and 50 cm) were averaged. As water uptake of Douglas-firs from 50 cm depth was not apparent (no change in SWC), we averaged RSW for the 10 and 30 cm soil depths.

Dendrometer and tree water deficit To study variations in stem growth and water dynamics, electronic point dendrometers (DD-S, Ecomatik, Dachau, Germany; accuracy of $\pm 1.5 \mu\text{m}$ and

temperature coefficient $<1 \mu\text{m K}^{-1}$) were installed, with the dendrometer head on the outer bark, at 5–10 cm stem height on two to three trees per treatment and species, in June 2013. The sensors were measured half-hourly, connected to data loggers via multiplexers (see above).

Measurements were corrected for temperature sensitivity following the manufacturer; however, the effect was negligible. Fluctuations in stem diameter are due to growth and/or water related-shrinking or swelling of elastic tissues in the bark. To estimate water dynamics from dendrometer data, the growth signal therefore needs to be subtracted (Zweifel et al. 2005). In brief, we started at the maximum value of each seasonal dendrometer curve, when growth was saturated, which occurred generally between September and October. From there, we drew a horizontal line with the slope decreasing until the next (earlier) maximum point. Following this procedure the line was connected to the next maximum point until the earliest data point of the curve was reached. The differences between the resulting growth line and the dendrometer data are a result of bark water deficit and can be interpreted as a direct measure of tree water deficit (for details see Zweifel et al. 2005).

To assess overall changes in stem growth, we estimated a basal area increment per tree, calculated from stem diameter measured at sensor height (black locust: 13 ± 1 mm and Douglas-fir: 12 ± 1 mm), assuming a circular area to which dendrometer increments were added. Tree heights were measured in spring and autumn 2013.

Sap flow Tree transpiration was measured with heat-balance sap flow sensors (EMS 62, EMS, Brno, Czech Republic) installed between 10 and 30 cm stem height. To minimize temperature fluctuations, sensors were shielded with aluminium bubble foil, in addition to the factory supplied foam enclosing. We installed three sensors per treatment and species but, unfortunately, sap flow data from Douglas-firs were extremely noisy (likely due to low sap flux in these young trees) and could not be used for further analysis.

To derive actual sap flow from the measurements, it is necessary to subtract the difference between the heated and unheated probe at zero sap flux. Because nocturnal sap flow occurs in most tree species, especially during warm nights, we only assumed night-time transpiration to be zero for nights with average VPD <0.2 kPa and linearly interpolated zero-flow between those nights.

Leaf gas exchange and biomass Photosynthesis (A_{sat}) and related transpiration (T_{leaf}) as well as stomatal conductance (g_s) and leaf internal CO_2 concentrations (C_i) were measured on each tree, per treatment and species, during late morning (10 am) on a weekly basis. To assess diurnal changes, additional measurement campaigns were conducted in each of the heat and recovery periods during mornings (9 am), middays (12 pm)

and afternoons (3 pm). Because we tried to keep campaigns short (<2 h), and therefore temporal variations at a minimum, Douglas-fir and black locust trees were measured on consecutive days. Foliage gas exchange was measured using a Li-6400 (LI-COR Inc., Lincoln, NE, USA) equipped with a light source (Li-6400-02B LED) under saturated light conditions ($\text{PAR} = 1300 \mu\text{mol m}^{-2} \text{s}^{-1}$, pre-determined from light response curves). After the foliage was clamped in the chamber, we allowed the fluxes to stabilize for at least 2 min before measurement. Leaf chamber temperature and relative humidity were set to mimic the conditions in the greenhouse. For black locust, we continued to measure the same leaves (at ~ 1 m vegetation height) throughout the experiment, but changed leaves if they turned yellow (mainly in the heat and heat-drought treatment). Because black locust trees grew quickly in the absence of stress, previously sun-lit leaves could become increasingly shaded with continuation of the experiment. For Douglas-fir the same needles were measured throughout the experiment (a 3 cm long section was marked on one side of a branch at ~ 50 – 80 cm tree height). Half-sided leaf and needle areas were calculated from pictures using ImageJ v1.48 (Schneider et al. 2012).

Leaf biomass in Douglas-fir was not quantified, because needle senescence hardly occurred, and trees were not harvested, as the experiment was designed to continue over another growing period. In black locust, leaf biomass was quantified from litter fall. Generally, litter fall during the growing period was negligible, but it was pronounced during stress in the heat and heat-drought treatment. Dried or yellow leaves were collected in mobile litter traps, from pots or directly harvested from the trees until mid-November, when all leaves were senescent. The leaves were subsequently dried at 60°C for 48 h. Half-sided leaf area was calculated from leaf biomass and pre-determined specific leaf area (SLA, measured once for each black locust tree after the first heat wave). Treatment averages of SLA differed significantly ($F = 8.23$, $P < 0.01$), with smaller SLA in heat-drought ($185 \pm 16 \text{ cm}^2 \text{ g dw}^{-1}$) and drought ($183 \pm 6 \text{ cm}^2 \text{ g dw}^{-1}$) compared with control ($234 \pm 12 \text{ cm}^2 \text{ g dw}^{-1}$) and heat ($208 \pm 12 \text{ cm}^2 \text{ g dw}^{-1}$) trees. Leaf area per tree was thus calculated using treatment-specific SLA.

Experimental conditions

The two intermittent heat waves affected air temperature marginally on the annual scale ($+0.9^\circ\text{C}$), but short-term effects during the campaigns were more pronounced (Figure 1c and d). Under heat, daily averaged air temperature (29.2°C) was almost $+10^\circ\text{C}$ above ambient conditions (Figure 1c). This affected VPD, which increased exponentially with temperature and reached a maximum of 7.6 kPa (Figure 1b) during the two hottest days. The warmer air temperatures transferred to deeper soil layers with some delay, due to automated cooling at the bottom of the pots (Figure 1d).

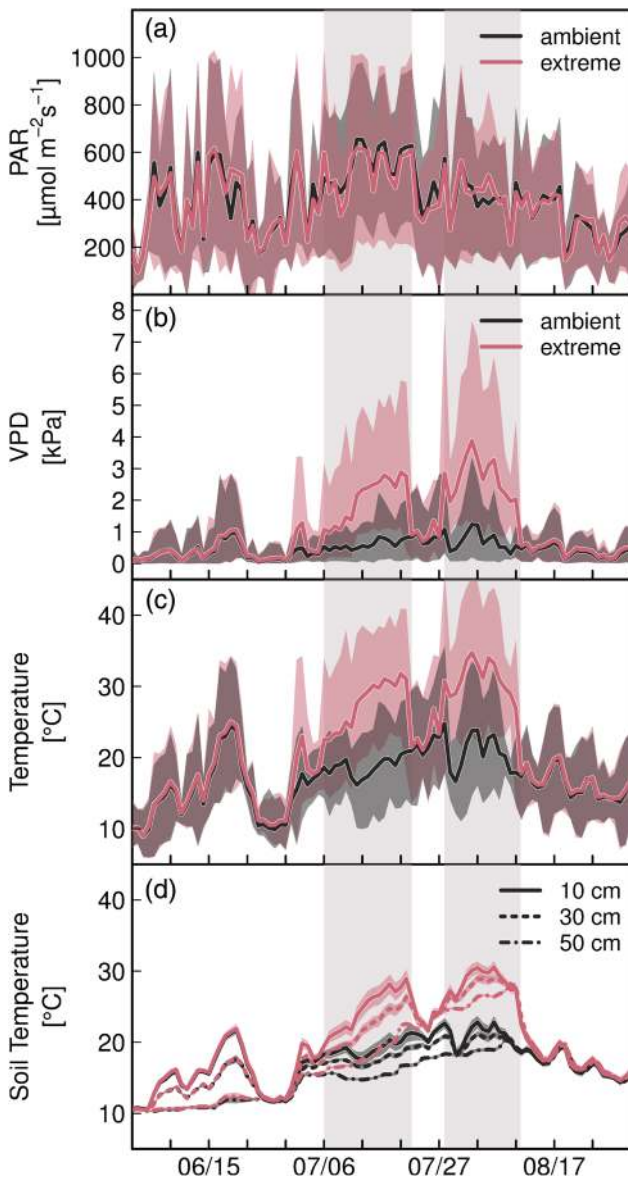


Figure 1. Dynamics of environmental drivers under ambient and extreme conditions. Shown are daily averaged PAR during daytime (a), VPD (b), air temperatures (c) and soil temperatures (d). The shaded areas around the mean represent daily minima and maxima. The two heat waves are depicted by the light grey areas. Note that a 2-day heat event was accidentally initiated at the beginning of July.

With the exception of the heat wave periods, differences in the environmental conditions between the two greenhouse compartments were minimal. Daily averaged air temperature, soil temperature, relative humidity, PAR and carbon dioxide concentrations differed by <2% on average. Differences in environmental drivers within compartments were <1% during the heat wave periods. Given the close agreement in environmental conditions, an effect on trees due to placement in the greenhouse ('chamber effect') should be minimal. To totally overcome such concerns and the possibility of 'pseudoreplication', pots can be moved in and between chambers (as enacted in similar experiments, e.g.,

Bauweraerts et al. 2013). In our case, this was not possible due to instrumentation and soil cooling equipment. However, a modified power analysis showed that small differences (i.e., <15%) are unlikely to be detected (<10%), and that differences between treatments need to be larger than 40% to be identified as statistically significant ($P \leq 0.05$), with a probability of 80%. Thus, in agreement with the close environmental control, we can consider it as highly unlikely that chamber effects could have influenced statistical significance. We therefore considered each tree as replicate and applied inferential statistics as suggested by Oksanen (2001).

Data analysis and statistics

To interpret treatment differences in the context of before, during and after stress variations, a before–after control–impact (BACI) analysis was implemented, using linear mixed-effects models (McDonald et al. 2000). Treatment (control, drought, heat and heat–drought) and period (before stress, during stress and recovery) were assigned as fixed effects, and trees as random effect (to account for the repeated measure design). The interaction term of treatment and period provides information on the significance of the impact by comparing it with changes in the control treatment set within the context of pre-stress conditions. The significance of the overall interaction term was additionally tested by likelihood ratio tests. Differences between treatments and control per sampling point were assessed by analysis of variance (ANOVA). Treatment effects were calculated as $\%effect = 100 \times (treatment - control) / control$. To reveal dependencies of physiological parameters with biotic and abiotic drivers, linear models and nonlinear least squares regressions were applied. All data processing, analysis and statistics were carried out using R 3.2.1 (R Core Team 2013) and extended with the nlme package (Pinheiro et al. 2010) for mixed-effects modelling.

Results

Effects of heat stress and water availability

Soil water availability in black locust was affected by heat and drought, but also declined under control conditions because of large transpiration rates and irrigation that was not adapted to keep soil water at a pre-defined level. Thus, relative extractable soil water content (RSW) declined sharply in response to reduced irrigation (drought and heat–drought) and heat stress (Figure 2a). The sharp decrease in RSW in the heat treatment is explained by even larger tree transpiration rates in comparison with the control, as long as water supply was sufficient to meet the increased atmospheric demand (Figure 2g). The quick depletion of water reserves in heat-stressed black locust trees became further apparent on viewing the large daily variations in transpiration (Figure 2a and g) resulting from changes in water availability due to every-other-day irrigation (outside the recovery

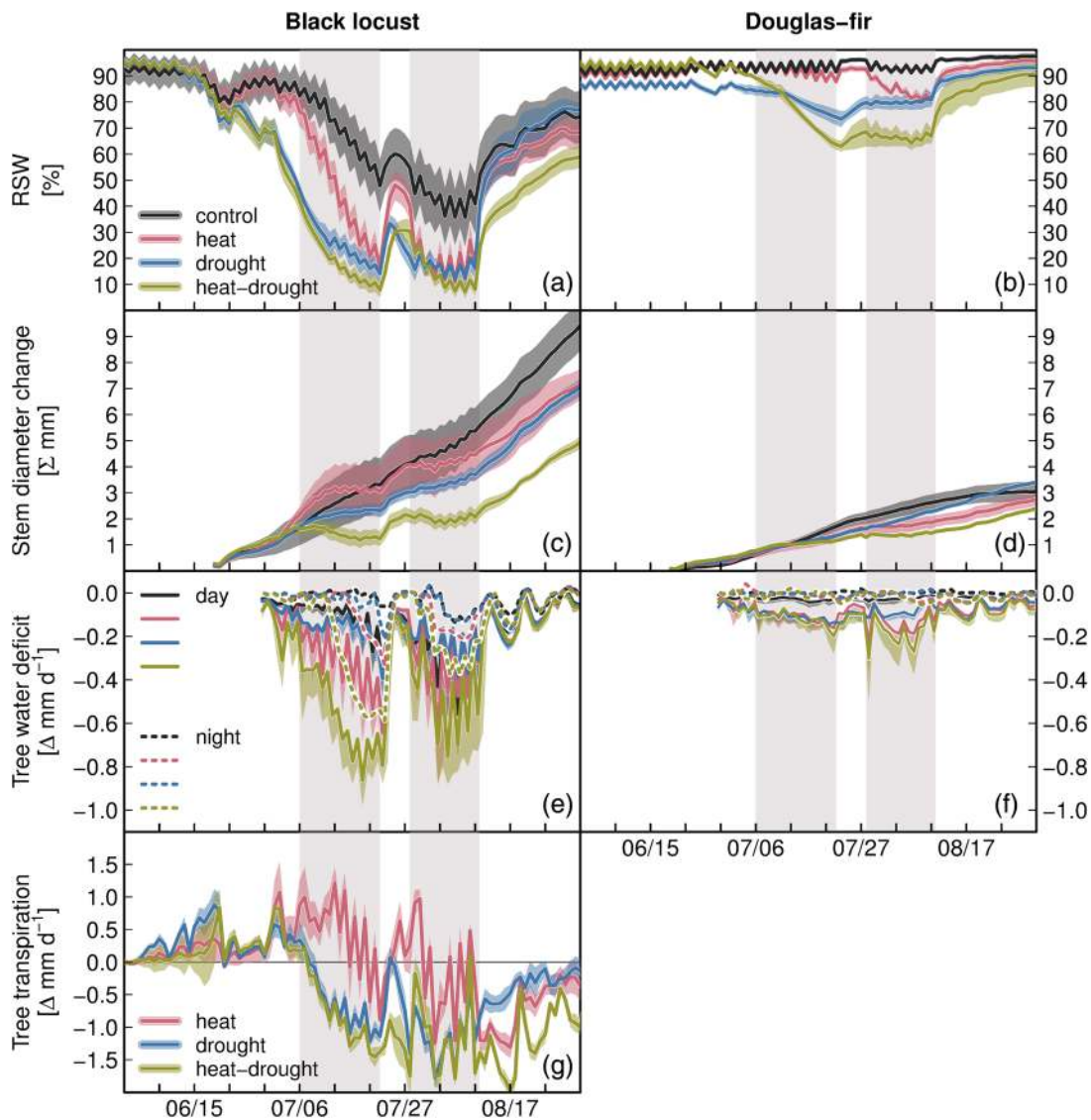


Figure 2. Dynamics of soil water, stem diameter change and transpiration for the four treatments. Shown are daily averaged relative extractable soil water content (RSW; a and b), cumulative stem diameter change (c and d) and tree water deficit (e and f) for black locust and Douglas-fir, as well as transpiration for black locust trees only (g), shown as differing from the control. Note that tree water deficit is separated into daytime showing maximum dehydration (shaded area) and night-time with minimum water deficit indicating the degree of refilling (full rehydration occurred when night-time water deficit reached zero). The shaded areas around the mean represent the standard error between pots. The two heat waves are depicted by the light grey areas.

period). This day-to-day variation in transpiration was less pronounced under heat-drought, and transpiration declined steadily with decreasing RSW, similar to that of drought-treated black locust (Figure 2g). Interestingly, soil water availability did not directly relate to tree water deficit (derived from stem diameter change), and a pronounced tree water deficit occurred only under heat and heat-drought conditions (Figure 2e). An explanation, in addition to differences in water use, could be refilling of tree water reserves at night (derived from minimum tree water deficit). In heat-drought trees, stem nocturnal rehydration was impaired 7 days after the onset of the first heat wave and then steadily declined (Figure 2e, dashed lines), while larger water availability in the heat treatment mitigated tree water

deficit. Moderate temperatures, combined with drought, affected tree water deficit the least, with full rehydration continuing most nights (i.e., tree water deficit reached zero during the night). A similar pattern was reflected in stem increment, which continued at a steady but slowed rate under drought, while it immediately ceased in the heat-drought treatment (Figure 2c).

In Douglas-fir, we found the effect of the experimental treatments on soil water availability to be small: RSW remained close to saturation in the heat treatment, and declined slightly in response to reduced irrigation, with the largest reduction in the heat-drought treatment (Figure 2b). Concurrently, impairment of stem growth and tree water deficit were largest under heat and heat-drought stress (Figure 2d). Water supply was likely

sufficient, however, and night-time refilling of daytime tree water deficit occurred in all treatments throughout the experiment (Figure 2f). Interestingly, tree water deficit peaked in the heat and heat–drought treatment on the hottest day (28 July, maximum air temperature of 46 °C and VPD of 7.6 kPa), when we also observed wilting of short second-flush needle sections (approximately <1% needle biomass; Figure S4 available as Supplementary Data at *Tree Physiology* Online).

Leaf gas exchange was significantly affected by the treatments (Figure 3 and Table 1). We found the largest decline in light-saturated photosynthesis (A_{sat}) in response to heat stress coupled with low water availability, consistent in both species (Figure 3a and b). A_{sat} and leaf transpiration varied largely in phase under drought and heat–drought conditions, but showed out-of-phase responses in the heat treatment (Figure 3a–d). Despite contrasting treatment effects on transpiration, stomata opening appeared to be one of the limiting factors for C assimilation as indicated by the close (exponentially saturated) relationship of A_{sat} with stomatal conductance in black locust (heat: $R^2 = 0.82$, drought: $R^2 = 0.90$, heat–drought: $R^2 = 0.82$; see Figure 5e) and

Douglas-fir (heat: $R^2 = 0.75$, drought: $R^2 = 0.71$, heat–drought: $R^2 = 0.82$; see Figure 5f). A predominant role of stomatal aperture on photosynthesis could be inferred from the concurrent decline in the C_i/C_a ratio (i.e., intercellular $[\text{CO}_2]$ /atmospheric $[\text{CO}_2]$; see Figure S2 available as Supplementary Data at *Tree Physiology* Online), until a value of $\sim 0.04 \text{ mol m}^{-2} \text{ s}^{-1}$ was reached. At stomatal conductances below this value, C_i/C_a values tended to increase again, especially in black locust and, to some degree, also in heat–drought treated Douglas-fir trees.

The combined effects of reduced C uptake and low water availability (i.e., low turgor for growth processes) translated into reduced growth of basal area, which was by far lowest under heat–drought stress (Figure 4a and b, hatched areas). In black locust (but not in Douglas-fir) we additionally observed pronounced leaf shedding during stress (heat: 12% and heat–drought: 18% of total end-of-season leaf area; Figure 4c, hatched areas). Overall, the combination of heat and drought led to a large reduction in leaf biomass (–46%), basal area (–43%) and tree height (–31%) compared with control trees. This also indicates that, due to the reductions in leaf area, the effects on

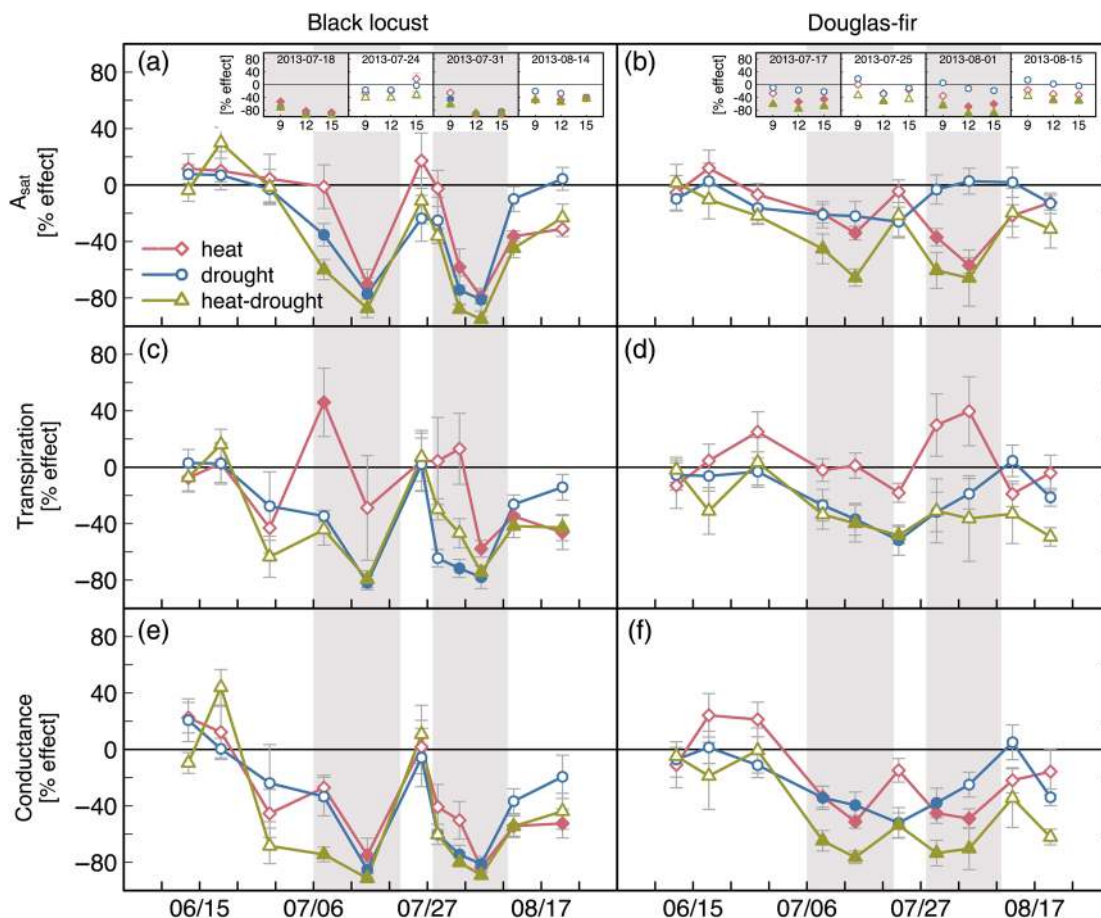


Figure 3. Treatment effects of leaf photosynthesis (A_{sat}), transpiration and conductance, in heat, drought and heat–drought treated trees during the course of the experiment in 2013. The insets show diurnals of A_{sat} measured during periods of stress and recovery. Closed symbols indicate significant treatment effects compared with the control, derived through analysis of variance per time point ($P \leq 0.05$). The error bars are $\pm 1\text{SE}$. The two heat waves are depicted by the light grey area.

Table 1. Results of linear mixed-effects models testing for treatment differences in leaf gas exchange and tree transpiration during the experimental periods (before stress, during heat stress and recovery). The interaction terms test for treatment differences during stress and recovery by accounting for changes in the control treatment relative to before-stress conditions. Means, standard error (SE) and *t*-values of fixed effects for photosynthesis (A_{sat} , $\mu\text{mol m}^{-2} \text{s}^{-1}$), stomatal conductance (g_s , $\text{mol m}^{-2} \text{s}^{-1}$), leaf transpiration (T_{leaf} , $\text{mmol m}^{-2} \text{s}^{-1}$) and tree transpiration (T_{tree} , mm day^{-1}) are given. Significant values ($P \leq 0.05$) of interactions indicating the before–after impact on treatments are given in bold. Note that likelihood ratio tests of the overall interaction term (treatment \times period) were significant in all models.

| | A_{sat} | | | g_s | | | T_{leaf} | | | T_{tree} | | |
|--------------------------------|------------------|------|----------|--------------|------|----------|-------------------|------|----------|-------------------|------|----------|
| | Value | SE | <i>t</i> | Value | SE | <i>t</i> | Value | SE | <i>t</i> | Value | SE | <i>t</i> |
| Black locust | | | | | | | | | | | | |
| Intercept | 9.80 | 1.00 | 9.66 | 0.13 | 0.02 | 6.32 | 1.36 | 0.18 | 7.50 | 0.53 | 0.16 | 3.35 |
| Drought | 0.46 | 1.42 | 0.32 | -0.01 | 0.03 | -0.23 | -0.05 | 0.25 | -0.21 | 0.21 | 0.22 | 0.96 |
| Heat | 0.99 | 1.43 | 0.69 | -0.01 | 0.03 | -0.42 | -0.12 | 0.26 | -0.45 | 0.11 | 0.22 | 0.48 |
| Heat–drought | 0.91 | 1.42 | 0.64 | -0.03 | 0.03 | -0.85 | -0.13 | 0.25 | -0.50 | 0.12 | 0.22 | 0.52 |
| Stress periods | -1.19 | 0.96 | -1.24 | -0.03 | 0.02 | -1.22 | 0.12 | 0.19 | 0.62 | 1.11 | 0.08 | 13.82 |
| Recovery periods | 2.16 | 1.00 | 2.14 | 0.05 | 0.02 | 2.17 | 0.04 | 0.20 | 0.18 | 1.15 | 0.08 | 13.71 |
| Drought \times stress | -5.23 | 1.35 | -3.86 | -0.06 | 0.03 | -1.98 | -0.95 | 0.27 | -3.46 | -0.89 | 0.11 | -7.90 |
| Heat \times stress | -4.53 | 1.36 | -3.33 | -0.05 | 0.03 | -1.48 | -0.01 | 0.27 | -0.04 | 0.29 | 0.11 | 2.53 |
| Heat–drought \times stress | -6.95 | 1.37 | -5.07 | -0.06 | 0.03 | -1.80 | -0.68 | 0.28 | -2.44 | -0.87 | 0.11 | -7.70 |
| Drought \times recovery | -1.32 | 1.41 | -0.94 | -0.03 | 0.03 | -0.96 | -0.18 | 0.28 | -0.62 | -0.28 | 0.12 | -2.38 |
| Heat \times recovery | -3.47 | 1.44 | -2.41 | -0.05 | 0.03 | -1.59 | -0.30 | 0.29 | -1.03 | -0.32 | 0.12 | -2.66 |
| Heat–drought \times recovery | -4.21 | 1.42 | 2.96 | -0.02 | 0.03 | -0.52 | -0.23 | 0.29 | -0.81 | -0.96 | 0.12 | -8.04 |
| Douglas-fir | | | | | | | | | | | | |
| Intercept | 7.66 | 1.26 | 6.08 | 0.07 | 0.02 | 3.66 | 0.89 | 0.24 | 3.68 | | | |
| Drought | -0.50 | 1.78 | -0.28 | 0.00 | 0.03 | 0.11 | 0.04 | 0.34 | 0.12 | | | |
| Heat | -0.44 | 1.78 | -0.25 | 0.01 | 0.03 | 0.48 | 0.07 | 0.34 | 0.21 | | | |
| Heat–drought | -0.55 | 1.99 | -0.28 | 0.00 | 0.03 | 0.05 | -0.11 | 0.38 | -0.28 | | | |
| Stress periods | 3.23 | 0.63 | 5.13 | 0.07 | 0.02 | 4.20 | 1.13 | 0.20 | 5.53 | | | |
| Recovery periods | 3.73 | 0.68 | 5.46 | 0.08 | 0.02 | 4.57 | 0.53 | 0.22 | 2.41 | | | |
| Drought \times stress | -0.36 | 0.89 | -0.40 | -0.05 | 0.02 | -2.23 | -0.58 | 0.29 | -2.03 | | | |
| Heat \times stress | -3.35 | 0.89 | -3.76 | -0.08 | 0.02 | -3.41 | 0.31 | 0.29 | 1.06 | | | |
| Heat–drought \times stress | -5.79 | 1.01 | 5.72 | -0.11 | 0.03 | -4.06 | -0.64 | 0.33 | -1.95 | | | |
| Drought \times recovery | -0.25 | 0.96 | -0.26 | -0.04 | 0.03 | -1.50 | -0.38 | 0.31 | -1.20 | | | |
| Heat \times recovery | -0.72 | 0.96 | -0.75 | -0.04 | 0.03 | -1.52 | -0.27 | 0.31 | -0.88 | | | |
| Heat–drought \times recovery | -2.06 | 1.09 | -1.89 | -0.08 | 0.03 | -2.92 | -0.52 | 0.35 | -1.47 | | | |

overall tree C uptake were larger than individual leaf gas-exchange measurements had implied.

Recovery from stress

Increased water supply and moderate-to-cool temperatures during post-stress recovery resulted in quick refilling of tree water deficit (Figure 2e and f). Stem growth (Figure 2c and d) and leaf photosynthesis (Figure 3a and b) simultaneously increased. Nevertheless, lagged stress effects became apparent, as indicated by significant interaction terms of treatment and recovery in linear mixed-effects model analysis (Table 1). In heat-stressed black locust, leaf photosynthesis reached control values quickly after the first heat wave, while A_{sat} remained below control rates following the second heat wave (Figure 3a). The impaired recovery of leaf assimilation (ranging from -41 to -23% of control rates) was accompanied by significantly lower transpiration (-41%) and stomatal conductance (-52%) than in control trees, during the first 2 weeks after the second heat wave (Figure 3c and e). Accordingly, overall tree transpiration (-46%) and stem growth (-40%) of post-heat stressed black locust

remained below control rates (Table 1 and Figure 4). In the third week after the second stress phase, transpiration and conductance rates finally reached control values, but not photosynthesis. Also, stem growth continued to be lower until the end of the season (Figure 4a).

The pattern of recovery in heat–drought treated black locust largely followed that of the heat treatment, but a more severe impairment became apparent. For instance, tree transpiration remained lowest in heat–drought trees throughout the recovery (Table 1). Together with smallest leaf biomass, this indicates a continual decline in overall tree C uptake. Interestingly, reduced irrigation alone had a small, although significant impact on overall tree transpiration during recovery, but longer lasting effects on leaf gas exchange and growth were not apparent (Figures 2e and 3a and c).

In Douglas-fir, we found that previously heat-stressed trees generally recovered quickly. In contrast, combined heat–drought stress apparently caused some lasting effects: stomatal conductance remained below the control (Table 1 and Figure 3d) and A_{sat} stayed significantly lower during midday and afternoon,

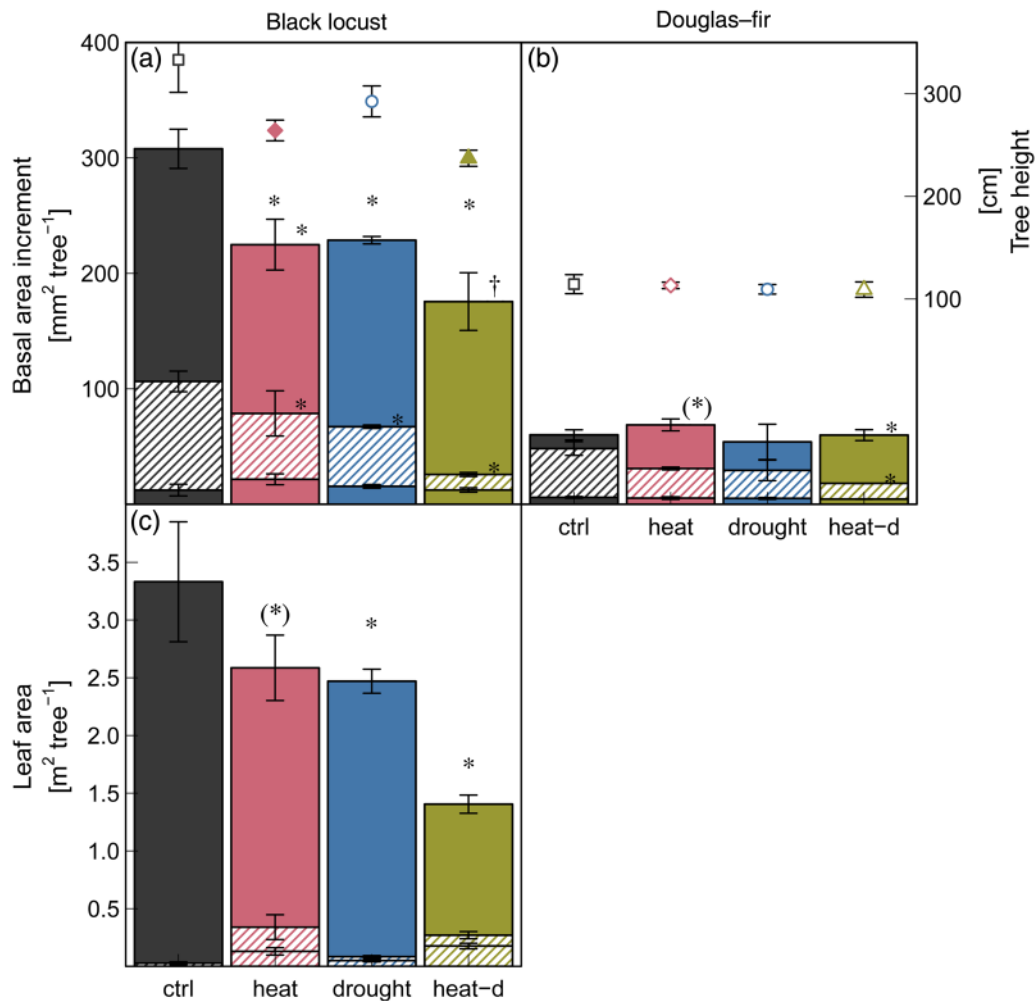


Figure 4. Treatment averages of basal area increment, leaf production and tree heights. Basal area growth from July to November 2013 is divided into before heat stress (filled areas, bottom), during heat waves including first recovery (hatched areas) and post-stress (filled areas, top); end-of growing season tree heights are represented by symbols and, if filled, significantly deviate from the control (a and b). Leaf area production for black locust is distinguished in litter fall during the first and second heat wave (hatched areas; from bottom) and end-of-season litter fall (filled areas; c). The error bars are ± 1 SE. Overall change in basal area increment between control and treatments was tested by analysis of variance (significance levels above error bars). Treatment differences in daily growth rates during sub-periods were analysed by linear mixed effect models (* $P \leq 0.05$; (*) $0.05 < P < 0.1$).

5 days after the end of stress, but not when measured earlier during the day (Figure 3b, inset). An unexpected result was that post-stress stem growth compensated for heat- and drought-imposed growth declines (Figure 4b, filled area). This was because stem increment continued in the previously stress-exposed trees until the end of September, whereas it had already flattened in control trees by the end of August.

Environmental drivers and WUE

Changes in leaf photosynthesis in black locust were correlated most strongly with soil water content. The close relation of A_{sat} with water availability in black locust was likely caused by the tremendous 'thirst' of this species under high temperature conditions, quickly resulting in water limitation and stomatal closure (Figure 5e and f). In Douglas-firs growing under higher soil

water content than black locust trees, a strong polynomial relationship with leaf temperature emerged (Figure 5a and b; the relationship with VPD was less significant and is not shown). The rapid decline in A_{sat} with temperatures above 30 °C in Douglas-fir indicates an apparent temperature limitation of photosynthetic processes. Changes in C_i/C_a values with increasing temperatures show stomatal closure to be one of the predominant factors, but also that C_i/C_a tends to increase above 35 °C in heat-drought treated trees (see Figure S3 available as Supplementary Data at *Tree Physiology* Online), pointing towards non-stomatal limitation. Interestingly, across all treatments, reductions in A_{sat} were highly related to same and next day stem growth in black locust, and to a lesser extent in Douglas-fir (Figure 6). This indicates that growth and C uptake may respond similarly to environmental conditions.

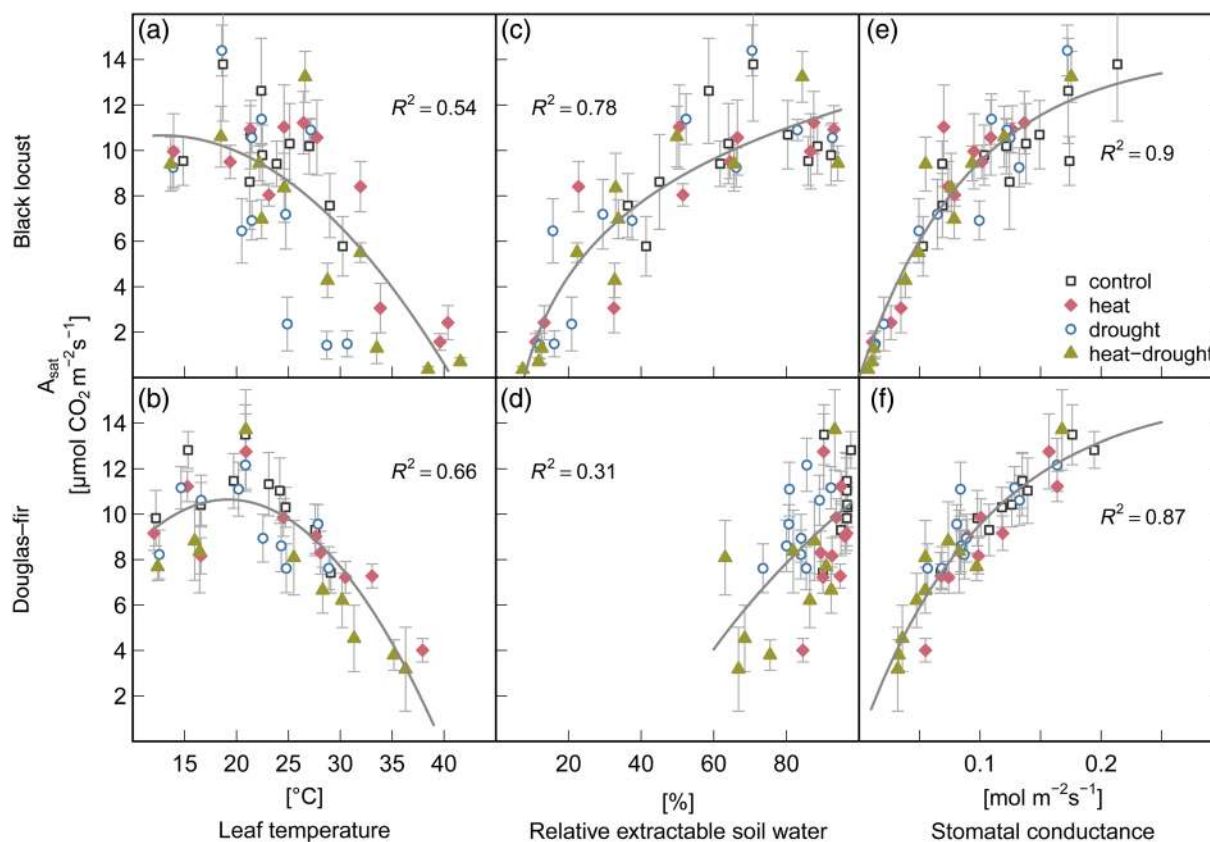


Figure 5. Relationship of leaf photosynthesis (A_{sat}) with temperature (a and b), relative extractable soil water (c and d) and stomatal conductance (e and f) for black locust and Douglas-fir. Shown are treatment averages and error bars $\pm 1\text{SE}$. The relationship of A_{sat} with temperature is indicated by polynomial functions and with relative extractable soil water by logarithmic functions. The relationship of A_{sat} with stomatal conductance was best described by an exponential saturation model. The coefficients of determination (R^2) of the relationships are given ($P < 0.01$).

In addition, both species showed a close relationship of WUE (calculated from leaf-level photosynthesis and transpiration) with leaf temperature and VPD (Figure 7), with little or no variation between treatments. We found that the largest WUE was at leaf temperatures below 25 °C (VPD < 1 kPa), and declined quickly at higher temperatures. For example, WUE in both species decreased by ~25% when temperature increased from 25 to 30 °C. The often observed water-saving effect of drought became apparent in heat-drought treated Douglas-firs, which experienced slightly higher WUE than control and heat trees under similar VPD conditions ($t = 2.8$, $P = 0.01$; Figure 7d). This indicates an imbalance between C gain and water loss under increasing summer temperatures.

Discussion

The main purpose of the study was not to directly compare heat responses in Douglas-fir and black locust, but rather to investigate how each individual species would respond to preset levels of air temperature and humidity, light, and irrigation. We found water availability exerted a dominant control on leaf gas exchange and growth during the two heat waves, and that

under the experimental conditions applied here, heat caused greater stress than moderate drought on the tree hydraulic system. Further, stress severity determined the rate and amount of tree recovery, although compensation might be possible, as seen in the later culmination of stem increment in previously stressed Douglas-firs. With regard to heat-related changes in WUE, we found a large decline of WUE with increasing leaf temperature and VPD in both species, largely independent of soil water content.

Tree water relations during heat and drought

High temperatures, along with large evaporative demand, affected tree and soil water dynamics. Because we did not adapt irrigation to match pre-defined levels of soil water content, tree available soil water decreased with heat progression. At the onset of heat, however, when soil water content was still high, transpiration initially increased in heat-treated black locust, followed by a progressive build-up of tree water deficit. Although irrigation and soil water availability were higher under heat compared with drought conditions, the heat-stressed black locust trees experienced a larger water deficit. This indicates pronounced stress on the tree hydraulic system under heat, and

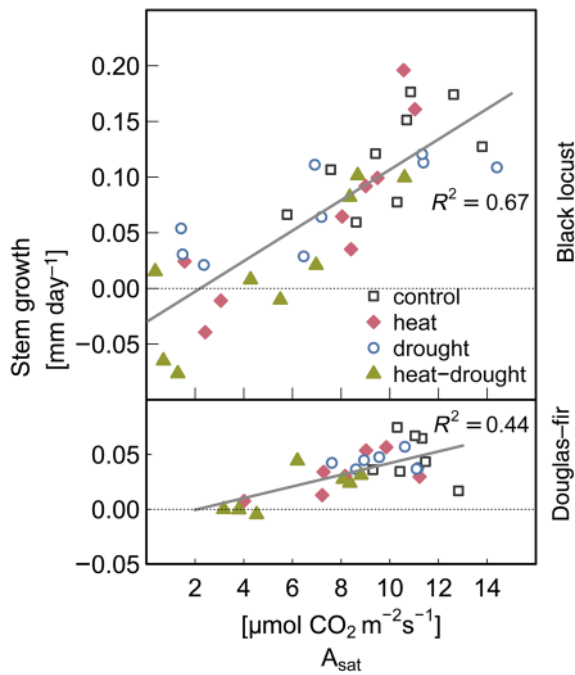


Figure 6. Relationship of daily stem diameter growth with photosynthesis (A_{sat}) in black locust and Douglas-fir. Shown are treatment averages between June and August 2013. Stem growth was calculated as the change in night-time maximum stem diameter from two consecutive days (during and following A_{sat} measurements) but with rehydration subtracted (calculated from changes in night-time tree water deficit). 'Negative growth' values are caused by water loss-induced stem shrinking. The coefficients of determination (R^2) of the relationships are given ($P < 0.01$).

confirms the dominant role of VPD and temperature within tree water relations (Zweifel et al. 2005, Barron-Gafford et al. 2007, Ruehr et al. 2012). In addition, the impairment of night-time tree rehydration points towards the accumulation of embolisms and reductions in hydraulic conductivity. Likely reasons for the large water deficit in black locust exposed to heat may include continued transpiration as a function of evaporative cooling, and the lack of full stomata closure ($0.005\text{--}0.02\text{ mol m}^{-2}\text{ s}^{-1}$ at $>35\text{ }^\circ\text{C}$ and $\text{VPD} >4.5\text{ kPa}$), as well as enhanced non-stomatal water loss caused by increasing cuticular permeability at high temperatures (Riederer and Schreiber 2001). As a result of such excessive water loss, leaf drying and shedding occurred, which may protect the hydraulic system from much larger failure (Bucci et al. 2012).

In Douglas-fir, tree water deficit was much less affected by heat and drought, with night-time rehydration continuing throughout the experiment. Likely explanations may be the comparatively smaller bark thickness (i.e., less volume to rehydrate; personal observation) and relatively high soil water availability ($\text{RSW} >60\%$). Interestingly, while we did not find a significant depletion in tree water status at temperatures below $40\text{ }^\circ\text{C}$ ($\text{VPD} <7\text{ kPa}$), the three hottest days stand out, with large water deficits in the heat and heat-drought treatments, and wilting of second-flush needles. Such responses provide insights into the

water-use strategies of Douglas-fir, indicating that a generally conservative resource use exists if water becomes limited (Smit and Van Den Driessche 1992). This strategy does not seem enough, however, to protect the species from an increasing risk of hydraulic failure under extreme atmospheric conditions. The wilting of second-flush needles on the hottest day (air temperature $>45\text{ }^\circ\text{C}$ and $\text{VPD} >7\text{ kPa}$) was likely induced by dehydration due to continuing water loss through undeveloped cuticles. In black locust, we did not find such an immediate response, likely because at the highest temperatures the leaves were folded (paraheliotropism) to expose a reflective, hairy underside, which helps to protect them from excessive heating (Arena et al. 2008).

Responses of photosynthesis and growth

Soil and atmospheric water deficit as well as high temperatures reduced photosynthesis and growth. Thus we may assume that a close relationship exists between tree water deficit and leaf-level gas exchange, which was apparent in Douglas-fir, but less obvious in black locust. A likely reason might be that we did not scale leaf-level gas exchange to the whole tree in black locust (due to the difficulty in assessing undetermined leaf growth) and, as such, missed the effects of leaf shedding and reduced growth on C uptake. Indeed, stress impacts on canopy conductance (calculated from tree transpiration, data not shown) were in line with tree water deficit (lowest under heat-drought, followed by the heat and drought treatments), thereby indicating a close relationship between water status and gas exchange at the canopy level. This was further confirmed by leaf-level relationships. Here we found that stomatal conductance may control temperature-related changes in photosynthesis. The feedforward control (Farquhar and Sharkey 1982) of conductance on photosynthesis at moderate to severe stress levels became clear from the evident decrease in the C_i/C_a ratio with stomatal closure (see Figure S2 available as Supplementary Data at *Tree Physiology* Online). However, under high temperature stress ($>35\text{ }^\circ\text{C}$) and low stomatal conductance ($<0.04\text{ mol m}^{-2}\text{ s}^{-1}$), the ratio of C_i/C_a tended to increase again, pointing towards a decrease in photochemistry and the onset of non-reversible photoinhibition (Brodribb 1996, Flexas and Medrano 2002). For oak leaves exposed to high temperatures under optimal water supply, non-stomatal limitation became apparent with a rapid increase of C_i above $40\text{ }^\circ\text{C}$, accompanied by an inhibition in photochemistry (Haldemann and Feller 2004). Drought experiments reveal similar patterns: rainfall exclusion studies in a *Quercus ilex* forest found that stomatal limitations largely dominated photosynthesis during mild to more severe drought stress (Limousin et al. 2010), but that, under extreme stress, biochemical and mesophyll limitations can exceed stomatal control (Misson et al. 2010). In contrast, a study with heat-stressed oak and pine trees, which were exposed to higher temperatures than the trees in our study, showed no decline in stomatal aperture, but an

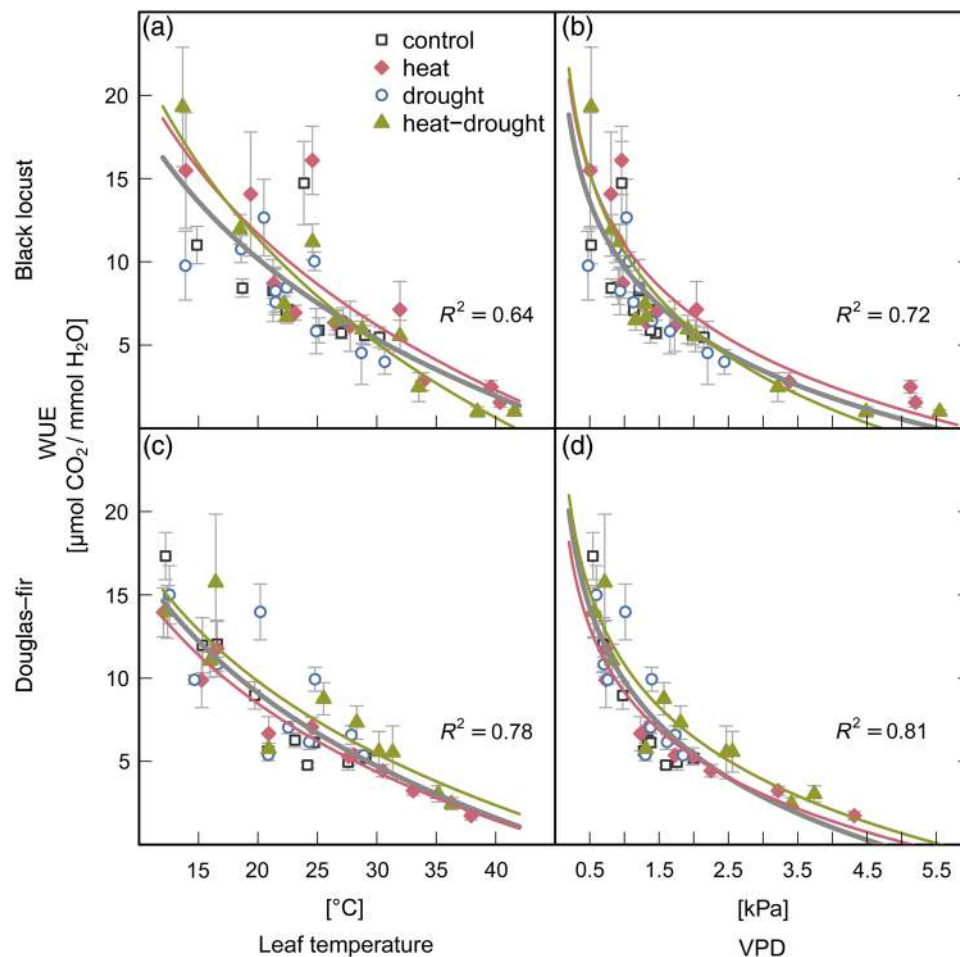


Figure 7. Relationship of WUE ($= A_{\text{sat}}/T_{\text{leaf}}$) with leaf temperature (a and c) and VPD (b and d) for black locust and Douglas-fir. Shown are treatment averages with standard errors of each measurement campaign. The relationship of WUE with temperature and VPD is explained by logarithmic functions and is shown for all treatments combined, as well as separately for the heat and heat-drought treatments. The coefficients of determination (R^2) of the relationships are given. Note that the regression of WUE with VPD in heat-drought treated Douglas-firs differed significantly from the control ($P < 0.01$).

impairment of the photosynthetic apparatus (Ameje et al. 2012). A lack of stomatal response to high temperature and VPD may be explained by intensive watering (in Ameje et al. (2012), trees were irrigated four times a day to field capacity) preventing soil and tree water deficit even at a high rate of transpirational water loss. This agrees well with a study on *Thuja occidentalis*, in which the largest stomatal conductance was found in the high temperature treatment as long as water supply was sufficient, followed by a sharp decline with soil water depletion (Zhao et al. 2013). Another heat wave experiment on oak trees grown under reduced irrigation showed, similar to our results, that the overall conductance of heat-treated trees decreased (Bauweraerts et al. 2013). However, a different diurnal picture emerged during the heat waves with high conductance rates in the mornings followed by decreases in the afternoons (Bauweraerts et al. 2013), something we did not observe in our own study. Such contrasting patterns indicate that a detailed understanding of stomatal behaviour to the

combined effects of extreme temperature and water deficit is needed, in order to predict photosynthetic responses of trees to heat waves.

In both species, we found stem growth to cease during heat, and markedly so when coupled with drought stress. A dominant impact of water availability on tree growth during heat waves has recently been confirmed in pine and oak trees (Bauweraerts et al. 2014). The reasons could be direct weakening of cell expansion and division due to reduced water potential (Hsiao 1973), as well as a decrease in available C due to low photosynthesis and increased respiration rates (Ameje et al. 2012, Bauweraerts et al. 2014). A close link between assimilation and growth in plants is well known (Smith and Stitt 2007), but growth has also been shown to decline faster than photosynthesis in response to water deficit (Muller et al. 2011). Thus, it is not clear if the relationship between assimilation and stem diameter increment, as found in our study, points towards C limitation of growth or just indicates that similar environmental drivers exist.

Recovery and acclimation

We found that heat, especially in combination with drought, evoked stress responses that lasted longer than the actual duration of the extreme event. Most interestingly, we noted the absence of treatment effects on stem expansion in Douglas-fir at the end of the experiment, which was accompanied, however, by different growth rates during and after stress. While stem growth declined during the heat waves, it continued in previously stressed trees until September, when it had already ceased in the control. Diameter growth patterns in Douglas-fir from the Northwest Pacific seem to be relatively plastic, as stem increment has been reported to culminate between August and September (Lassoie 1979, Devine and Harrington 2011), but was found to last until October in mature trees that experienced growth reductions during the summer drought season (Beedlow et al. 2013). However, a compensation of stress-induced stem-growth decline under field conditions was not apparent, as growth rates following drought release were much lower. Nevertheless, changes in growth patterns and allocation following stress have been reported in other field studies and other species: for example, an acceleration of root growth after drought stress was reported in balsam fir trees (Olesinski et al. 2011). Such post-stress growth compensation might be at the expense of other processes if C uptake does not increase simultaneously, thereby resulting in lagged effects that are eventually more difficult to detect (Walter et al. 2013).

The capacity of Douglas-fir to deal with heat stress also became clear in the quick recovery of photosynthesis. Indeed, Douglas-fir seems to be well adapted to heat, as revealed by changes in stress-protecting compounds that indicate a metabolic acclimation to high temperatures (Jansen et al. 2014). An apparent acclimation to water deficit was visible in Douglas-fir, with decreased stomatal conductance accompanied by increased WUE in the heat–drought treatment, continuing past the actual stress period. Increase in WUE as a result of reduced stomatal conductance and transpiration during post-stress recovery has also been observed in studies on bean plants (Miyashita et al. 2005) and beech saplings (Gallé and Feller 2007) in the weeks following a period of drought. It remains to be tested whether or not such an acclimation lasts into the following growing season.

Black locust trees were affected more severely by the experimental conditions because soil water content reached much lower levels than in Douglas-firs, and full recovery was not observed during the experimental period. While tree water deficit refilled instantaneously upon re-watering, and leaf-level stomatal conductance recovered, photosynthesis in the heat and heat–drought treated trees did not reach control values, pointing towards irreversible photoinhibition. The observed increase in C_i/C_a at very low stomatal conductance confirms this assumption. In a meta-analysis on C_3 species, Flexas and Medrano (2002) found that irreversible damage to the photosystem, marked by the C_i inflexion point, can be expected at stomatal

conductance values below $0.05 \text{ mol m}^{-2} \text{ s}^{-1}$, similar to what we have observed in our study. Such lasting impairment of photosynthesis has also been observed in other deciduous tree species recovering from heat (Ameje et al. 2012) and drought (Gallé and Feller 2007). As a consequence, we did not observe an acclimation in WUE during or following water limitation, in line with another study on black locust trees exposed to drought (Mantovani et al. 2014). Nevertheless, because reductions in leaf area were not compensated for, water demand (i.e., tree transpiration) of previously heat–drought stressed black locust trees remained significantly below control rates during the remainder of the growing season. This acclimation to low water availability (Bréda et al. 2006) reduces C uptake and growth potential, with as yet unknown consequences for the tree's resilience in future stress events.

Conclusions

Two consecutive summer heat waves that resulted in $+1 \text{ }^\circ\text{C}$ annually had large impacts on C and water relations in Douglas-fir and black locust, with stress severity being clearly affected by water availability, evaporative demand and temperature. Further, by studying heat and drought individually as well as in combination, we observed that combined heat–drought effects were mostly larger than the sum of single stresses. Interestingly, acclimation processes relating to water savings became apparent in the heat–drought treatments, caused either by increases in WUE (Douglas-fir) or reductions in leaf area (black locust). Post-stress responses of trees revealed that stress severity affected both the timing and amount of recovery, but also that compensation for, or offsetting of, growth suppression following heat stress might be possible. In summary, our study clearly showed the paramount importance of water availability during heat, and confirmed that plant responses to combined heat–drought stress are not simply the sum of single stresses. Because heat waves and drought naturally co-occur, reliable predictions of plant responses to a future climate can only be made if changes in temperature and water availability are studied in parallel, both during and beyond an extreme event.

Supplementary data

Supplementary data for this article are available at *Tree Physiology* Online.

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Conflict of interest

None declared.

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