

# Heat waves imposed during early pod development in soybean (*Glycine max*) cause significant yield loss despite a rapid recovery from oxidative stress

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## Abstract

Heat waves already have a large impact on crops and are predicted to become more intense and more frequent in the future. In this study, heat waves were imposed on soybean using infrared heating technology in a fully open-air field experiment. Five separate heat waves were applied to field-grown soybean (*Glycine max*) in central Illinois, three in 2010 and two in 2011. Thirty years of historical weather data from Illinois were analyzed to determine the length and intensity of a regionally realistic heat wave resulting in experimental heat wave treatments during which day and night canopy temperatures were elevated 6 °C above ambient for 3 days. Heat waves were applied during early or late reproductive stages to determine whether and when heat waves had an impact on carbon metabolism and seed yield. By the third day of each heat wave, net photosynthesis (*A*), specific leaf weight (SLW), and leaf total nonstructural carbohydrate concentration (TNC) were decreased, while leaf oxidative stress was increased. However, *A*, SLW, TNC, and measures of oxidative stress were no different than the control ca. 12 h after the heat waves ended, indicating rapid physiological recovery from the high-temperature stress. That end of season seed yield was reduced (~10%) only when heat waves were applied during early pod developmental stages indicates the yield loss had more to do with direct impacts of the heat waves on reproductive process than on photosynthesis. Soybean was unable to mitigate yield loss after heat waves given during late reproductive stages. This study shows that short high-temperature stress events that reduce photosynthesis and increase oxidative stress resulted in significant losses to soybean production in the Midwest, U.S. The study also suggests that to mitigate heat wave-induced yield loss, soybean needs improved reproductive and photosynthetic tolerance to high but increasingly common temperatures.

**Keywords:** heat stress, heat waves, oxidative stress, photosynthesis, soybean, yield

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## Introduction

Heat waves can dramatically impact crop and forest productivity (Ciais *et al.*, 2005; Bauweraerts *et al.*, 2013; Filewod & Thomas, 2014). In 2003, a heat wave in Europe caused an estimated 13 billion dollars in agricultural loss (Parry *et al.*, 2007). During a heat wave that covered most of eastern Russia in 2010, wildfires emitted 10 Tg of CO<sub>2</sub> (Konovalov *et al.*, 2011). Heat waves are predicted to increase in frequency and intensity with global climate change (Meehl & Tebaldi, 2004; Seneviratne *et al.*, 2012). However, few experimental efforts have been made to investigate how heat waves

impact crop physiology and quantify their impact on yield.

Soybean (*Glycine max*) is the fourth most important commodity crop globally and is grown on over 67 million acres of the United States (National Agricultural Statistics Service, 2011). Soybean is sensitive to periodic temperature increases and in the Midwestern United States may currently be growing close to or even above its average optimum seasonal temperature (Ferris *et al.*, 1999; Lobell & Asner, 2003; Kucharik & Serbin, 2008). Additionally, soybean reproductive development typically has a lower temperature optimum (ca. 26 °C) than vegetative growth (ca. 30 °C; Hatfield *et al.*, 2011). Thus, heat waves both current and future can be expected to have large physiological and economic effects on soybean.

There are a number of ways that heat wave temperatures might affect plant productivity. In soybean, two of the processes that are the most sensitive to

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temperature and central to yield and productivity are net photosynthesis ( $A$ ) and pod set (Zinn *et al.*, 2010). Photosynthesis can be affected by temperature in direct or indirect ways. Indirectly, high-temperature events increase the vapor pressure deficit and evaporative demand (DeBoeck *et al.*, 2010), which more rapidly depletes soil moisture causing stomatal closure, and leading to decreased rates of  $A$ . The largest direct high-temperature effect on C3 plants such as soybean is the decrease in net  $A$  due to the increase in the rate of photorespiration at elevated temperature. Higher temperatures alter the enzymatic properties of rubisco and decrease the solubility of  $\text{CO}_2$  relative to  $\text{O}_2$ . Both of those factors favor increased oxygenation of ribulose-1,5-bisphosphate (RuBP) by rubisco at elevated temperature (Badger & Andrews, 1974). Rubisco activase can also be directly affected by elevated temperature and has been linked to declines in photosynthesis at high temperatures (Salvucci & Crafts-Brander, 2004). The permeability of the thylakoid membrane increases with temperature, which compromises the membrane's ability to maintain transmembrane ion gradients necessary for photosynthesis (Schrader *et al.*, 2004). The potential for damage to the water evolving complex and photosystem II (PSII) is also greater as temperatures increase beyond optimum, which can be detected as a decrease in the operating efficiency of photosystem II ( $\phi\text{PSII}$ ) using fluorescence techniques (Baker, 2008). The hardiness of PSII and rubisco activase to resisting temperature stress is species dependent, but generally the operating efficiency of PSII begins to decline above 35 °C in nearly all species (Gamon & Pearcy, 1990).

The transition from a fertile flower to a developing pod is known as pod set. As in all legumes, pod set in soybean is highly sensitive to temperature and photoassimilate availability (Boote *et al.*, 2005). The rates of pod set and seed growth in soybean are relatively consistent from 22 to 30 °C but decrease sharply at higher temperatures (Egli & Wardlaw, 1980).

Many temperate plants have an extensive capacity to mitigate damage during and recover from high temperature events. High temperatures have been observed to stimulate the development of flowers and pods on secondary and tertiary inflorescences in soybean and thus moderate yield reduction (Zheng *et al.*, 2002; Koti *et al.*, 2005). Furthermore, soybean has an extended flowering period producing flowers for 20–30 days (Smith *et al.*, 1988; Castro *et al.*, 2009), which implies a potential for yield recovery following a heat wave by increased pod set. Photosynthetic and respiratory acclimation can also occur in response to high temperatures (Rosenthal *et al.*, 2014). This acclimation can include but is not limited to: decreased daytime respiration, increased

electron transport capacity, and the synthesis of a more heat stable rubisco activase (Sage & Kubien, 2007; Way & Yamori, 2014). Increased oxidative stress, which is frequently associated with high-temperature events, is expected to slow the repair of the photosynthetic machinery (Nishiyama *et al.*, 2001). Although recovery is not instantaneous, damage to PSII usually recovers after heat stress.

We hypothesized that if leaf temperatures within the heat wave treatments consistently exceed optimal temperatures, then (i) photosynthesis will be decreased in the short term due to stomatal closure and increasing the proportion of photorespiration relative to carbon assimilation, and (ii) damage, which compromises  $\phi\text{PSII}$ , will cause reductions in carbon assimilation that will persist for a period after the end of the heat wave and affect season-long yield. Also, given the length of the heat wave treatments relative to the length of the developmental periods over which they were applied, (iii) any damage caused to reproductive structures or processes during the heat waves will have negligible impact on yield due to the long flowering period for soybean and its consequent ability to compensate for yield loss. To investigate these predictions, we elevated plant canopy temperatures in field-grown soybean by 6 °C for 72-h periods at discrete crop developmental stages over two growing seasons to determine the effects of heat waves in central Illinois on soybean physiology and productivity.

## Methods

### Site and heating infrastructure description

Field-grown soybean (*Glycine max* cv. Pioneer 93B15) was exposed to three heat waves during 2010 and two heat waves during 2011 using arrays of infrared lamps at the SoyFACE research facility located on the University of Illinois research farm, Savoy, IL, USA. The 32-ha field site is planted half in corn and half in soybean, and these crops are rotated yearly. The site is tile-drained and employs standard agronomic practices for the region. Soybeans were planted in 0.75-m row spacing at ~350 000 plants per hectare in 2010 and 0.4-m row spacing at ~350 000 plants per hectare in 2011. Within a linear, 1-m stretch of a 0.75-m spaced row in 2010, there was an average of 27 plants. In 2011, there was an average of 17 soybean plants in a linear, 1-m stretch of 0.4-m spaced rows.

The IR heating method in this experiment was modified from Kimball (2005) and fully described in Ruiz-Vera *et al.* (2013). The circular heated area of each heat wave subplot was 7 m<sup>2</sup>. In 2010, the heat wave treatments covered a width of 5–6 rows of plants (0.75-m row spacing) and 11–13 rows in 2011 (0.4-m row spacing). Heaters (Salamander Ceramic Infrared Heaters with Aluminum Extrusion Reflector Assembly Housings; Mor Electric Heating Association Inc., Comstock Park, MI, USA) were hung in a hexagonal

arrangement 1.2 m above the canopy and mounted at a 45° angle facing into the plot. Each heater contained four ceramic heating elements (Mor-FTE 1000W heaters; Mor Electric Heating Association Inc.). Two infrared radiometers (IRRs) (SI-121; Apogee Instruments, Inc., Logan, UT, USA) were positioned in each block at the height of the heaters and monitored the canopy surface temperature in the heat wave plot and the control plot. The IRR canopy temperature data were relayed to a central computer. The electrical output to the heaters was continuously modulated to maintain surface canopy temperatures in the heat wave plots at the target temperature difference using a PID-controlled dimmer system (Model LCED-2484, 240V, 35A; Kalglo Electronics Co., Inc., Bethlehem, PA, USA).

The experiment was a randomized complete block design ( $n = 3$  in 2010,  $n = 4$  in 2011). In 2010, when heat waves were applied at three different crop developmental stages, each block contained four subplots: the control subplot and three treatment subplots (one for each of the three heat wave treatments). The subplots were arranged side by side. The order of the subplots was randomized within each block. An additional 4.6-m-wide row of soybean at the same row spacing was planted around each rectangular block as a buffer zone. In 2010, this consisted of 6 buffer rows of plants at 0.75-m row spacing and 12 buffer rows of plants at 0.4-m row spacing in 2011. Blocks were designed the same way in 2011, but two instead of three heat wave treatments were conducted.

The heating infrastructures were relocated during the growing season to the different heat wave plots, such that each set of subplots was heated only a single time but at different developmental stages. Heaters and IRRs were installed 1 week prior to each heat wave and removed 3 days after the heat wave ended. Table 1 provides the timing of each heat wave for both years. Heat wave one in 2010 is referred to as Wv10.1, the second as Wv10.2, and the third as Wv10.3. The two heat waves in 2011 are designated as Wv11.1 and Wv11.2.

### *Establishing the characteristics of a historically representative heat wave*

To define a heat wave in central Illinois, 30 years of historical weather data from June, July, and August were analyzed to identify a target temperature and duration that

qualified as a realistic heat wave for central Illinois. We determined the frequency of days that were 2–8 °C above the 30-years mean for that month (MRCC Applied Climate System; <http://mrcc.isws.illinois.edu/MACS/>). Temperature aberrations that were 7 or 8 °C above the 30-years mean were too infrequent to be considered historically representative. Over the 30-years period from 1970 to 2000, <1% of days in June, July, or August experienced a day with 7 or 8 °C above the 30-years mean. Events where the temperature was 5 degrees above the 30-years mean were deemed too common: ca. 10% of days in June, July, and August from 1970 to 2000 had mean daily temperatures 5 °C above the monthly mean. Focusing on events where the temperature was 6 °C above the monthly mean, 2-day-long heat waves were deemed too frequent. There were 18 occasions between 1970 and 2000 when there was a 2-day-long 6 °C heat wave, meaning there was a better than 50% chance each year of experiencing such an event. Three-day-long 6 °C heat wave events occurred approximately once in every 5 years and were thus qualified as a representative heat wave for central Illinois.

During each heat wave treatment, infrared heaters were turned on at 4:30 hours. The target temperature for heated subplots was 6 °C above ambient temperatures except under two circumstances. First, if the canopy temperature dropped below the Fourier-transformed 30-years mean at any time during a heat wave treatment, the canopy temperature was elevated 6 °C above the 30-years mean. This ensured credible heat wave treatments even on days that were cooler than average, which happened on day two of the second heat wave in 2010 (Wv10.2). Second, to ensure that leaves or plants were not killed, heat wave subplots were not heated above 40 °C. This upper limit temperature threshold was not necessary to invoke during any heat wave reported in this study.

### *Development*

In 2010, the developmental stage of five plants in each plot was measured 2 days before and 2 days after each heat wave. In 2011, five plants were tagged in each plot, and their development was tracked over the course of the growing season. Observations were made every 2–3 days. The vegetative and reproductive stage classifications followed Fehr & Caviness (1977).

### *Pod painting*

In 2011 to track temporal pod development throughout the canopy, ten neighboring plants within the area designated for final harvest in the control and Wv11.2 subplots were flagged for pod painting. Beginning at R2, each flagged plant was checked for newly developed pods every 2–3 days. Pods were marked during early development (pod length = 10 mm) and dotted with water-based acrylic paint on the proximal and distal tips (Egli & Bruening, 2006). The two colors used to paint each pod represented the number of days the pod entered early development after the beginning of full flowering (R2). For example, white was used to represent the number zero and dark blue

**Table 1** The reproductive timing of each heat wave and its abbreviation used in the text

Year	Start Date/ (DOY)	Vegetative stage	Reproductive stage	Name
2010	July 3 (184)	V6	10% of plants in R1	Wv10.1
2010	July 27 (208)	V14	R3 (beginning pod)	Wv10.2
2010	Aug 13 (225)	V18	R5 (seed fill)	Wv10.3
2011	July 14 (195)	V7	10% of plants in R1	Wv11.1
2011	Aug 19 (231)	V15	R4 (full pod)	Wv11.2

was used for the number nine. At final harvest, a pod bearing a white acrylic mark on its proximal tip and a dark blue mark on its distal end reached 10 mm and had been marked 9 days after the beginning of R2. Additionally, the node placement and height was recorded for each painted pod.

### Gas exchange and chlorophyll fluorescence

Midday and diurnal gas exchange and chlorophyll fluorescence measurements were taken *in situ* on the youngest most fully expanded leaf using open-path infrared gas analyzers (LI-COR-6400, LiCOR, Lincoln, NE, USA) equipped with fluorescence heads. Midday gas exchange and accompanying chlorophyll fluorescence measurements were taken between 11:30 and 13:00 hours on the day preceding each heat wave (day 0), the first day of the heat wave (day 1), the final day of each heat wave (day 3), and the day after the heat wave (day 4/recovery). Midday gas exchange data for these time points were extracted from diurnal gas exchange data taken on days 3 and 4. Diurnal gas exchange and leaf fluorescence measurements were taken on days 3 and 4 to assess recovery. Due to rain, the Wv11.2 recovery diurnal was carried out 2 days after the end of the treatment. Photosynthetic photon flux density (PPFD) in the leaf chamber was adjusted to match ambient light intensity, which was measured at an on-site weather station described previously (Leakey *et al.*, 2004). Block temperatures were adjusted before each time point so that leaf temperatures within the gas exchange chambers matched average values measured by the IRRs in each plot. The measurement chamber relative humidity ranged between approximately 50% and 70%.

In 2011, photosynthesis vs. intercellular  $[CO_2]$  ( $A/C_i$ ) curves were measured using the same instrumentation described for midday and diurnal gas exchange measurements. The youngest most fully expanded leaves from 2 plants per plot were harvested predawn and then taken back to the laboratory, where the petioles were recut under water and stored in low light with the recut petiole in water until measured. This protocol was favored over in field measurements on attached leaves because of the greater precision of the laboratory measurements and to ensure that any photoinhibition or downregulation of photosynthesis due to photoprotection was fully relaxed. Leaves were allowed to acclimate (1700  $\mu\text{mol}$  PPFD for 10 min) before beginning  $A/C_i$  measurements. The light level for all measurements was 1700  $\mu\text{mol}$  PPFD, and the block temperature of the gas exchange chamber was set to 25 °C. Plots of  $A$  vs.  $C_i$  were used to solve for  $V_{\text{cmax}}$  and  $J_{\text{max}}$  using the equations of Farquhar *et al.* (1980).  $CO_2$  concentrations in the leaf chamber were adjusted in the following order: 400, 300, 200, 100, 50, 400, 600, 800, 1000, 1200, 1500 ppm. When necessary, measurements were corrected to leaf temperatures of 25 °C using the temperature responses of Bernacchi *et al.* (2001).  $\phi\text{PSII}$  was measured and calculated as described by Baker (2008).

### Respiration

In 2011, the dark-adapted rate of leaf respiration was measured as  $CO_2$  efflux *in situ* from the youngest most fully

expanded leaf on the second night of Wv11.1 (DOY 195) and Wv11.2 (DOY 233). Measurements started 2 h after sunset and were completed by 2:00 hours. Two subsamples were measured per plot. Whole-leaf respiration rates were measured with LiCor 6400 instruments using a customized leaf chamber that enclosed an entire trifoliolate (Gillespie *et al.*, 2012). Leaf temperatures inside the chamber were kept at the temperature measured by the IRR for the control or heat wave plots. After leaf respiration reached a uniform rate, approximately 10 min after clamping onto a leaf, the values were logged and the measured leaf was tagged, removed from the plant, and stored in a sealed plastic bag for leaf area determination. Immediately after the gas exchange measurements, trifoliolate area was determined (LiCOR-3100 leaf area meter; LI-COR, Inc.) and then used to normalize respiration rates to leaf area.

### Leaf water potential

Leaf water potential (WP) was measured on three 0.5-cm<sup>2</sup> leaf disks collected from the youngest fully expanded leaf. Samples were taken at the same time as midday gas exchange measurements. Leaf disks were sealed into steel chambers containing thermocouple psychrometers (C-30, Wescor Inc., Logan, UT, USA) and taken back to the laboratory where they equilibrated at 25 °C (Leakey *et al.*, 2006). After thermal equilibration, WP was measured using an integrated dew point micro-voltmeter (HR-33T; Wescor) after which the chambers were submerged in liquid nitrogen for 60 s and the thermal equilibration and measurement were repeated to determine osmotic potential (OP). The turgor potential (TP) was calculated as WP–OP. The thermocouples were calibrated independently each year using sucrose solutions ranging in concentration from 0 to 1.60 M; this curve was used to correct raw data from the thermocouple psychrometers.

### Soil moisture

Soil volumetric water content was measured in 2010 and 2011 using a time domain reflectometry soil moisture meter with 20-cm rods (Field Scout TDR 300, Spectrum Technologies Inc., Aurora, IL, USA). Soil moisture was measured the day preceding, the first day, the third day, and the day after each heat wave. On each of these days, six soil moisture readings were taken per subplot (three between row and three within row) between noon and 14:00 hours.

### Tissue biochemical analysis

Specific leaf weight (SLW; g m<sup>-2</sup>) was determined on three leaf disks sampled at midday, dried in an oven at 50 °C for 1 week, and weighed. Leaf disks were always sampled from the middle leaflet, avoiding the mid-vein. Leaf total nonstructural carbohydrates (TNC) was determined using ~30 mg of tissue from single leaflets of five plants per plot that were combined and ground in liquid nitrogen.

Glucose, fructose, and sucrose were extracted in ethanol and measured as glucose equivalents with a continuous enzymatic substrate assay (Jones *et al.*, 1977). Starch content in the

remaining pellets of the ethanol extracts was converted to glucose by incubation with exo- and endoamylases (Geigenberger *et al.*, 1996). Starch was subsequently measured as glucose equivalents. For analysis of ascorbate content, one leaf disk (1.34 cm<sup>2</sup>) was sampled from 3 plants per plot and immediately frozen in the field in liquid nitrogen and then stored at -80 °C. Leaf ascorbate content, including reduced, oxidized, and total ascorbate, was determined using the methods of Gillespie & Ainsworth (2007).

### *Final harvest*

In 2010 and 2011, soybean plants were harvested from rectangular, two-row sections of each subplot (0.6 m<sup>2</sup>) that were not touched by destructive sampling during the season. There were two buffer rows of plants between edge of the treatment and the area that was sampled for final harvest. On each plant, the number of pods was counted and the stems and pods were separated. The tissues were dried in an oven at 65 °C for a week and weighed, and then, the pods were mechanically threshed to determine seed yield. Two hundred randomly selected seeds were counted out from each subplot and weighed to determine average individual seed weight. In addition, to examine yield components by height in 2011, ten extra plants from Wv11.2 and the control plots were harvested. The pods in each 10-cm increment were counted and dried, and the seeds were counted and weighed.

### *Statistics*

Analyses were performed on subplot means ( $n = 3$ , 2010;  $n = 4$ , 2011) in SAS using the MIXED procedure. Heat waves were analyzed independently as a mixed model ANOVA. In all cases, block was considered a random effect and the effect of a heat wave was fixed. For development and midday gas exchange data, DOY was included in the model as a repeated measure. Also, for the temperature profile and yield component by height data, measurement height was included in the model as a repeated measure. *In situ* gas exchange data and temperature profile data were analyzed separately for each DOY. *T*-tests were performed on SLW, TNC, and yield measurements.

## **Results**

### *Background meteorological conditions were variable and canopy heating treatments were successful, affecting leaf water potential only when soil moisture was low*

During each heat wave, the infrared heating system successfully elevated canopy temperatures. Wv10.1, Wv10.3, Wv11.1, and Wv11.2 were all within 15% (i.e.,  $\pm 0.9$  °C) of the target temperature difference 85% of the time. Wv10.2 was not controlled as well as the other heat waves. It was within 15% of the target 47% of the time. However, Wv10.2 was still within 2 °C of the target temperature ~70% of the time. Nighttime tempera-

tures were lower than the Fourier-transformed 30-years mean (see Methods) during Wv10.1 and Wv10.2, leading to nighttime temperature differentials of 10 °C during Wv10.1 and 8 °C during Wv10.2 (Table 2). The larger canopy volume during Wv10.2 relative to Wv10.1 and the lower-than-average nighttime temperatures contributed to the less successful application of the Wv10.2 heat wave treatment, as the IR heaters had difficulty maintaining greater canopy temperature differentials.

Soil moisture was only affected by Wv10.1, when the heaters at 1.2 m above the canopy were the closest to the ground and the row spacing was wider resulting in more soil infrared absorption than any of the other heat waves. The decreased soil moisture during Wv10.1 did not lead to changes in leaf water potential (Table 3). Midday leaf water potentials were affected in two of the five heat waves, both of which coincided with the driest periods of the growing seasons (Table 3). Compared to the control, leaf water potential was significantly reduced by 0.29 MPa on the day 3 of Wv10.3 ( $P = 0.0082$ ) and 0.16 MPa on day 3 of Wv11.2 ( $P = 0.02$ ).

### *Heat waves reduced primary metabolism, but the reductions were transient*

Midday gas exchange measurements in 2010 and 2011 were similar in that there were consistent reductions in  $A$  and  $g_s$  during the heat wave treatment later in the season when background conditions were hotter and drier (Fig. 1, Table 3). However, even when the heat wave treatments caused reductions in midday  $A$ , there was typically full recovery of photosynthesis on the day following the heat wave. During Wv10.3, there was a significant day  $\times$  treatment effect on  $A$ ,  $g_s$ , and  $\phi$ PSII (Fig. 1j, k, l). These parameters all decreased during Wv10.3, but then recovered completely by the day following the heat wave treatment.

The heat wave caused reductions in  $\phi$ PSII only during Wv10.3 and Wv11.2, which coincided with the hottest and driest portions of the growing seasons. The  $\phi$ PSII reduction was significant during Wv10.3 and Wv11.2, and multiple statistical comparisons of the least squared means showed that there were no differences between the heated and control plots at midday on the day following the heat waves (data not shown). Furthermore, any differences in  $\phi$ PSII at midday were transient and did not persist on day four, the day after the heat wave (Fig. 1d, h, l, p, t).

Reductions in  $A$  are typically associated with decreases in SLW (Oren *et al.*, 1986) and that was the case in our study. SLW was significantly decreased ( $P < 0.1$ ) on the first and third day of each heat wave but was never significantly different from the control

on the day after the heat wave (Fig. 2c, f, i, l, o). This included an initial average decrease of 13% on the first day of the treatment relative to control. By the last day of the heat wave, the decreases in SLW were less pronounced, but still significant. Therefore, it was unlikely that permanent structural changes in the leaf were altered by the heat wave. To investigate the mechanism underlying the decrease in SLW, we monitored changes in leaf carbohydrate content throughout the heat waves. Decreased amounts of leaf total nonstructural carbohydrate (TNC) content were observed in all five of the heat waves (Fig. 2a, d, g, j, m). Similar to the changes for SLW, the decrease in TNC content was evident only during the heat wave treatment and again returned to control levels by the day following the conclusion of the heat wave.

*Rapid change in oxidative stress and primary metabolism were followed by a quick recovery*

To assess the impact of the heat waves on the oxidative stress status of leaves, we quantified the concentration of reduced and oxidized ascorbate in the leaf. Although ascorbate levels are not a comprehensive monitor of all aspects of plant oxidative stress, ascorbate is a major antioxidant that plants use to detoxify harmful reactive oxygen species (ROS) generated by oxidative stress. The percent of reduced ascorbate was significantly decreased during every heat wave except Wv10.2 (Fig. 2b, e, h, k, n), indicating that for these heat waves, the treatment was sufficient to induce strong oxidative stress. In addition, total ascorbate levels (i.e., reduced plus oxidized) were decreased by day three in every heat wave except Wv10.2 (data not shown). Similar to SLW and TNC, reduced ascorbate in the heat wave treatments returned to control levels during the day following the heat wave.

*Leaf photosynthetic physiology was altered, but there were no differences in whole-leaf respiration*

To better understand the mechanisms that drove the decrease in  $A$ , SLW, and TNC seen during the heat

waves,  $A$  vs.  $C_i$  curves were measured on day two of Wv11.1 and Wv11.2, and nighttime respirations rates were measured on night two of Wv11.1 and Wv11.2 (Fig. 3a, b). During Wv11.1, there was a significant increase in  $V_{cmax}$  and no change to  $J_{max}$  (Fig. 3a). Wv11.2 saw a reduction in both  $V_{cmax}$  and  $J_{max}$  relative to the control. Nighttime respiration rates were no different than the control in either Wv11.1 or Wv11.2 (Fig 3b).

*Heat waves did not speed or slow development but caused reductions in yield when applied during pod set*

The heat waves in 2010 or 2011 did not alter plant development, neither detectably speeding nor slowing reproductive or vegetative growth. Although the heat waves were applied during a small window of season-long growth, they could span significant portions of a given reproductive stage; for instance, although Wv11.2 spanned only 5% of all reproductive growth (R1-R5), ~30% of R4 occurred during Wv11.2.

There was a consistent ca. 10% reduction in yield for heat waves applied during reproductive phases that coincided with early pod set: 10% during Wv10.2 ( $P = 0.07$ ) and 17% in Wv11.2 ( $P = 0.06$ ) (Table 4). Although the decrease in yield in Wv10.3 was similar in magnitude to Wv10.2, it was not statistically significant ( $P = 0.58$ ) due to increased variance in yield in the Wv10.3 subplots. The decrease in yield for Wv11.2 and Wv10.2 can be attributed to a statistically significant decrease in the number of pods per  $m^2$  of land area (Table 4). Within each year, individual seed weight and the number of seeds per pod were unaffected by the heat wave treatment (Table 4; Fig. 4a, b).

From the ten subsampled plants in the pod painting analysis, there were 1234 pods in the control plots and 1098 pods in the Wv11.2 plots where 90% of those pods had distinguishable marks. There were 1122 marked pods from the control and 998 marked pods in the Wv11.2 plot. The application of acrylic paint used to mark pods had a varied effect on pod development. Compared to the plants used in the

**Table 2** Nighttime and daytime infrared radiometer (IRR) canopy temperatures during each heat wave. Temperatures are reported in degrees Celsius. ' $T_{diff}$ ' is the average temperature difference between the heated and control plots

Wv	Control		Heat wave		$T_{diff}$
	Day	Night	Day	Night	
10.1	23.6 ± 0.8	13.3 ± 0.3	31.3 ± 0.4	23.6 ± 0.1	8.7 ± 0.4
10.2	25.6 ± 0.1	19.1 ± 0.1	32.6 ± 0.02	27.1 ± 0.1	7.4 ± 0.1
10.3	27.8 ± 0.1	21.2 ± 0.1	33.8 ± 0.1	27.4 ± 0.1	6.1 ± 0.2
11.1	27.8 ± 0.4	18.1 ± 0.3	33.7 ± 0.4	25.4 ± 0.1	6.3 ± 0.2
11.2	25.3 ± 0.2	17.6 ± 0.9	31.5 ± 0.3	27.2 ± 1.7	7.0 ± 0.2

**Table 3** Soil moisture concentration and leaf water potential during each heat wave. This table shows the mean soil volumetric water content by percentage (left columns) and the leaf water potential (WP, right columns) in megapascals (MPa) on the day before each heat wave started (day 0) and the last day of each heat wave (day 3) in the control and heat wave subplots as well as the standard error (SE). The ANOVA column tests for statistical differences between the heat wave and the control plot; 'day' indicates a significant effect of day, 'trt' is a significant effect of heat wave, and 'day\*trt' is a significant day by heat wave interaction ( $P < 0.1$ )

Wv	Day	Soil Moisture (%)				Leaf Water Potential (MPa)			
		Control	Heat wave	SE	ANOVA	Control	Heat wave	SE	ANOVA
10.1	Day 0	41.4	40.7	±2.7	Day	-0.71	-0.87	±0.09	-
	Day 3	34.8	32.8		Day*trt	-0.8	-0.85		
10.2	Day 0	31.4	29	±1.5	Day	-0.61	-0.66	±0.05	-
	Day 3	39.4	36.1			-0.69	-0.74		
10.3	Day 0	23.3	22	±2.3	Day	-0.81	-0.76	±0.08	trt
	Day 3	24.1	16.5			-0.77	-1.07		
11.1	Day 0	30.2	31	±4.2	Day	-0.6	-0.61	±0.03	-
	Day 3	30.3	32.3			-0.52	-0.59		
11.2	Day 0	19.3		±1.1		-0.69	-0.7	±0.06	trt
	Day 3	23.2				-0.75	-0.86		

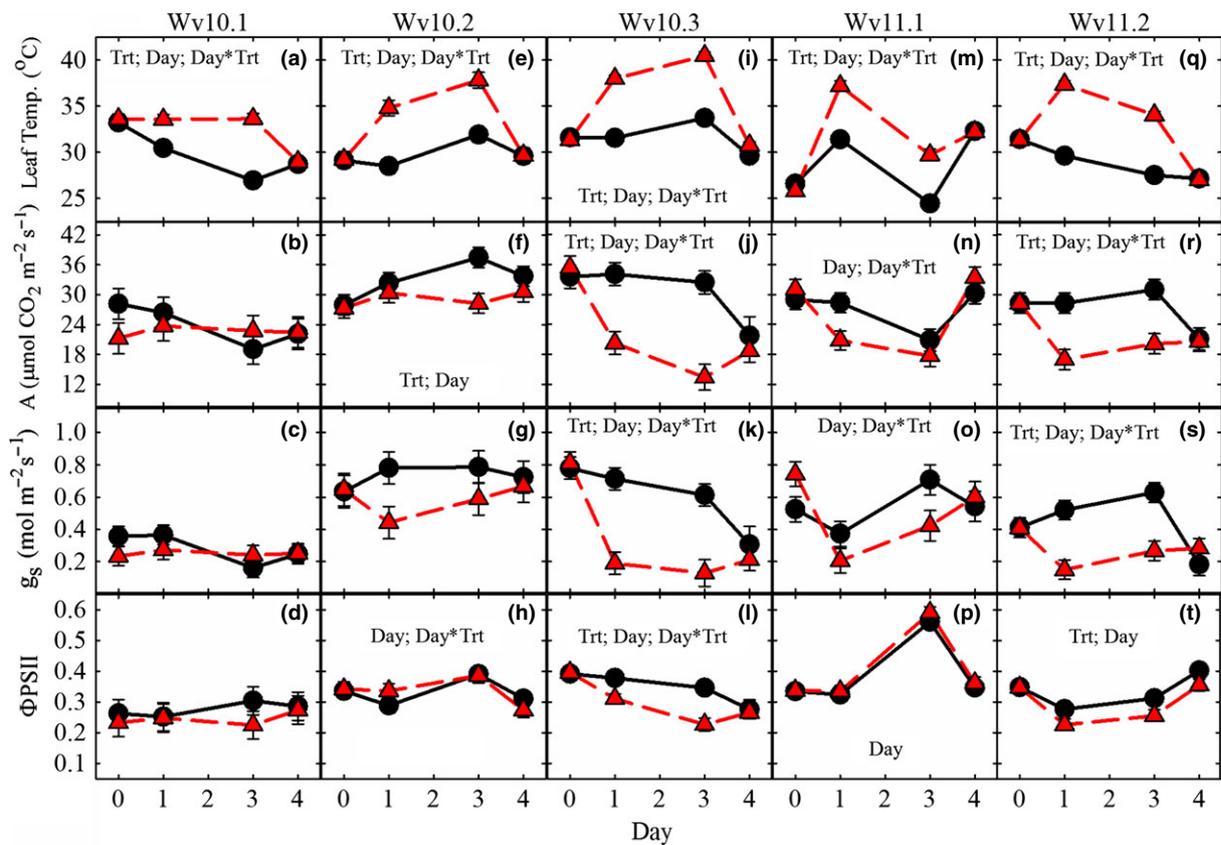
yield component by height analysis, which were not marked with acrylic paint, there were 6.3 fewer pods per plant in the control of the pod painting experiment ( $P = 0.05$ ). However, there was no difference in the number of pods per plant between painted vs. unpainted plants from the heat wave treatment ( $P = 0.24$ ). Plants began setting pods on the lower regions first, closest to the ground, and set the most pods 8–12 days after R2 (Fig. 5a–f). During the Wv11.2 heat wave, 18–21 days after the onset of R2, there was a reduction ( $P = 0.05$ ) in the number of pods produced relative to the control (Fig. 5e). The significant reduction was driven by a 54% decrease in the number of pods 30–50 cm from the ground. No other yield component was significantly affected by Wv11.2 (Table 4; Fig. 4a, b).

## Discussion

This is the first field experiment to use IR heaters to study the effects of a regionally defined heat wave on any crop. A +6 °C, 3-day heat wave applied to soybean strongly impacted leaf physiology (e.g.,  $A$ ,  $g_s$ ,  $\phi$  PSII, TNC, and oxidative stress) especially when background soil moisture levels were low (Wv11.2). In most cases, there was rapid recovery for all these physiological parameters within 24 h of the end of the heat wave.  $V_{\text{cmax}}$  was increased during Wv11.1, but both  $V_{\text{cmax}}$  and  $J_{\text{max}}$  decreased during Wv11.2. Heat waves did not alter the rate of reproductive or vegetative development. However, there were significant reductions in yield when heat waves were applied during reproductive stages that coincided with early pod development. The fact that yield was only reduced during early pod

set, despite large leaf physiological and photosynthetic responses during every heat wave, suggests that a significant portion of the yield reduction was due to effects of the high temperature directly on reproductive processes.

The first hypothesis that photosynthesis would be decreased in the short term in response to heat waves due to reduced stomatal conductance and increasing the proportion of photorespiration relative to carbon assimilation was supported. Except for Wv11.1, midday  $A$  and  $g_s$  were significantly reduced on all days during every heat wave. The greater stimulation of  $A$  by elevated  $[\text{CO}_2]$  observed for the heat wave plants in  $A$  vs.  $C_i$  analysis taken during Wv11.1 and 11.2 (data not shown) confirmed the expected increase in the proportion of rubisco-catalyzed oxygenation relative to carboxylation at higher temperature. The second hypothesis that damage, which compromises  $\phi$ PSII, will cause reductions in carbon assimilation that will persist for a period after the end of the heat wave and affect season-long yield was partially rejected. Oxidative stress and damage to the photosynthetic machinery did not cause a long-term decrease in photosynthesis. Observed reductions to  $\phi$ PSII did not result in any lingering reductions in photosynthesis after the heat waves. The rapid recovery of  $\phi$ PSII after the heat wave treatment was surprising since drought and an increased amount of oxidative stress are expected to slow the repair of the photosynthetic machinery (Nishiyama *et al.*, 2001; Souza *et al.*, 2004) possibly signifying changes in  $\phi$ PSII during heat waves were driven more by photoprotection than by damage to PSII. Overall, the data indicated that reduced  $\phi$ PSII



**Fig. 1** Midday leaf physiological data. Including: leaf temperature (Leaf Temp.; a, e, i, m, q), photosynthetic rate ( $A$ ; b, f, j, n, r), stomatal conductance ( $g_s$ ; c, g, k, o, s), and the operating efficiency of photosystem II ( $\Phi$ PSII; d, h, l, p, t). The columns from left to right are each of the five heat waves in chronological order across the 2 years of the experiment from the beginning of the 2010 to the end of 2011 growing season. Day 0 is the day before the heat wave. Days 1–3 are during the heat wave, and day four is the day after the heat treatment was turned off. The red triangles and the dashed lines are measurements from the heat wave plots. The black circles and solid lines are the control. Text within each graph indicates significant repeated-measures terms ( $P < 0.1$ ). 'Trt' indicates a significant heat wave effect. 'Day' is a significant effect of day, and 'Day\*Trt' notes significant treatment by day interactions.

was only an ancillary component of the impacts of effects heat waves on soybean. Carbohydrate deprivation experiments in soybean suggest that it takes prolonged reductions (7–14 days) in  $A$  to cause pod abortion (Egli & Bruening, 2006). The significant reduction in seed yield during relatively short heat waves suggests that yield loss was due primarily to direct impacts on reproductive processes.

The third hypothesis that any direct damage caused to reproductive structures or processes will have negligible impact on yield due to the long flowering period for soybean and its consequent capacity to compensate for yield loss was partially supported. Reproductive development spanned a long enough period to tolerate heat waves that occurred during early flowering stages (R1) without significant yield loss. However, heat waves had a significant effect on end of season yield when applied during early pod development: Wv10.2 was applied during R3 and Wv11.2 was applied during R4. This observation is consistent with historical

soybean yield data, showing that yield is negatively correlated to temperature increases above the 30-years mean in July and August (Tannura *et al.*, 2008). The simplest explanation for the decrease in seed yield during R3 (Wv10.2) and R4 (Wv11.2) is small pod availability; pod abortion is most sensitive while pods are in early development and less than their maximum size (Egli & Bruening, 2006). Assuming that the pattern of pod development was the same in 2010 and 2011, early heat waves (Wv10.1 and Wv11.1) did not cause yield loss because they were applied during R1, before the development of small pods (e.g., Fig. 5a). Furthermore, the latest heat wave (Wv10.3) did not cause yield reductions during R5 because plants were past the point where they were producing small pods and the treatment duration was not sufficiently long to decrease long-term  $A$  and assimilate availability (e.g., Fig. 5f). These results suggest that short heat waves will have the largest impact on seed yield when they occur during the developmental periods that coincide with the

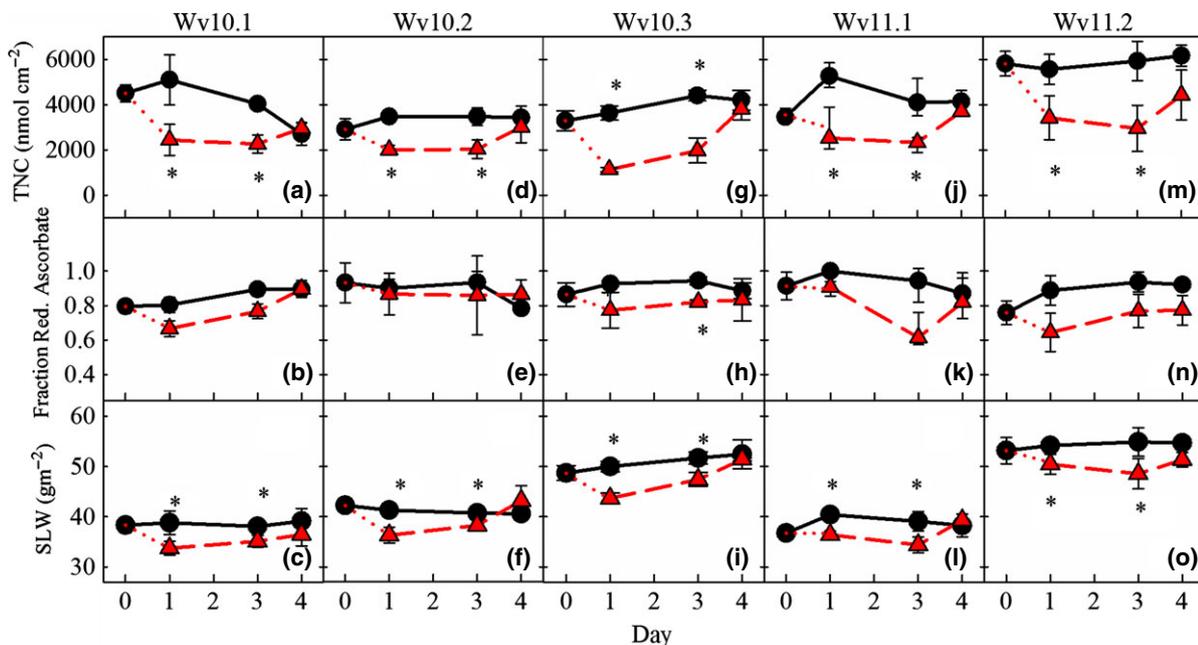


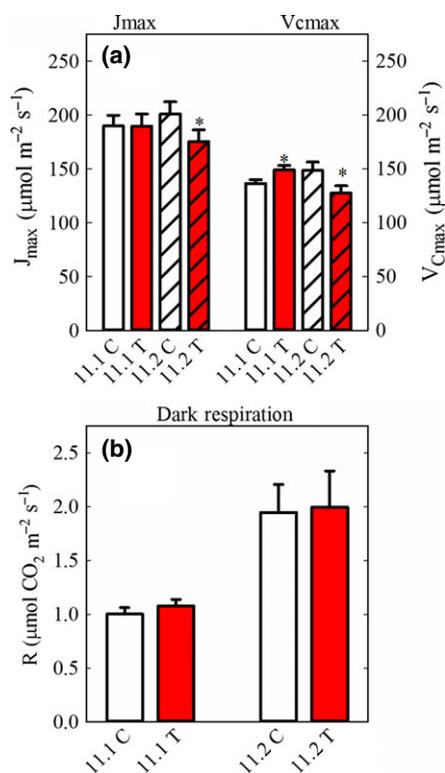
Fig. 2 Heat waves induced changes in total leaf nonstructural carbohydrate (TNC) concentration, the percent of reduced ascorbate, and specific leaf weight (SLW) at midday over the course of the heat wave. The *x*-axis is the day of the heat wave. The black circles are measurements taken from the control plots and the red triangles are from the heat wave plots. Asterisks indicated significant differences ( $P < 0.1$ ) between the heat wave and the control treatment within a day.

greatest rates of small pod development (e.g., Fig. 5c). Another possible explanation for the decrease in yield during R3 and R4 is that early soybean flowers (R1) abort at a much lower rate than flowers that develop later (R3) (Heitholt *et al.*, 1986). Heat wave treatments during early reproductive stages, Wv11.1 and Wv10.1, had no effect on yield. It is possible that plants in early flowering were able to moderate yield loss because they were still setting pods from early flowers, even after the heat wave. However, due to the high abortion rate of later developing flowers, few pods would be able to set after heat waves applied during later reproductive stages.

Soybeans have the ability to compensate for the loss of pods by increasing the seed size (Munier-Jolain *et al.*, 1998), measured in this study as individual seed weight (Table 4, Fig. 5b). In experiments where soybean plants were manually depodded, although there was a decrease in the total number of seeds per plant at final harvest, the individual seed weight of the depodded plants was increased relative to the control (Munier-Jolain *et al.*, 1998). As the heat wave treatments were relatively short and reduced pods without affecting *A* long term, it is somewhat surprising that there was no compensatory increase in seed weight. However, the extent to which soybean can adjust to pod loss is limited and seed weight does not always increase after depodding. For example, after 90% of soybean pods were experimentally removed 6 days after R2, there was no change

in final, individual seed dry weight despite the fact that the sucrose concentrations within the depodded seeds were greater than the control (Egli & Bruening, 2001).

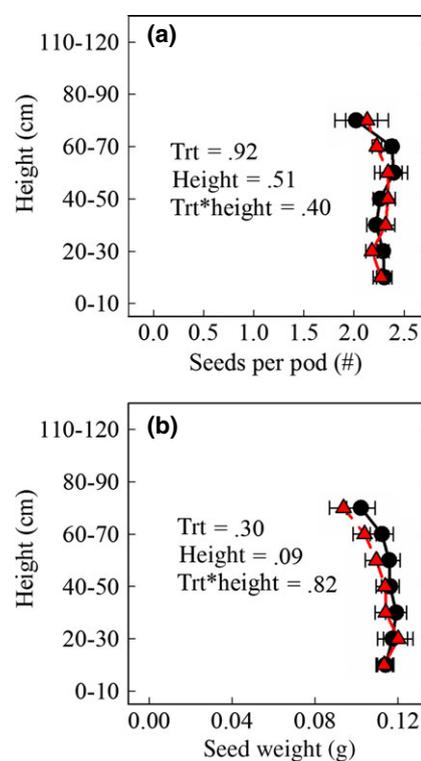
Differential acclimation of photosynthesis may also explain why there was no reduction in yield caused by early reproductive heat waves. Thermal acclimation has been observed within 2 days of exposure to elevated temperatures (Sage & Kubien, 2007). During Wv11.1,  $V_{\text{cmax}}$  was increased compared to the control. However, acclimation of  $V_{\text{cmax}}$  during Wv11.1 did not lead to persistently higher rates of *A* or increased biomass. It is likely and expected that acclimation occurred only in leaves that experienced the treatment and not in leaves that formed after the heat wave. In 2010, the effect of each heat wave on *A* was tracked throughout the season on later cohorts of leaves that did not directly experience the heat wave treatment. During Wv10.3, there was no difference in *A* between the control and plants that experienced Wv10.1 (data not shown). Later in the season, when the acclimated leaves became shaded, photosynthesis would be limited by electron transport (i.e., light limited), negating the impact any persistent increase in  $V_{\text{cmax}}$  would have on *A*. Wv11.1 was applied during V7 (seven fully developed trifoliolate leaves), whereas at the end of the season plants had ca.18 trifoliate leaves on average.  $V_{\text{cmax}}$  may have decreased during Wv11.2 due to the remobilization of nitrogen in rubisco for seed fill (Schiltz *et al.*, 2004).



**Fig. 3** The effect of 2011 heat waves on  $V_{c,max}$  and  $J_{max}$  and nighttime leaf respiration (R). (a) shows  $V_{c,max}$  and  $J_{max}$  values from  $A$  vs.  $C_i$  curves taken during Wv11.1 and 11.2. An asterisk denotes a significant difference from the control ( $P < 0.1$ ). (b) shows the rates of nighttime respiration in the control (C) and heat wave plots (T).

Although long-term  $A$  was not decreased in this experiment, multiple heat waves within a season or longer heat waves have the potential to reduce yield by reducing carbon uptake. The heat waves in this experiment were short compared to the European heat wave of 2003 and the Russian heat wave of 2010 (Fink *et al.*, 2004; Barriopedro *et al.*, 2011). During those events, month-long temperatures were 6 °C higher than average.

Respiration measurements were not taken during each heat wave, but it is clear that elevated nighttime respiration during the heat wave plots could also con-

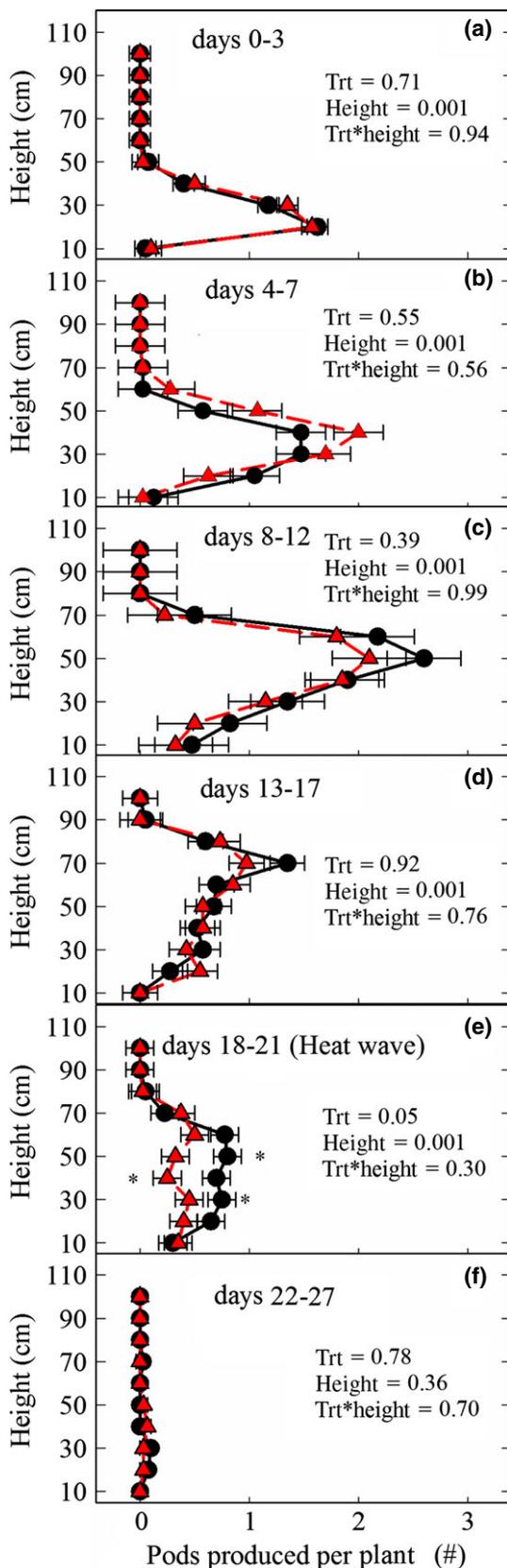


**Fig. 4** Analysis of Wv11.2 yield components at varying height classes. The text within each graph shows the results of a repeated-measures statistical analysis. The black circles depict measurements from the control and the red triangles are from the heat wave plots. An asterisk represents a significant difference between the heat wave and the control treatment within a height class ( $P < 0.01$ ).

tribute to loss in carbon gain. A number of variables including light and water availability can alter leaf respiration rates, but plant mitochondrial respiration responds most consistently to temperature (Atkin *et al.*, 2005). Nighttime respiration is also correlated to daily photosynthesis and soluble sugar and starch concentration (Whitehead *et al.*, 2004). Wv11.1 and Wv11.2 nighttime  $\text{CO}_2$  fluxes were the same in the heated and control plots (Fig. 3b). It is likely that the expected suppression of nighttime respiration rates due to decreases in midday  $A$ , SLW, and TNC in Wv11.1 and Wv11.2

**Table 4** Seed yield and yield component data from each heat wave and its control in 2010 and 2011. The parentheses beside the treatment mean contain the  $P$ -value of the statistical comparison made against the control for that year

Wv	Seed yield ( $\text{g m}^{-2}$ )	Pods per $\text{m}^2$ (#)	Seeds per pod (#)	Seed weight (g)
10c	341.2 $\pm$ 7.3	1065.9 $\pm$ 12	2.76 $\pm$ 0.05	0.139 $\pm$ 0.003
10.1	337.7 $\pm$ 35 (0.93)	1038.7 $\pm$ 48 (0.62)	2.76 $\pm$ 0.04 (0.97)	0.14 $\pm$ 0.009 (0.86)
10.2	306 $\pm$ 9.7 (0.07)	934.4 $\pm$ 48 (0.06)	2.76 $\pm$ 0.02 (0.4)	0.141 $\pm$ 0.007 (0.51)
10.3	313.2 $\pm$ 46 (0.36)	1043 $\pm$ 81.8 (0.79)	2.64 $\pm$ 0.06 (0.18)	0.137 $\pm$ 0.018 (0.9)
11c	340.3 $\pm$ 20	1499.6 $\pm$ 40.2	2.11 $\pm$ 0.03	0.11 $\pm$ 0.004
11.1	345.4 $\pm$ 45 (0.92)	1499 $\pm$ 154 (1)	2.34 $\pm$ 0.22 (0.39)	0.11 $\pm$ 0.004 (0.88)
11.2	280.8 $\pm$ 7 (0.06)	1245 $\pm$ 49.9 (0.02)	1.95 $\pm$ 0.14 (0.29)	0.109 $\pm$ 0.00 (0.84)



**Fig. 5** Spatial and temporal changes in pod set during Wv11.2. Panels a-f are from the beginning of R2 (day 0) to the beginning of R5 (day 27). The heat wave was applied on days 18–21. Statistical tests are the same as in Fig. 4.

was compensated for by the stimulation due to the higher temperature, resulting in no difference in nighttime respiration rates between the control and heat wave plants. Prolonged decreases in  $A$  coupled with enhanced rates of respiration could be one mechanism by which heat waves decrease productivity and yield. Furthermore, when considering future responses to heat waves, plant respiration is increased under elevated  $\text{CO}_2$  (Leakey *et al.*, 2009), but it is uncertain how respiration in plants under elevated  $\text{CO}_2$  will respond to heat waves.

This study illustrates the important impact that short-term high-temperature events can have on plant productivity. Despite rapid recoveries of  $A$ ,  $\phi$  PSII, TNC, and SLW within 24 h of the end of a heat wave, heat waves that coincided with early pod developmental (R3, R4) stages led to a 10% reduction of yield. As climate models predict an increase in the frequency and intensity of heat waves (Meehl & Tebaldi, 2004), it is important to identify the process in plants that need to be adapted to tolerate climate extremes under a variety of field situations. Further research is needed to explain how heat waves affect yield under varying degrees of drought stress as well as interactions with increasing atmospheric  $[\text{CO}_2]$ . High-temperature-tolerant pod set and development in soybean should also be considered a target for introgression to improve capability of salvaging yield after extreme events.

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### References

- Atkin OK, Bruhn D, Hurry VM, Tjoelker MG (2005) The hot and the cold: unraveling the variable response of plant respiration to temperature. *Functional Plant Biology*, **32**, 87–105.
- Badger MR, Andrews TJ (1974) Effects of  $\text{CO}_2$ ,  $\text{O}_2$  and temperature on high-affinity form of ribulose diphosphate carboxylase-oxygenase from spinach. *Biochemical and Biophysical Research Communications*, **60**, 204–210.
- Baker NR (2008) Chlorophyll fluorescence: a probe of photosynthesis *in vivo*. *Annual Review of Plant Biology*, **59**, 89–113.
- Barriopedro D, Fischer EM, Luterbacher J, Trigo RM, Garcia Herrera R (2011) The hot summer of 2010: redrawing the temperature record map of Europe. *Science*, **332**, 220–224.

- Bauweraerts I, Wertin TM, Ameye M, McGuire MA, Teskey RO, Steppe K (2013) The effects of heat waves, elevated [CO<sub>2</sub>] and low soil water availability on northern red oak (*Quercus rubra* L.) seedlings. *Global Change Biology*, **19**, 517–528.
- Bernacchi CJ, Singsaas EL, Pimentel C, Portis AR, Long SP (2001) Improved temperature response functions for models of rubisco-limited photosynthesis. *Plant Cell & Environment*, **24**, 253–259.
- Boote KJ, Allen LH, Prasad PVV *et al.* (2005) Elevated temperature and CO<sub>2</sub> impacts pollination, reproductive growth, and yield of several globally important crops. *Journal of Agricultural Meteorology*, **60**, 469–474.
- Castro JC, Dohleman FG, Bernacchi CJ, Long SP (2009) Elevated CO<sub>2</sub> significantly delays reproductive development of soybean under free-air concentration enrichment (FACE). *Journal of Experimental Botany*, **60**, 2945–2951.
- Ciais P, Reichstein M, Viovy N *et al.* (2005) Europe-wide reduction in primary productivity caused by the heat and drought in 2003. *Nature*, **437**, 529–533.
- DeBoeck HJ, Dreesen FE, Janssens IA, Nijs I (2010) Climatic characteristics of heat waves and their simulation in plant experiments. *Global Change Biology*, **16**, 1992–2000.
- Egli DB, Bruening WP (2001) Source-sink relationships, seed sucrose levels and seed growth rates in soybean. *Annals of Botany*, **88**, 235–242.
- Egli DB, Bruening WP (2006) Temporal profiles of pod production and pod set in soybean. *European Journal of Agronomy*, **24**, 11–18.
- Egli DB, Wardlaw IF (1980) Temperature response of seed growth characteristics of soybeans. *Agronomy Journal*, **72**, 560–564.
- Farquhar GD, von Cammerer S, Berry JA (1980) A biochemical model of photosynthetic CO<sub>2</sub> assimilation in leaves of C<sub>3</sub> species. *Planta*, **149**, 78–90.
- Fehr WR, Caviness CE (1977) *Stages of Soybean Development*. pp. 1–12. Iowa State University of Science and Technology, Ames, IA.
- Ferris R, Wheeler TR, Ellis RH, Hadley P (1999) Seed yield after environmental stress in soybean grown under elevated CO<sub>2</sub>. *Crop Science*, **39**, 710–718.
- Filewod B, Thomas SC (2014) Impacts of a spring heat wave on canopy processes in a northern hardwood forest. *Global Change Biology*, **20**, 360–371.
- Fink AH, Bücher T, Krüger A, Leckebusch G, Pinto J, Ulbrich U (2004) The 2003 summer heat waves and drought – synoptic diagnosis and impacts. *Weather*, **59**, 209–216.
- Gamon JA, Pearcy RW (1990) Photoinhibition in *Vitis californica*: the role of temperature during high-light treatment. *Plant Physiology*, **92**, 487–494.
- Geigenberger P, Lerchl J, Stitt M, Sonnwald U (1996) Phloem-specific expression of pyrophosphatase inhibits long-distance transport of carbohydrates and amino acids in tobacco plants. *Plant Cell & Environment*, **19**, 43–55.
- Gillespie KM, Ainsworth EA (2007) Measurement of reduced, oxidized and total ascorbate content in plants. *Nature Protocols*, **2**, 871–874.
- Gillespie KM, Xu F, Richter KT *et al.* (2012) Greater antioxidant and respiratory metabolism in field-grown soybean exposed to elevated O<sub>3</sub> under both ambient and elevated CO<sub>2</sub>. *Plant Cell & Environment*, **35**, 169–184.
- Hatfield JL, Boote KJ, Kimball BA *et al.* (2011) Climate impacts on agriculture: implications for crop production. *Agronomy Journal*, **103**, 351–370.
- Heitholt JJ, Egli DB, Leggett JE, MacKown CT (1986) Role of assimilate and carbon-14 photosynthate partitioning in soybean reproductive abortion. *Crop Science*, **26**, 999–1004.
- Jones MGK, Outlaw WH, Lowry OH (1977) Enzymatic assay of 10<sup>-7</sup> to 10<sup>-14</sup> moles of sucrose in plant tissues. *Plant Physiology*, **60**, 379–383.
- Kimball BA (2005) Theory and performance of an infrared heater for ecosystem warming. *Global Change Biology*, **11**, 2041–2056.
- Konovalov B, Beekmann M, Kuznetsova IN, Yurova A, Zvyagintsev A (2011) Atmospheric impacts of the 2010 Russian wildfires: integrating modelling measurements of an extreme air pollution episode in the Moscow region. *Atmospheric Chemistry and Physics*, **11**, 10031–10056.
- Koti S, Reddy KR, Reddy VR, Kakani VG, Zhao D (2005) Interactive effects of carbon dioxide, temperature and ultraviolet-B radiation on soybean (*Glycine max* L.) flower and pollen morphology, pollen production, germination and tube lengths. *Journal of Experimental Botany*, **56**, 725–736.
- Kucharik CJ, Serbin S (2008) Impacts of recent climate change on Wisconsin corn and soybean yield trends. *Environmental Research Letters*, **3**, 1–10.
- Leakey ABD, Bernacchi CJ, Dohleman FG, Ort DR, Long SP (2004) Will photosynthesis of maize (*Zea mays*) in the US Corn Belt increase in future [CO<sub>2</sub>] rich atmospheres? An analysis of diurnal courses of CO<sub>2</sub> uptake under free-air concentration enrichment (FACE). *Global Change Biology*, **10**, 951–962.
- Leakey ABD, Uribelarrea M, Ainsworth EA, Naidu SL, Rogers A, Ort DR, Long SP (2006) Photosynthesis, productivity and yield of maize are not affected by open-air elevation of CO<sub>2</sub> concentration in the absence of drought. *Plant Physiology*, **140**, 779–790.
- Leakey ABD, Xu F, Gillespie KM, McGrath JM, Ainsworth EA, Ort DR (2009) Genomic basis for simulated respiration by plants growing under elevated carbon dioxide. *Proceedings of the National Academy of Sciences*, **106**, 3597–3602.
- Lobell DB, Asner GP (2003) Climate and management contributions to recent trends in U.S. agricultural yields. *Science*, **299**, 1032.
- Meehl GA, Tebaldi C (2004) More intense, more frequent and longer lasting heat waves in the 21st century. *Science*, **305**, 994–997.
- Munier-Jolain NG, Munier-Jolain NM, Roche R, Ney B, Duthion C (1998) Seed growth rate in grain legumes I. The effect of photoassimilate availability on seed growth rate. *Journal of Experimental Botany*, **49**, 1963–1969.
- National Agricultural Statistics Service (2011) Acreage. US Department of Agriculture. Available at: <http://usda.mannlib.cornell.edu/MannUsda/viewDocumentInfo.do?documentID=1000> (accessed 14 November 2013).
- Nishiyama Y, Yamamoto H, Ailakhverdiev SI, Inaba M, Yokota A, Murata N (2001) Oxidative stress inhibits the repair of photodamage to the photosynthetic machinery. *European Molecular Biology Organization Journal*, **20**, 5587–5594.
- Oren R, Schulze E-D, Matyssek R, Zimmermann R (1986) Estimating photosynthetic rate and annual carbon gain in conifers from specific leaf weight and leaf biomass. *Oecologia*, **70**, 187–193.
- Parry ML, Canziani OF, Palutikof JP, Van der Linden PJ, Hanson CE, eds. (2007) *Cross-chapter case study. Climate Change 2007: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. pp. 843–868. Cambridge University Press, Cambridge, UK.
- Rosenthal DM, Ruiz-Vera UM, Siebers MH, Gray SB, Bernacchi CJ, Ort DR (2014) Biochemical acclimation, stomatal limitation and precipitation patterns underlie decreases in photosynthetic stimulation of soybean (*Glycine max*) at elevated [CO<sub>2</sub>] and temperatures under fully open air field conditions. *Plant Science*, **226**, 136–146.
- Ruiz-Vera UM, Siebers M, Gray SB *et al.* (2013) Global warming can negate the expected CO<sub>2</sub> stimulation in photosynthesis and productivity for soybean grown in the Midwestern United States. *Plant Physiology*, **161**, 410–423.
- Sage RF, Kubien DS (2007) The temperature response of C<sub>3</sub> and C<sub>4</sub> photosynthesis. *Plant Cell & Environment*, **30**, 1086–1106.
- Salvucci ME, Crafts-Brander SJ (2004) Mechanism for deactivation of rubisco under moderate heat stress. *Physiologia Plantarum*, **122**, 513–519.
- Schiltz S, Gallardo K, Huart M, Negroni L, Sommerer N, Burstin J (2004) Proteome reference maps of vegetative tissues in pea. An investigation of nitrogen mobilization from leaves during seed fill. *Plant Physiology*, **135**, 2241–2260.
- Schrader SM, Wise RR, Wacholtz WF, Ort DR, Sharkey TD (2004) Thylakoid membrane responses to moderately high leaf temperature in Pima cotton. *Plant Cell & Environment*, **27**, 725–735.
- Seneviratne SI, Nicholls N, Easterling D *et al.* (2012) *A Special Report of Working Groups I and II of the Intergovernmental Panel on Climate Change (IPCC)*. pp. 109–230. Cambridge University Press, Cambridge, UK, and New York, NY, USA.
- Smith JR, Nelson RL, Vasilas BL (1988) Variation among soybean breeding lines in relation to yield and seed-fill duration. *Agronomy Journal*, **80**, 825–829.
- Souza RP, Machado EC, Silva JAB, Lagôa AMMA, Silveria JAG (2004) Photosynthetic gas exchange in cowpea (*Vigna unguiculata*) during water stress and recovery. *Environmental and Experimental Botany*, **51**, 45–56.
- Tannura M, Irwin S, Good D. (2008) Weather, Technology, and Corn and Soybean Yields in the U.S. Corn Belt. Marketing and Outlook Research Report 2008-01, Department of Agricultural and Consumer Economics, University of Illinois at Urbana-Champaign, Feb. 2008. Available at: [http://www.farmdoc.uiuc.edu/marketing/morr/morr\\_archive.html](http://www.farmdoc.uiuc.edu/marketing/morr/morr_archive.html) (accessed January 2013).
- Way D, Yamori W (2014) Thermal acclimation of photosynthesis: on the importance of adjusting our definitions and accounting for thermal acclimation of respiration. *Photosynthesis Research*, **119**, 89–100.
- Whitehead D, Griffin KL, Turnbull MH *et al.* (2004) Response of total night-time respiration to differences in total daily photosynthesis for leaves in a *Quercus rubra* L. canopy: implication for modeling canopy CO<sub>2</sub> exchange. *Global Change Biology*, **10**, 925–938.
- Zheng S, Nakamoto H, Yoshikawa K, Furuya T, Fukuyama M (2002) Influences of high night time temperature on flower and pod setting in soybean. *Plant Production Science*, **5**, 215–218.
- Zinn KE, Tunc-Ozdemir M, Harper JF (2010) Temperature stress and plant sexual reproduction: uncovering the weakest links. *Journal of Experimental Botany*, **61**, 1959–1968.

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