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Plant Science 172 (2007) 1113–1123
PLANT SCIENCE
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Heat stress induced ethylene production in developing wheat grains induces kernel abortion and increased maturation in a susceptible cultivar

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Received 21 November 2006; received in revised form 8 March 2007; accepted 9 March 2007
Available online 15 March 2007

Abstract

In this work we present evidence that the heat stress induced kernel abortion and suppression of grain maturation in a representative heat susceptible hard red winter wheat (*Triticum aestivum* L.) cultivar is regulated by heat stress induced ethylene production. Exposure to heat stress (38 °C) during early kernel development (10 DAP) resulted in a 6-fold increase in ethylene production in developing kernels of the heat susceptible hard red winter wheat cultivar 'Karl 92'. A similar 7-fold increase in ethylene production in embryos and 12-fold increase in ethylene production in the flag leaf of heat stressed plants of 'Karl 92' was also found. In contrast, no change in ethylene production was observed in the heat tolerant hard white spring wheat cultivar 'Halberd'. In an effort to link the heat stress induced ethylene production to the observed increase in kernel abortion and reduced kernel weight in the heat susceptible 'Karl 92', plants were treated with the ethylene receptor inhibitor 1-methylcyclopropane (1-MCP) prior to exposure to heat stress. Inhibiting ethylene perception in the heat susceptible 'Karl 92' in this manner blocked heat stress induced kernel abortion and reduction in kernel weight and demonstrated a clear link between ethylene in regulating susceptibility to heat stress or perception of high temperatures as a timing signal for transitioning to developmental arrest and senescence in certain wheat genotype classes.
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Keywords: Wheat; Triticum aestivum; Heat stress; Seed; Ethylene

1. Introduction

Heat stress during reproductive development is a primary constraint to wheat (*Triticum aestivum* L.) production and profitability in many wheat-growing regions of the world. Additional yield reductions from the predicted rise in global temperatures and rising farming input costs will add further strain to food supplies, the economic viability of farming and related farm-based businesses. In a recent international consultancy, leaders of national wheat programs identified heat tolerance as one of their major priorities [1]. In heat susceptible wheat genotypes, heat stress during reproductive development reduces photosynthesis and promotes premature senescence. As a consequence, heat stress reduces yields by inducing pollen sterility and seed abortion and subsequently lowers seed weight, flour yield, and dough quality. Susceptible wheat genotypes exhibit a 3% reduction in yield for every degree rise above 15 °C [2]. Wheat genotypes with high levels of tolerance to high temperatures have been identified and are defined by maintenance of photosynthesis, chlorophyll content, and high stem carbohydrate reserves and yield through higher seed set, grain weight, and an extended grain filling duration even at elevated temperatures [3]. While these phenotypic traits have been associated with the quantitative inheritance of heat tolerance, a fundamental understanding of the molecular and physiological basis of the heat stress susceptibility response is lacking.

Heat stress impacts a multitude of plant and cellular functions. High temperatures are known to alter membrane fluidity [4,5] and impair enzyme function in many instances via denaturation [6,7]. Both heat stress induced membrane and protein damage can result in elevated reactive oxygen species that cause oxidative stress [8–10]. Heat stress can also induce programmed cell death [11,12]. Collectively, these deleterious consequences lead to reduced photosynthesis, reduced assimilate translocation, altered growth and reproduction, and premature senescence [13].

Enhanced ethylene production has been associated with reduced growth, accelerated senescence, and responses to

Abbreviations: DAP, days after pollination; MCP, 1-methylcyclopropene

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0168-9452/$ – see front matter. Published by Elsevier Ireland Ltd.
doi:10.1016/j.plantsci.2007.03.004

environmental stress. In mature wheat plants, increased ethylene has been shown to shorten the grain filling period, decrease 1000 kernel weight, hasten maturity and trigger premature senescence [14]. A study of wheat in the Mir space station determined that plant growth was vigorous with 5 to 8 tillers compared to 3 to 5 on earth. However, plants grown in the Mir space station were sterile. The levels of ethylene measured on the Mir prior to and during flowering, were found in earth studies at normal gravity to cause male sterility [15–17]. Ethylene overproduction has also been found during or after recovery from water stress [18–20,14]. As well, paraquat-induced ethylene production was related to formation of reactive oxygen species, lipid peroxidation, and a reduction in membrane fluidity [21]. Increased ethylene production in shoots and roots also were recently associated with decreased salt tolerance in a susceptible versus tolerant wheat cultivar [22].

Since many of the symptoms associated with heat stress in heat susceptible wheat lines can be associated with a premature transition to senescence and maturity during reproductive development, and the strong influence that ethylene has been shown to impart on this process, we hypothesized that heat stress symptoms in susceptible lines that are common for many cultivars in the hard red winter wheat class [3] is regulated, at least in part by increased ethylene production. In this study we have compared ethylene production in response to heat stress in a heat susceptible versus a heat tolerant wheat genotype. We present evidence that ethylene is a likely regulator of heat stress induced seed abortion, reduction in 1000 kernel weight, and early transition to the dry seed stage in the heat susceptible cultivar investigated.

2. Materials and methods

2.1. Plant growth conditions

The heat tolerant 'Halberd' (hard white spring wheat) and a representative heat susceptible 'Karl 92' (hard red winter wheat) were used as examples of the differing responses to heat stress. Large sets of each cultivar were seeded in 36 cm pots with two plants per pot. Pots consisted of 3:1 Lufkin sand-soil, Metro Mix potting soil to maintain soil moisture. Pots were supplied with 5 g Osmocote™ and supplemented with Peters™ 20:20:20 and Peters Soluble Trace Element Mix at recommended rates every 3 weeks.

2.2. Heat stress treatment

In the case of analyzing ethylene evolution, 1000 kernel weight, and kernel number per main spike in response to heat stress, plants were grown in growth rooms with a 14 h day length beginning at (7:00 h (7:00 a.m.) day cycle start), 450 μmol m^{-2} s^{-1} photosynthetic photon flux (PPF) and a 20 °C/18 °C day/night temperature cycle. The 1°, 2°, and 3° spikes for each plant were tagged for day of pollination (at anther dehiscence). At 6:00 h 10 days after pollination (for the primary spike), 200 pots (400 plants) of each line were transferred to plant growth chambers with a 14 h day length, 450 μmol m^{-2} s^{-1} PPF and a 38 °C/25 °C day/night heat stress for 1 or 2 days. The heat cycle began at 9:00 h and ended at 18 h, 3 h prior to the end of the 14 h day cycle. At the same time, 200 pots (400 plants) of each line were transferred to an identical control growth chamber set at 14 h day length, illumination of 450 μmol m^{-2} s^{-1} with a 20 °C/18 °C day/night cycle for 1 or 2 days. Following the 1 or 2 days temperature treatment, plants were transferred back to growth rooms set at the original temperature and day length described above. Previous studies using 'Karl 92' have shown that at 10 DAP both kernel set and kernel weight were affected by high temperatures [23]. At later stages kernel number is set, while kernel weight is still negatively impacted by high temperatures [23]. We chose to use the rapid change in temperature stress to approximate the rapid temperature changes that occur during mid Spring reproductive development for hard red winter wheat (HRWW). Wheat grown in the Southern Great Plains. While extreme, it is a rigorous simplified approximation that Southern Great Plains HRRW cultivars need to be capable of coping with in terms of yield and end-use quality maintenance. In this region temperatures can change from the low to mid 20 °C to mid to upper thirties Celsius in the span of 5–7 days, a change we have observed that results in the senescence of numerous elite HRWW cultivars (http://uvalde.tamu.edu/weather/weather.php).

The effect of pre-conditioning at low-level heat stress prior to the 38 °C heat stress was also examined to determine its influence on 1000 kernel weight, and kernel number per main spike ear. In this case, plants were grown in growth rooms with a 14 h day length, 450 μmol m^{-2} s^{-1} PPF with a 20 °C/18 °C day/night temperature cycle. At Feekes 9 stage when the flag leaf was fully extended the plant growth temperatures were elevated to a preconditioning 30 °C/25 °C day/night cycle in the growth rooms for approximately 8–12 days prior to the high temperature stress treatment at 10 DAP. The same 14 h day cycle was maintained during the preconditioning treatment. The 1°, 2°, and 3° spikes for each plant were tagged for day of pollination (at anther dehiscence). At 10 days after pollination (for the primary spike), 25 pots (50 plants) of each line were transferred to growth chambers with the same 14 h day lengths, 450 μmol m^{-2} s^{-1} PPF and a 38 °C/25 °C day/night heat stress for 2 days. At the same time, 25 pots (50 plants) of each line was transferred to an identical control growth chambers set at 14 h day lengths with a 30 °C/25 °C day night cycle for 2 days. Following the 2 days temperature treatment, plants were transferred back to growth rooms set at the original 30 °C/25 °C day/night temperature and the same day length described above.

Developing kernels, dissected embryos, and flag leaves were sampled on regular time course intervals from the primary spikes of three heat-treated and control plants for ethylene measurements at each time point. Plants were watered daily during and after temperature treatments. Plants were watered on a regular cycle until all spikes reached maturity. All tagged spikes for each plant were harvested individually for the analysis of yield components. Spikes were mechanically

threshed and yield components of the primary spike ear was recorded for 20 individual plants for each treatment. These components included 1000 kernel weight and kernel number per primary spike. For ethylene production analysis, the data represent the mean ± S.E. ethylene measured from three individual plants for each treatment regime and time point.

2.3. Ethylene quantification

The methods used in the analysis of ethylene production have been previously described [24]. Briefly, developing kernels, dissected embryos, and flag leaves from control and heat-treated plants were placed in separate 10 ml syringes with the plunger adjusted to 2 ml. Fifteen embryos were dissected over a 5 min period following spike harvest and placed in 10 ml syringes in the same manner. A 1 ml gas sample was then transferred to a second gas tight syringe through a 3-way valve after 20 min (15 min for dissected embryos). Using different times for tissue incubation in the syringe and 1 ml head space sampling, it was found that the burst in wound induced ethylene production could not be detected in control or heat-treated plants until 30 min. There was no difference in wound ethylene production produced in control or heat-treated plants (not shown). The 1 ml gas sample was analyzed for ethylene concentration using a Photovac 10S Plus GC with a 3.2 mm × 2.45 m 60/80 Carbopack B column (Photovac, Markham, Canada) fitted with a photoionization detector. The data represent the mean ± S.E. ethylene measured from three individual plants for each treatment regime and time point.

2.4. 1-MCP ethylene inhibitor treatment

The ethylene receptor inhibitor 1-methylcyclopropane (1-MCP) (Agrofresh, Philadelphia, PA) was used to link ethylene production to the regulation of heat stress reduced kernel weights and kernel set. Plants (160 pots with 2 plants per pot for each 'Karl 92' and 'Halberd') were grown in growth rooms with a 14 h day length, 450 μmol m^{-2} s^{-1} PPF with a 20 °C/18 °C day/night temperature cycle. The 1°, 2°, and 3° spikes for each plant were tagged for day of pollination (at anther dehiscence). At 9 DAP or 1 day prior to heat stress at 10 DAP plants in 80 pots of each 'Karl 92' and 'Halberd' were treated with 1-MCP by applying 100 g AI/ha (3% active ingredient or 1 ppm) by dissolving the 1-MCP in a pre-prepared mixture of water with 0.25% DyneAmic™ an organosilicone adjuvant (Helena Chemical, USA) using a tank sprayer set a 40 PSI spray pressure. At the same time plants in 80 pots for each 'Karl 92' and Halberd' were treated with 0.25% DyneAmic™ as controls. Plants at 10 DAP were transferred to four separate but identical plant growth chambers set at a 14 h day length, and a 20 °C/18 °C day/night regime. After 12 h, the 1-MCP is thought to have passed through the cuticle or into stomates and bound to ethylene receptors (Mark Dahmer personnel communication, Agrofresh, Denver, CO). As such, at 6:00 h on 10 DAP 40 1-MCP treated plants were transferred to a chamber set at a 38 °C/25 °C day/night regime, 40 1-MCP treated plants were transferred to a second chamber set at 20 °C/18 °C day/night regime, 40 control treated plants were transferred to a third chamber set at a 38 °C/25 °C day/night regime, and 40 control untreated plants were transferred to a fourth chamber set at a 38 °C/25 °C day/night regime. All four plant growth chambers were identical and set at the same day length regime. After 2-days all plants were transferred back to the same growth room set at a 20 °C/18 °C day/night regime and 14 h day length. Three primary spikes were collected from individual plants for each treatment at regular intervals to determine changes in fresh and dry weight during development. The kernel number was determined by hand threshing eight primary spike ears collected from individual plants for each treatment.

2.5. Statistical analysis

Values are the mean ± standard error of three replicates. Standard errors and significant differences between samples were determined using the Student–Newmen–Keuls test and the SPSS software package (SPSS Inc., Chicago, IL).

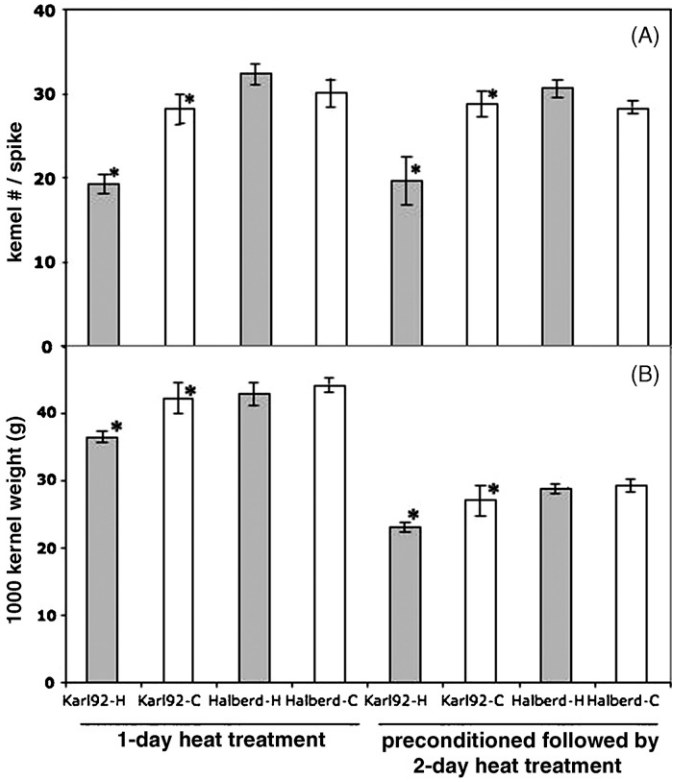

Fig. 1. Wheat kernel set and grain weight responses to heat stress of 38 °/25 °C day/night at 10 days after pollination (DAP) in a heat tolerant and susceptible wheat cultivar for 1 d with no preconditioning or 2 d following preconditioning with a 30 °/25 °C day/night regime beginning at Feekes 9 (flag leaf fully extended). (A) Kernel number per spike of the primary ears exposed to heat stress at 10 DAP. (B) 1000 kernel weight of the primary head exposed to heat stress at 10 DAP. Data are the mean of 1000 kernel weights and kernel numbers per primary ears of twenty plants for each treatment. Dark bars are means of heat-treated plants, white bars are means from control-treated plants. Error bars are ±S.E., asterisk above bars indicate significant difference between means of control vs. heat-treated for individual varieties at $P \leq 0.05$.

3. Results

3.1. Grain weight and grain set responses to heat stress during grain development

In the heat susceptible cultivar 'Karl 92', heat stress imposed as a 1 day 38 °C daytime treatment when the primary ear was 10 days after pollination (DAP) significantly reduced grain set per ear (Fig. 1A) and kernel weight (measured as 1000 kernel weight) (Fig. 1B) versus control. The significant reduction occurred when heat stressed and control plants were not preconditioned with mild temperature stress. A similar significant reduction in kernel number per primary ear and 1000 kernel weight also occurred when plants were preconditioned with a 30 °C/25 °C day/night treatment in plant growth rooms beginning when primary ear flag leaves were fully elongated (Feekes stage 9) prior to being transferred to a 2-day heat stress at 38 °C as described in Section 2 (Fig. 1A and B, respectively). Pretreatment alone did reduce 1000 kernel weight versus untreated control in the heat susceptible 'Karl 92' (Karl 92-C, preconditioned followed by 2-day heat treatment, Fig. 1B).

In contrast, the heat tolerant cultivar 'Halberd' exhibited no change in kernel number per primary spike or 1000-kernel weight when either preconditioned or maintained at ideal temperature conditions prior to being transferred to heat stress or control chambers for 1 or 2 days (Fig. 1A and B, respectively). Preconditioning did significantly lower grain weight for 'Halberd' versus non-preconditioning both for heat stress and control treated plants (Fig. 1B). This appeared to be due to the extended preconditioning temperature of 30 °C since both heat-treated and control plants responded the same.

3.2. Ethylene evolution in developing kernels exposed to heat stress

In response to heat stress imposed at 10 DAP the susceptible cultivar 'Karl 92' exhibited a significant 6-fold increase in ethylene production in the developing kernels at 11:00 h, 2 h after the beginning of the heat stress (Fig. 2B). The day cycle

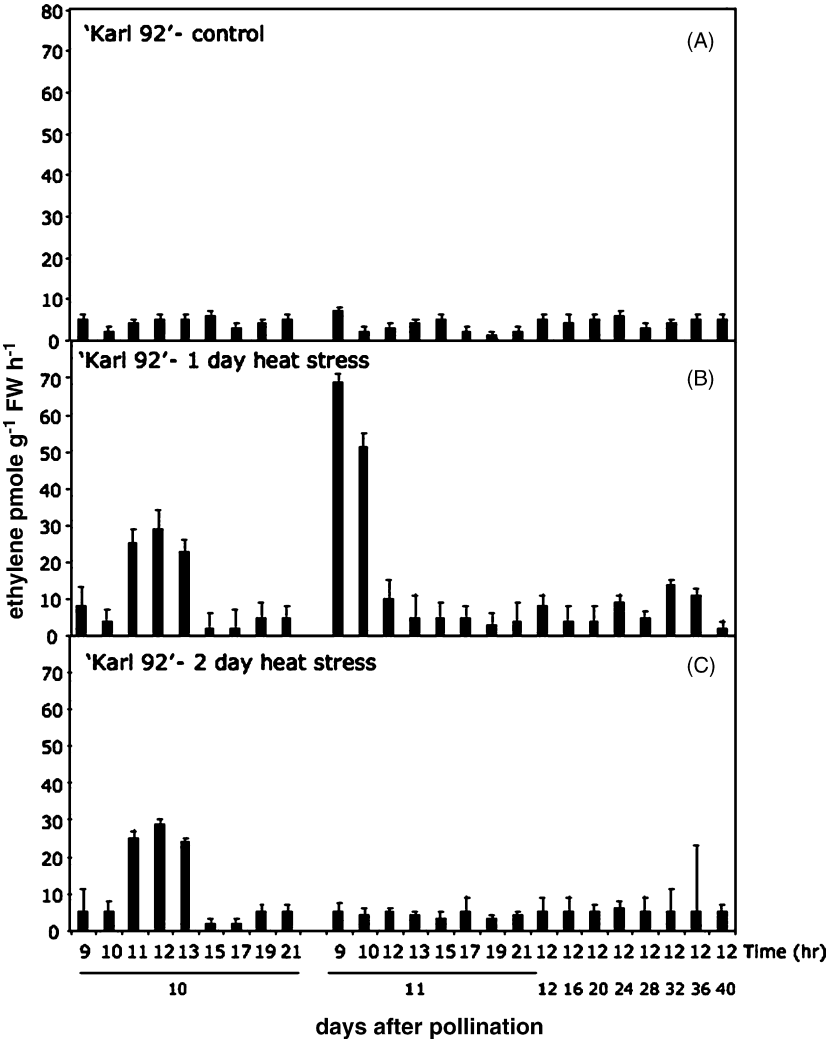

Fig. 2. Ethylene production in the developing kernels of wheat primary ears beginning at 10 DAP for plants maintained at ideal control conditions of 20 °C (A), or following a daytime heat stress of 38 °C imposed for 1 (B), or 2 days (C) for the heat susceptible wheat cultivar 'Karl 92'. Data are the mean of ethylene production from all kernels collected from each primary ear from three plants for each time point ($n = 3$). Error bars are ±S.E.

began at 7:00 h with the heat treatment beginning at 9:00 h. The elevated ethylene levels occurred over the next 3 h then dropped backed to levels similar to that found in the untreated control plants (Fig. 2B and A, respectively). A change or increase in ethylene was not observed over the same period in control treated plants that were transferred to an identical plant growth chamber set at 20 °C/18 °C day/night cycle (Fig. 2A). When plants that were heat stressed for 1 day were again maintained in a 20 °C/18 °C day/night cycle on 11 DAP, developing kernels from the heat-treated plants exhibited a 14-fold elevation in ethylene production versus ethylene production in kernels from control treated plants. This elevated ethylene production was first observed at 9:00 h, 2 h after the beginning of the day cycle on 11 DAP (Fig. 2B and A, respectively). When this increase in ethylene began is not known since no ethylene measurements were recorded between the end of the day cycle at 21:00 h on 10 DAP and 9:00 h on 11 DAP. However, the elevation in ethylene did not begin immediately following the cessation of the heat stress that ended at 18 h on 10 DAP. This elevation in ethylene levels on 11 DAP on the day following the 1 day heat stress treatment was short lived, continuing over 3 h then returning to control equivalent levels. The elevation in ethylene levels during the day following a day of heat stress only occurred in kernels from the set of plants that were exposed to 1 day of heat stress, and not in developing kernels of the separate set of plants exposed to 2 consecutive days of heat stress at 38 °C (Fig. 2C). Also, an elevation in ethylene was not observed on the second day of exposure to heat stress. The data represent the mean ± S.E. ethylene measured from three individual plants for each treatment regime and time point (refer to Section 2).

In contrast to what was observed in the heat susceptible cultivar 'Karl 92', no substantial changes in ethylene levels were observed in the heat tolerant cultivar 'Halberd' (Fig. 3). This was true both during the day of heat stress and during recovery on the following day (Fig. 3B and C).

3.3. Ethylene evolution in developing embryos from plants exposed to heat stress

We examined the origin of the increased ethylene production found in 'Karl 92' developing kernels to determine the

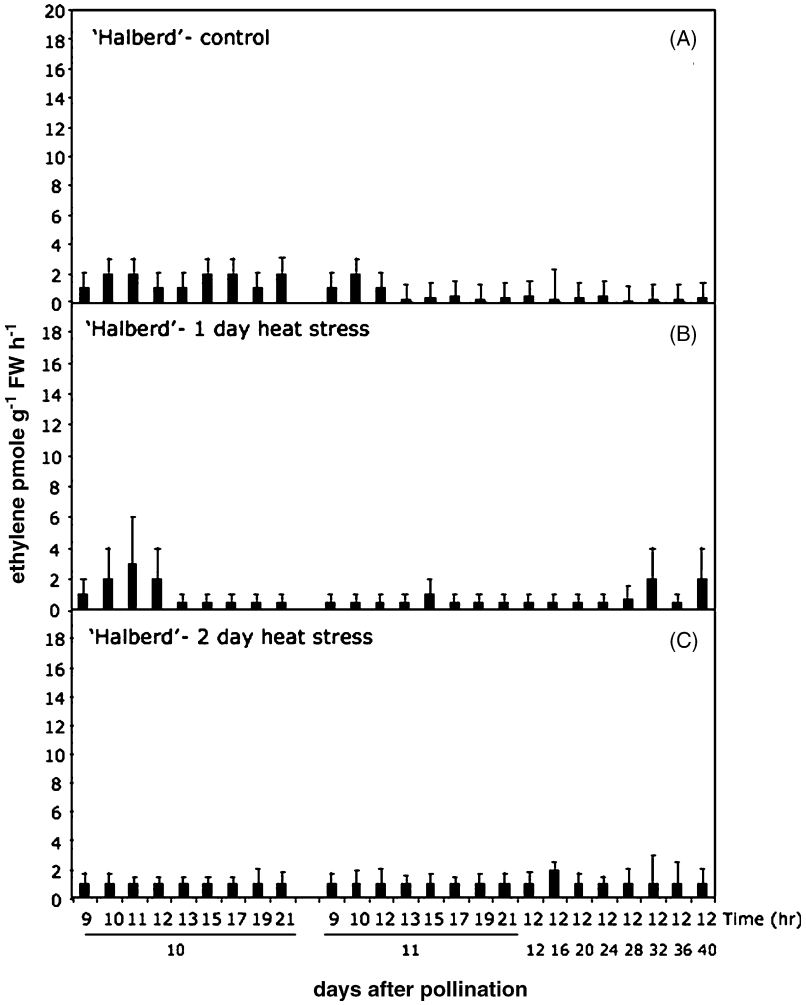

Fig. 3. Ethylene production in the developing kernels of wheat primary ears beginning at 10 DAP for plants maintained at ideal control conditions of 20 °C (A), or following a daytime heat stress of 38 °C imposed for 1 (B), or 2 days (C) for the heat tolerant wheat cultivar 'Halberd'. Data are the mean of ethylene production from all kernels collected from each primary ear from three plants for each time point ($n = 3$). Error bars are ±S.E.

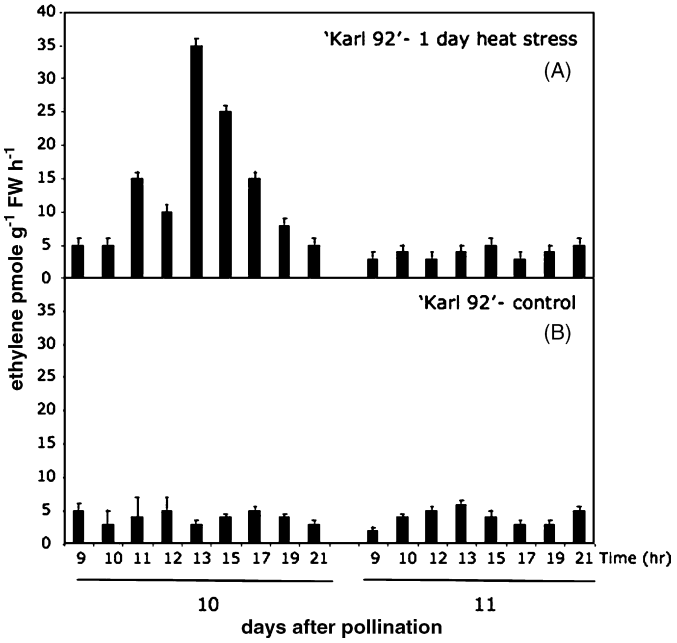

Fig. 4. Ethylene production in developing embryos dissected from wheat kernels of the primary ears beginning at 10 DAP for plants exposed to a 1 day daytime heat stress of 38 °C (A), or maintained at ideal control conditions of 20 °C (B) for the heat susceptible wheat cultivar 'Karl 92'. Data are the mean of ethylene content of 15 embryos dissected from kernels collected from each primary ear from three plants for each time point ($n = 3$). Error bars are ±S.E.

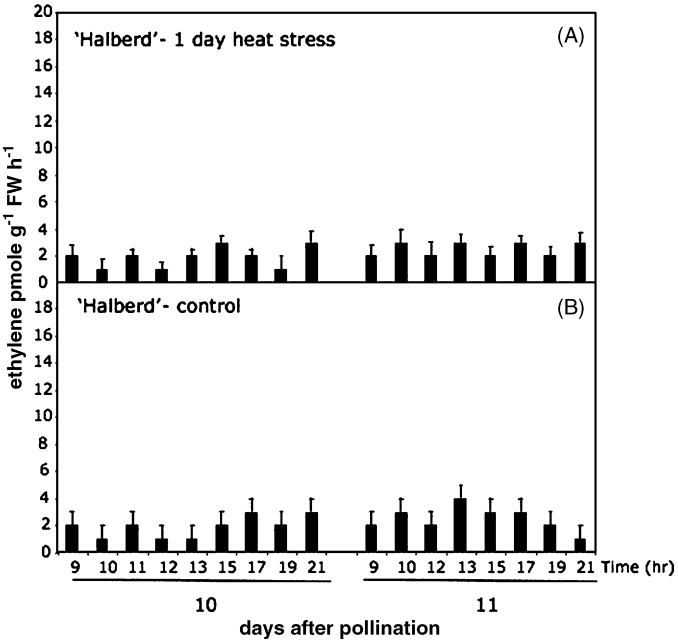

Fig. 5. Ethylene production in developing embryos dissected from wheat kernels of the primary ears beginning at 10 DAP for plants exposed to a 1 day daytime heat stress of 38 °C (A), or maintained at ideal control conditions of 20 °C (B) for the heat tolerant wheat cultivar 'Halberd'. Data are the mean of ethylene content of 15 embryos dissected from kernels collected from each primary ear from three plants for each time point ($n = 3$). Error bars are ±S.E.

contribution that was derived from the developing embryo. Developing embryos were dissected from the same set of plants used to analyze ethylene in developing whole kernels from heat stressed and control treated plants (see above). Plants with tagged ears were exposed to heat stress beginning at 10 DAP. The heat stress began at 9:00 h, 2 h after the start of the day cycle, by ramping the plant growth chamber temperature to 38 °C over 30 min. An increase in ethylene evolution from developing embryos began 2 h after the beginning of the heat treatment. The increase in ethylene production continued, peaking to 7-fold above embryos from control treated plants at 4 h after beginning the heat treatment (Fig. 4A and B, respectively). Following this peak at 13:00 h, ethylene levels gradually declined, yet remained elevated above embryos from control treated plants for 6 h (Fig. 4A). No change in ethylene levels were observed in 'Karl 92' embryos following the 1 day heat treatment when the growth chamber temperature was again set at an ideal 20 °C/18 °C day/night temperature cycle.

As was observed in the analysis of ethylene production from developing kernels in response to heat stress for the heat tolerant cultivar 'Halberd', there were also no changes in ethylene production in developing embryos of 'Halberd' from heat-treated plants either during or after the 1 day heat stress of 38 °C (Fig. 5A).

3.4. Ethylene production in flag leaves in response to heat stress

Given the importance of the flag leaf as a source of photoassimilates for developing ears, ethylene production in flag leaves of primary ears was also examined at the same time that ethylene levels were determined in developing kernels and embryos in response to heat stress (Figs. 6 and 7). For the heat susceptible 'Karl 92', a 6-fold increase in ethylene levels relative to control treated plants was observed at 9–10 h following exposure to heat stress that began at 9:00 h (Fig. 6B and A, respectively). As was observed in developing kernels of 'Karl 92', a recovery increase in ethylene was only observed in flag leaves following release from 1 day of elevated temperatures (Fig. 6B) and not on the second day of heat stress following the second day of heat stress (Fig. 6C). Interestingly, the increase in flag leaf ethylene production during the initial day of heat stress was late in comparison to the elevation in ethylene that was observed in the same plants in the developing kernels and embryos, where the ethylene increase occurred 6 h earlier. A 9-fold increase in ethylene levels did occur in flag leaves of control plants of 'Karl 92' and 1 and 2 day heat-stressed 'Halberd'. However, these increases occurred late or at 36 DAP, both in the heat susceptible 'Karl 92' and the heat tolerant 'Halberd' (Figs. 6A, 7B and C, respectively).

3.5. Ethylene receptor inhibitors block heat stress loss in kernel weight and abortion

Given our observations that heat stress induced a rapid increase in ethylene production in developing kernels, the embryo itself and later the source flag leaf, we suspected that ethylene was a principal regulator of lowering kernel weight, and inducing kernel abortion in the susceptible 'Karl 92'. In an effort to test this hypothesis, plants were treated with the

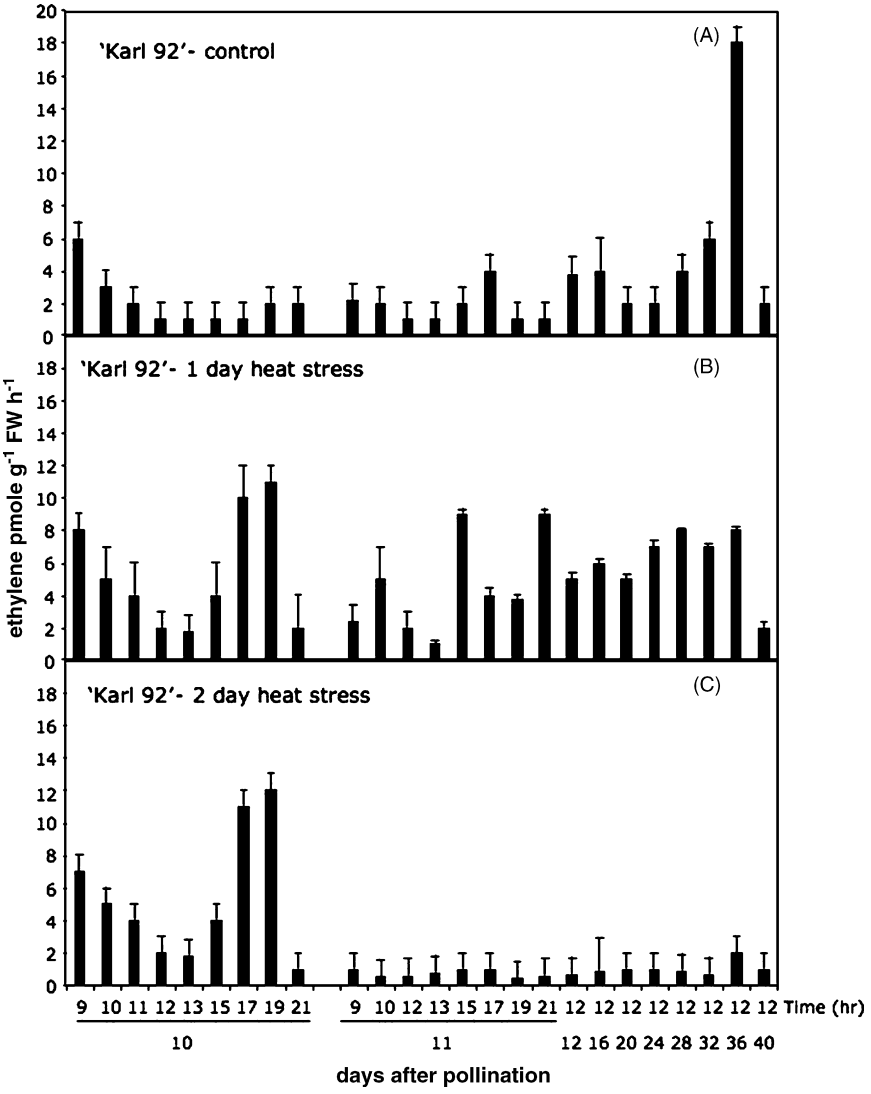

Fig. 6. Ethylene production in flag leaves of wheat primary ears beginning at 10 DAP for plants maintained at ideal control conditions of 20 °C (A), or following a daytime heat stress of 38 °C imposed for 1 (B), or 2 days (C) for the heat susceptible wheat cultivar 'Karl 92'. Data are the mean of ethylene content from flag leaves collected from each primary ear from three plants for each time point ($n = 3$). Error bars are ±S.E.

ethylene receptor inhibitor 1-MCP at 9 DAP for the primary ear prior to exposure to 2 days of 38 °C heat stress at 10 DAP. Following removal from growth chambers and returning heat-treated and control treated plants to plant growth rooms set at 20 °C/18 °C day/night temperature regime, developing kernels were harvested from three plants for each treatment group at regular intervals to monitor changes in kernel weight gain. The fresh weight of 'Karl 92' kernels increased to 27 DAP and then declined as seeds transitioned to the dry seed stage (Fig. 8A). Plants that were heat stressed without being previously treated with 1 ppm 1-MCP exhibited a 34% reduction in fresh weight and dry weight during development (Fig. 8A and B, respectively). The kernel number per primary spike ear was also significantly reduced to 8 kernels per ear versus 24–25 kernels per ear (Fig. 10A). Treatment of plants with 1 ppm 1-MCP prior to exposure to heat stress blocked the reduction in kernel fresh and dry weight gain during development (Fig. 8A and B), and it blocked heat stress induced kernel abortions with kernel set equivalent to control treated plants (Fig. 10A). An identical treatment with 1-MCP had no effect on kernel weight and number for the heat tolerant 'Halberd' (Figs. 9A, B, and 10B, respectively).

4. Discussion

Increased ethylene production in response to water stress has been well documented [18–20,14]. Overproduction of ethylene has frequently been related to fruit abortion in dicots such as cotton (*Gossypium hirsutum*) during drought stress [25], ovule abortion in a flowering tree *Syzygium cuminii* (L.) [26], and *Arabidopsis* in response to salt stress [27]. Similar phenomena have been found in monocots such as ethylene induced grain weight reduction in rice [28–30] and wheat in response to water stress [31,32,14] and maize (*Zea Mays*) [33]. In wheat, extended and short duration heat stress has been demonstrated to reduce kernel weight and kernel set, a consequence of

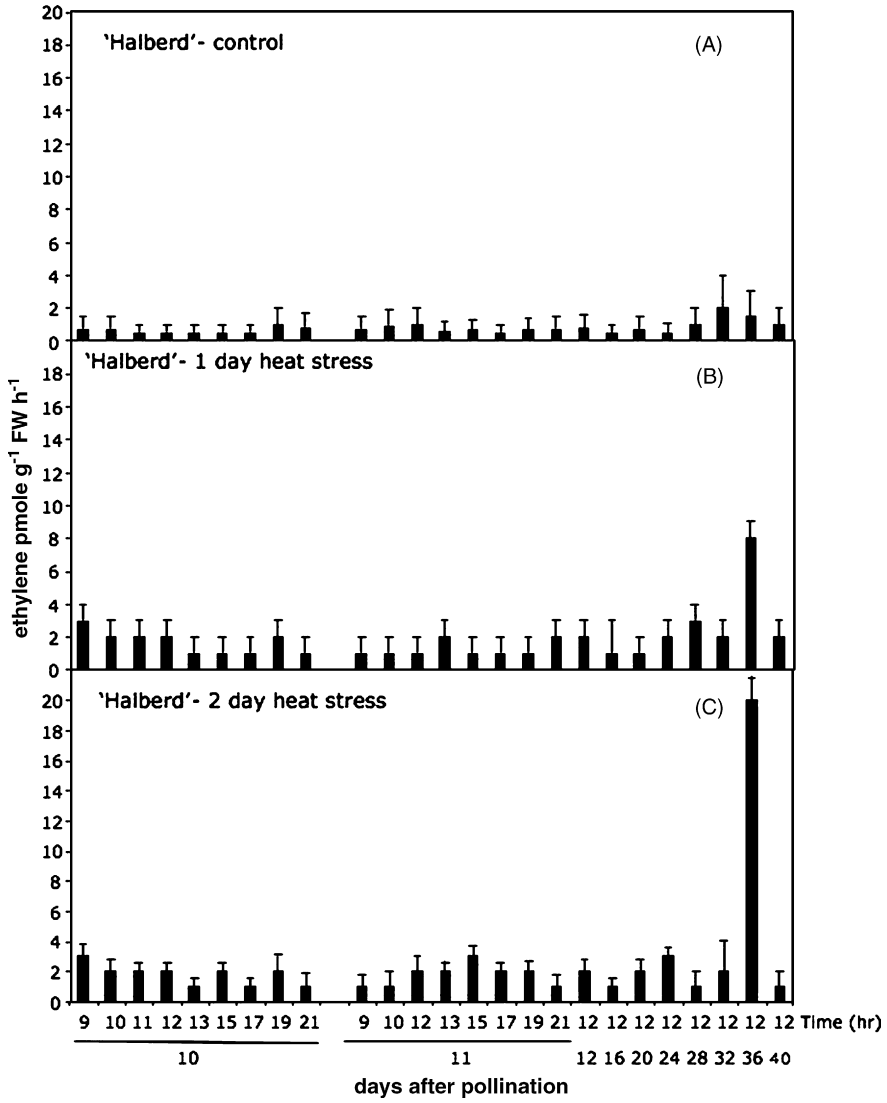

Fig. 7. Ethylene production in flag leaves of wheat primary ears beginning at 10 DAP for plants maintained at ideal control conditions of 20 °C (A), or following a daytime heat stress of 38 °C imposed for 1 (B), or 2 days (C) for the heat tolerant wheat cultivar 'Halberd'. Data are the mean of ethylene content from flag leaves collected from each primary ear from three plants for each time point ($n = 3$). Error bars are ±S.E.

increased seed abortion [3]. Both of these consequences of heat stress result from an early senescence and seed desiccation event. While the increased seed abortion and reduction in kernel weight is genotype dependent, we and others have found this response to heat stress to be common in the hard red winter wheat class such as 'Karl 92' [3], while a number of genotypes from other programs and wheat classes can be found that do not exhibit decreased kernel weight and increased seed abortion in response to heat stress. The cultivar 'Halberd' is just one cultivar we have found that does not respond to short durations of high temperature stress as a signal to senesce. Senescence is an actively programmed phenomenon in plants, as is the duration of seed development and maturation. Yet both of these phenomena can be accelerated by exogenous abiotic and biotic signals [34–36]. For some genotypes episodes of high temperatures may be one such signal used for timing the transition to developmental arrest. Unfortunately, yield and end-use quality are often compromised when heat stress accelerates senescence and seed desiccation. We hypothesized that given the well-documented evidence for the role that ethylene plays in reducing seed set in cereals [37,14,31] and regulating senescence; heat stress induced ethylene too was a likely candidate for regulating the acceleration of this event.

We found strong evidence that this is the case. Two hours following exposure to heat stress (38 °C) increased ethylene levels were detected in developing kernels and the developing embryos of the heat susceptible 'Karl 92' (Figs. 2B and 4A, respectively). However, this elevation in ethylene was not observed in the heat tolerant 'Halberd' (Figs. 3 and 5, respectively). It is interesting to note that the increase in ethylene observed in the developing embryos preceded an increase in ethylene found in the kernels and flag leaf of the same ears used to measure ethylene in the developing embryo. We speculate that the initial elevation in ethylene in the developing embryos may function to turn off sink signaling in the embryo itself and surrounding endosperm, followed by

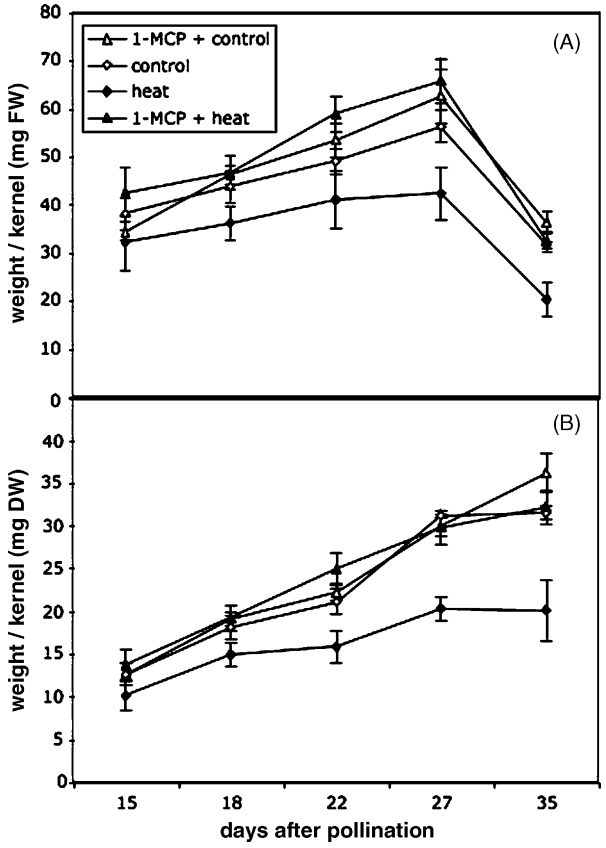

Fig. 8. Grain weight changes during kernel development in response to heat stress imposed at 10 days after pollination (DAP) in the susceptible cultivar 'Karl 92' pretreated with the ethylene receptor inhibitor 1-MCP. Plants were treated with 1 ppm 1-MCP in water with 0.25% DyneAmic™ surfactant or water with 0.25% DyneAmic™ surfactant (for controls) 1 day prior to imposing a daytime heat stress of 38 °C on plants for 2 days or maintained at ideal control conditions of 20 °C. (A) Kernel fresh weight and (B) kernel dry weight. Data are the mean kernel weight of all individual kernels per primary ears of four plants per time point. Error bars are ±S.E.

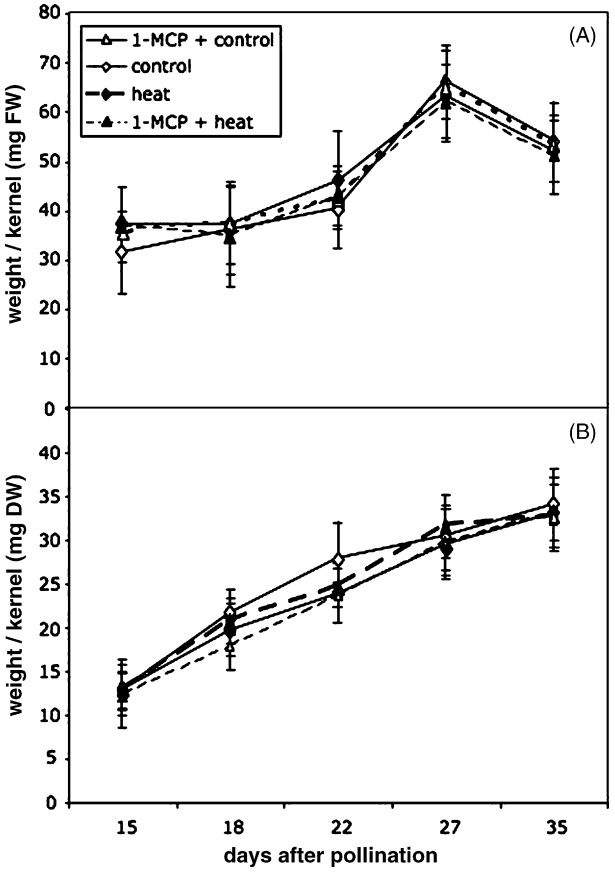

Fig. 9. Grain weight changes during kernel development in response to heat stress imposed at 10 days after pollination (DAP) in the tolerant cultivar 'Halberd' pretreated with the ethylene receptor inhibitor 1-MCP. Plants were treated with 1 ppm 1-MCP in water with 0.25% DyneAmic™ surfactant or water with 0.25% DyneAmic™ surfactant (for controls) 1 day prior to imposing a daytime heat stress of 38 °C on plants for 2 days or maintained at ideal control conditions of 20 °C. (A) Kernel fresh weight and (B) kernel dry weight. Data are the mean kernel weight of all individual kernels per primary ears of four plants per time point. Error bars are ±S.E.

feedback signals that result in elevated ethylene levels in the source flag leaf, resulting in flag leaf senescence. Ethylene levels also significantly increased in developing kernels of the heat susceptible 'Karl 92' on the day following the heat stress treatment only in plants exposed to 1 day of heat stress and not in plants exposed to 2 days of heat stress (Fig. 2C). This result is puzzling, yet may be a function of as yet unknown acquired thermotolerance mechanisms that suppress additional seed abortion events during extended high temperature events. The observation that seeds weights at harvest were reduced by the short duration heat stress is also puzzling given that reductions in seed set are typically associated with increase in seed weight [38]. However, short duration heat stress imposed early in seed development has been shown to decrease seed set, seed weight and photosynthetic activity in susceptible wheat for extended periods following the heat stress event [3,23]. The expression of genes involved in starch deposition in wheat endosperm also are down-regulated in response to heat stress [39]. Similar heat treatments of 40 °C in barley for 5 days halted grain growth, possibly by irreversible inactivation of enzymes involved in starch synthesis [40]. As well, drought stress and ethylene have been shown to reduce the enzymatic activity of sucrose synthase, ADP glucose pyrophosphorylase, and soluble starch synthase, grain filling rate and grain weight of wheat, an outcome that could be inhibited by an inhibitor of ethylene synthesis (cobalt ion) or promoted by an ethylene releasing agent (ethephon) [31]. Thus ethylene may alter seed weight and grain filling in wheat in part at the level of starch deposition.

The present results demonstrate that in the heat susceptible genotype 'Karl 92' (a representative heat susceptible hard red winter wheat) ethylene functions as a key signaling hormone for senescence, the early transition to the dry seed stage and seed abortion in response to periods of high temperature stress. The use of the ethylene receptor inhibitor 1-MCP demonstrates that this signal is required for these phenomena, yet it is short lived and can be deferred (Figs. 8 and 10) preserving normal kernel development and kernel set. We hypothesize that the late stage increase (36 DAP) in ethylene found in flag leaves of control treated 'Karl 92' and heat stressed 'Halberd' may serve to regulate developmental arrest in kernels and leaf senescence under ideal growth conditions (Figs. 6 and 7, respectively). Thus, ethylene may function as a timing signal for temporal

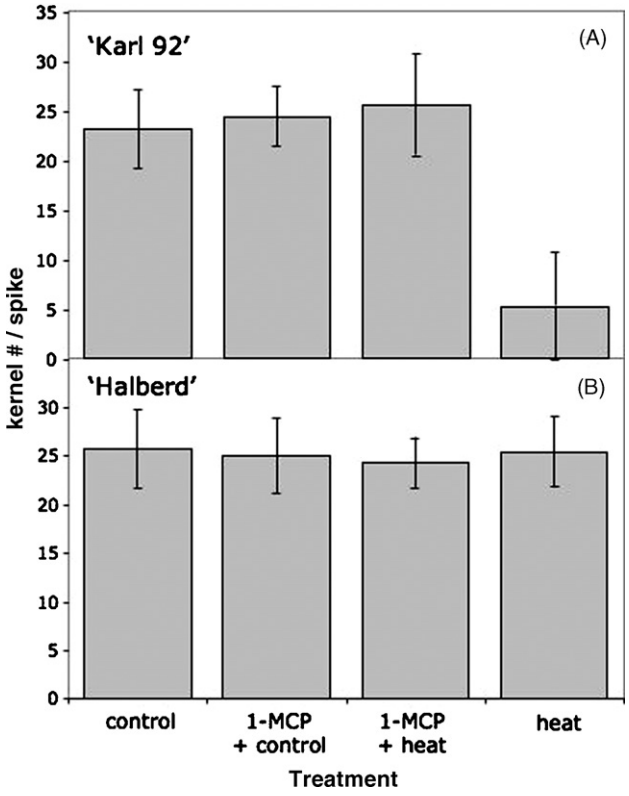

Fig. 10. Wheat kernel set responses to heat stress imposed at 10 days after pollination (DAP) in the heat susceptible cultivar Karl 92 (A) and heat tolerant cultivar 'Halberd' (B) pretreated with the ethylene receptor inhibitor 1-MCP. Plants were treated with 1 ppm 1-MCP in water with 0.25% DyneAmic™ surfactant or water with 0.25% DyneAmic™ surfactant 1 day prior to imposing a daytime heat stress of 38 °C on plants for 2 days or maintained at ideal control conditions of 20 °C. Data are the mean kernel number of the primary ears of 8 plants for each treatment. Error bars are ±S.E.

kernel/embryo development duration. The elevation in ethylene levels in response to heat stress is being verified in other heat susceptible hard red winter wheat cultivars. Whether 'Karl 92' and other related hard red winter wheat cultivars are heat susceptible or, as hypothesized, simply use high temperatures as a timing signal to arrest development, is being tested. We are also exploring the molecular sensory mechanism for heat stress signaling and where ethylene functions in this sensory mechanism, and investigating the molecular nature of 'Halberd's' heat tolerance.

Acknowledgement

This research was supported by a research grant from the Texas Wheat Producers Board.

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