Impact of high temperatures in maize: phenology and yield components.

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Abstract

Heat stress is a main threat to current and future global maize production. Adaptation of maize to future warmer conditions requires improving our understanding of crop responses to elevated temperatures. For this purpose, the same short-season (FAO 300) maize hybrid PR37N01 was grown over three years of field experiments on three contrasting Spanish locations in terms of temperature regime. The information complemented three years of greenhouse experiments with the same hybrid, applying heat treatments at various critical moments of the crop cycle. Crop phenology, growth, grain yield, and yield components were monitored. An optimized beta function improved the calculation of thermal time compared to the linear-cutoff estimator with base and optimum temperatures of 8 and 34 °C, respectively. Our results showed that warmer temperatures accelerate development rate resulting in shorter vegetative and reproductive phases (*ca*. 30 days for the whole cycle). Heat stress did not cause silking delay in relation to anthesis (extended anthesis-silking interval), at least in the range of temperatures (maximum temperature up to 42.9 °C in the field and up to 52.5 °C in the greenhouse) considered in this study. Our results indicated that maize grain yield is reduced under heat stress mainly via pollen viability that in turn determines kernel

number, although a smaller but significant effect of the female component has been also detected.

Keywords

Heat stress; maize; kernel number; anthesis; beta function

Introduction

FAO has reported an improvement in food security in the last two decades, with a global reduction of undernourished people of 216 million in 2015 compared to 1990–92. These figures are especially encouraging in developing regions dropping from 23.3 % of the population undernourished in 1990–92 to 12.9 % in 2015 (FAO, et al., 2015). In spite of these positive data, the already observed and projected impacts of climate change on agriculture (IPCC, 2013) and their implications for the food security of current world population and of the 9 billion people foreseen by 2050 emphasize the urgent need for farmers to adapt to a changing climate (FAO, 2016). In addition, crops with high water requirements cultivated under semi-arid or arid conditions require to be adapted to the new climate conditions to increase water productivity and irrigation water efficiency (Molden et al., 2010) in an elevated temperature environment.

The major staple crops, such as maize (*Zea mays* L.), the cereal with greatest world production (in the period 2010-2014, average production was 932.7 Million Mg with an average yield of 5.27 Mg/ha, http://www.fao.org/faostat/en/) will need to adapt to the new conditions. Maize is cultivated in a wide range of climate conditions, following the rainy season in tropical regions and as a summer crop in temperate ones, with high irrigation requirements under semi-arid conditions. Maize adaptation should deal not only with changed climate averages, but also with the increased frequency and intensity of extreme events (IPCC, 2012). More specifically, several studies have identified heat stress as a main threat for future maize cultivation in several relevant production regions (e.g. Gourdji et al., 2013).

Kernel number, i.e. the size of the physiological sink of assimilates, is a key yield component to determine final maize grain yield (Fischer and Palmer, 1984; Andrade et

al., 2000). In turn, this component is closely related to the source of assimilates during a narrow time window of four or five week period around anthesis (Fischer and Palmer, 1984; Otegui and Bonhomme, 1998; Andrade et al., 1999). No clear dependency of the kernel number on growth rates during the occurrence of heat stress in pre-silking period has been found (Cicchino et al., 2010b). However, heat stress during the period around silking leads to high yield reduction (Cicchino et al., 2010b) affecting both plant sources and sinks. Source capacity is directly affected by a reduced synthesis of carbohydrates (Barnabás et al., 2008), in turn caused by decreased photosynthesis and escalated respiration rates (Rattalino-Edreira and Otegui, 2012; Wahid et al., 2007; Ordoñez et al., 2015). Sink capacity is affected by the disruption of the anthesis-silking synchrony, reduced ovule fertilization and increased kernel abortion. In turn, these effects disturbs pollination and kernel set and can result in severe yield losses (Herrero and Johnson, 1980; Rattalino-Edreira et al., 2011; Ordoñez et al., 2015; Dupuis and Dumas, 1990; Cicchino et al., 2010b). Also, recent studies (Rattalino-Edreira et al., 2011; Ordoñez et al., 2015) have found an important role of the female component of the sinks in the maize response to heat stress.

The upper optimum temperature for maize flowering has been considered to be between 29 and 37.3 ºC (Schlenker and Roberts, 2009; Gilmore and Rogers, 1958; Tollenaar et al., 1979; Cicchino et al., 2010b; Porter and Semenov, 2005; Sánchez et al., 2014). Some authors have explained partially this wide range by the experimental error coming from considering air temperature instead of canopy temperature (Craufurd et al., 2013; Siebert et al., 2014, 2017; Webber et al., 2016, Lobell et al., 2008) or plant profile temperature (Rattalino-Edreira and Otegui, 2012). Differences in vapor pressure deficit (VPD) may also affect these responses. On one hand, the difference between those temperatures can be especially large under irrigated conditions (up to 10 ºC according to Kimball et al., 2015), but even the smaller differences registered under rainfed conditions (*ca*. 2 ºC) can lead to underestimation of heat stress impact (Webber et al., 2016). Most of the previous experiments introduced modifications in temperature, gas exchange, wind profile and radiation not just in the greenhouse experiments but also in the field ones (e.g. by using polyethylene films as Cicchino et al., 2010a,b; Rattalino-Edreira et al., 2011; Ordóñez et al., 2015) to achieve fully or partially controlled heat stress conditions. On the other hand, increases in air temperature under field conditions

usually induce higher VPD, enhancing the demand for soil water and the effect of water deficits (Mittler, 2006), which in turn can raise canopy temperature.

The objective of this study was to improve the understanding of the response of maize development, growth and grain production to heat stress conditions. For that reason, our study combines data collection under controlled conditions (greenhouse) with field experiments under natural conditions with unperturbed wind, radiation, humidity, and temperature regimes. Also, data collection on the same hybrid under several field and controlled conditions across all years was crucial to remove the uncertainty linked to genotype variation.

Materials and Methods

Experimental conditions and design.

Field treatments

The study was conducted over three years (2014-2016) growing the short-season maize (*Zea mays* L.) hybrid PR37N01 (FAO-300) in three locations in Spain with a North-South thermal gradient (Candás in Northern Spain, Aranjuez a Central site, and Córdoba in the South, Figure 1a). The soils of the field experiments (Figure 1b), were fertilized according to soil analysis recommendations, typically with 250 kg N/ha split in two applications at V4 and V8, to avoid nutrient limitation. Irrigation was applied weekly or as required to maintain soil moisture near field capacity. The amounts varied according to the soil, year, and crop cycle based on the reference evapotranspiration (ET0), but typically 40 mm were applied weekly for a total seasonal irrigation of ca. 550-700 mm in the Center and South, less in the North. Crops were protected from pests, diseases and weeds, and management was adjusted to local conditions and practices.

Treatments for field experiments consisted of two sowing dates in each location (Table 1), aimed to cover a wide range of temperatures through the growing cycle. The experimental design was completely randomized, with four replications of plots 10-12 m length containing six rows 0.75 m apart, at a target plant population density of 5 plants m^{-2}.

Greenhouse treatments

In parallel, a greenhouse experiment was conducted over the same years and with the same hybrid in Madrid (within the Experimental Fields of the Technical University of Madrid, 40°26'14"N 3°44'10"W, 657 m above mean sea level). Two greenhouses, cool and hot, differing in target daytime temperature, provided the controlled environment to complement the information for this work. The controls were adjusted in the cool greenhouse to maintain the daytime temperature around 25° C, and above 35° C in the hot greenhouse. Late in the afternoon, heating and cooling systems were switched off and windows opened allowing both greenhouses to equilibrate with outside temperatures. Sensors measuring photosynthetically active radiation, PAR (QSO-S, Decagon, Pullman, Washington, USA) and temperature and relative humidity (PASS VP-3, Decagon, Pullman, Washington, USA) were located in the center of each greenhouse. The same hybrid used in the field experiments was sown in 15-L pots filled each year with a fresh mixture (1:1:1) of sand, peat, and compost. Three seeds per pot were planted on late May or early June during 2014, 2015, and 2016, and thinned to one plant at V3. Pots were irrigated twice a day, 8:30 am and 8:30 pm, during 2 minutes, and fertilized weekly with 5 g per pot of 15-15-15/18 (water soluble SO_3). The experimental unit consisted of three plants replicated three times, with two pollen sources used for pollination, for a total of 18 plants per treatment. All topmost ears were hand-pollinated at mid-morning three days after silking, half with pollen from the same treatment (local pollen) and half with fresh pollen collected in a nearby field. Heat treatments consisted of moving 18 plants of the corresponding treatment from the cool greenhouse to the hot greenhouse, and returning the plants back to the cool greenhouse seven days later. Heat treatments were applied at specific phenological times (Table 1). In 2015 and 2016 an additional treatment was incorporated by maintaining 18 plants in the hot greenhouse all the season. In 2016 the V4 treatment was changed. Two complete treatments were maintained in the hot and in the cool greenhouses until V4 and then the plants were switched reciprocally and maintained in the new location until the end of the season. Also in 2016, to further examine heat effect on pollen and pistils, we incorporated two additional treatments with plants growing all season in the hot and cool greenhouses. Half of the plants growing in the cool greenhouse were pollinated with pollen from plants growing in the hot greenhouse. Reciprocally, half of the plants growing in the hot greenhouse, were pollinated with pollen from the cool greenhouse.

169 The remaining plants as usual, were pollinated with fresh pollen. No grain filling (GF)
170 treatment was included in 2016. Therefore, there were six treatments in 2014 (108
171 plants from 18 plants x six treatments), seven in 2015 and nine in 2016. Treatments
172 were identified as follows: plants in the cool greenhouse all crop cycle or control (C),
173 heat at V4 (V4), in cool greenhouse up to V4, then moved to hot greenhouse (V4c), in
174 hot greenhouse up to V4, then moved to the cool greenhouse (V4h), heat at V9 (V9),
175 heat at anthesis (FL), heat at lag phase (LG), heat at early GF, heat all crop cycle (H),
176 plants in the cool greenhouse pollinated with pollen from the hot greenhouse (CxH),
177 and the corresponding opposite (HxC) .
178
179 Weather data
180
181 In the field experiments, automatic weather stations at each site provided daily records
182 of maximum and minimum temperature, precipitation and radiation for all sites, and
183 also relative humidity and wind speed for the Central and South sites. Weather station in
184 Cordoba is part of the Agroclimatic Information Network (RIA, in Spanish) described
185 in Gavilán et al. (2006).
186
187 In the greenhouse, the relative humidity, air temperature and PAR were recorded every
188 5 minutes separately for the cool and hot greenhouses.
189
190 Crop data
191
192 *Field measurements*
193 Plants were monitored every other day to determine emergence date (seedlings visible
194 above the soil surface), anthesis (anthers visible) and silking (silks visible). Leaf
195 number or stage and physiological maturity were monitored weekly. Plants in a
196 treatment were considered to be in a particular phenological stage when 50% of the
197 plants had reached that stage. Leaf stage was determined by numbering the leaf collars
198 (V-stage, Ritchie et al., 1993) and the visible leaf tips (T-stage). Anthesis or silking date
199 were estimated by fitting a sigmoidal curve as function of time (days after sowing,
200 DAS) to the average percentage of plants (P, %) with anthers or silks exposed:
201

$$P = \frac{Px}{1+\exp(-s(t-t_{50}))} \quad (1)$$

Where *Px* is the maximum percentage of plants with visible anthers or silks, parameter *s* determines the slope of the function, *t* is the current day in DAS and t_{50} is the DAS when 50% of the population reaches anthesis or silking. Data to fit the function was collected from a two-row sample of 10 m in each repetition. Physiological maturity was determined by weekly sampling of five ears per repetition. Next, 30 seeds were taken from the mid-section of each ear to determine average dry matter per seed and percentage of grain humidity in the laboratory. Physiological maturity was considered to happen when the mean seed dry weight stopped increasing. Crop cycle was calculated as the chronological time from emergence to physiological maturity.

Above ground biomass was measured twice, at silking and physiological maturity. Biomass was separated into leaves, stalks and ears (including seeds). In addition for the first sowing date, tassel size after pollen shed and Tassel Area Index (*TAI*) were measured sampling 3-5 tassels per repetition to estimate the amount of pollen produced (Fonseca, 2004). Since some authors have shown a relationship between the total expanded plant leaf area and ear leaf area (Lizaso et al., 2003), leaf area of the largest leaf (ear leaf) and final number of leaves were recorded for 3 consecutive tagged plants per repetition. The leaf area was estimated by Montgomery allometry.

After all plants in a treatment reached physiological maturity the plants of the two central rows per repetition were harvested. The grain yield components recorded were number of ears per plant, kernel number and weight.

Greenhouse measurements

Phenology was monitored in the same way than in field experiments. Above ground biomass was not monitored. At silking the apical ear was covered and hand pollinated tree days later with pollen either local (same treatment) or fresh (from a nearby field sown with the same hybrid). After pollination, ears were covered again. Yield and yield components were measured on every plant similarly to field experiments but harvesting only the topmost ear per plant.

Thermal time calculation

We calculated and compared two procedures to estimate thermal time using the daily mean temperature. The standard method assumes that the rate of development increases linearly between a base temperature of 8 ºC and an optimum temperature of 34 ºC. Temperatures above the optimum will result in a constant thermal time as at 34 ºC, therefore does not consider the reduction in development rate at supraoptimum temperatures. The simplified beta function (Eq. 2, Yan and Hunt, 1999) is calculated as:

$$TTd = TTx\left(\frac{Tx-T}{Tx-To}\right)\left(\frac{T}{To}\right)^{\frac{To}{Tx-To}} \quad (2)$$

Where *TTd* is the estimated daily thermal time and *T* is the daily mean temperature, *To* and *Tx* are the optimum and maximum temperatures, and *TTx* is the maximum thermal time at the optimum temperature *To*. The function assumes a gradual curvilinear increase in thermal time between *T* values of zero and *To*, and a corresponding drop between *To* and *Tx*.

Statistical analysis

Statistical analysis consisted of an ANOVA analysis applied per year considering the factors location and sowing and their interaction (location x sowing). A Duncan test at 0.05 significance level was applied to compare means and for the calculation of standard deviations per treatment. All calculations were done with SPSS software (IBM Corp. Released v. 20, 2011).

Results

Effects of heat stress on phenology

Field experiments

The three field experimental locations showed an increasing Southward gradient of temperatures. In our hottest year (2015) and location (South) the range of mean temperatures during the growing season was 12.5 – 32.4 ºC. Maximum temperatures (*Tmax*) registered in the North site were always below 30 ºC, while *Tmax* was over 35 ºC for several days in both Central and South locations, mostly in the middle of the crop

cycle, and over 40 ºC for some days in the summer of 2015 (Figure 2 for the 1st sowing date). These high temperatures matched anthesis and grain filling, particularly in the Southern location. *Tmax* in this site was over 35 ºC during the period from *ca*. 40 days after emergence nearly up to physiological maturity for the first sowing date of the three years. Weather conditions of the second sowing were very similar (Figure S1 in suppl. material). Diurnal temperature range was much smaller in the North place than in the other locations (Figure 2), and this site received *ca*. one third less radiation than the other ones. Radiation was similar in the Central and South sites during the whole cycle except for the last month of crop cycle, when radiation was lower in the Central site.

Crop cycle duration (in days after emergence, DAE) showed a decreasing Southward gradient, with mean yearly values of 121, 110 and 92 for North, Central and South locations, respectively (Figures 2 and 3). This spatial variability was greater (7 days of difference between locations) in 2015 and 2016. Besides, milder temperatures resulted in longer crop cycle in the North. In spite of these variations in crop cycle, the anthesis-silking interval (ASI) was similar in the three locations and therefore it was not affected by the hot conditions experienced in the Central and Southern locations.

Number of leaves was recorded only in 2015 and it was explained as a function of thermal units (degree-days, GDD) accumulation since emergence (Figure 4 a, b). Thermal time accumulation over a base temperature of 8 ºC did not result in a single rate of ligulated leaf (V stage) appearance at the three sites (Figure 4a). However, when the simplified beta function was applied (Eq. 2, Yan and Hunt, 1999), a single appearance rate of ligulated leaves (V stage) could be estimated for all locations (0.019 leaves GDD^{-1}) (Figure 4b). Although the R^2 coefficients were similar when the linear regressions were calculated separately for every treatment and location, when all data were pooled together the R^2 coefficient improved from 0,85 to 0,92 when the linear function was substituted by the simplified beta function (Table S1 in suppl. mat, for V-stages).

The three parameters of Eq. 2 (*TTx, To, Tx)* were optimized with the Simulated Annealing algorithm (Goffe et al., 1994), keeping separated the vegetative and the reproductive phases. Two sets of parameters were adjusted: For the sowing to anthesis

period, *TTx* was set to 16.28 GDD, *To* was 30.04 ºC and *Tx* was 47.63 ºC, while for the anthesis to maturity period *TTx* was set to 15.2 GDD, *To* was 24.95 ºC and *Tx* was 46.94 ºC (a more detailed description of the parameter optimization and results of this improvement on phenology modelling can be found in Lizaso et al., 2017b, this special issue).

Greenhouse

Cool greenhouse temperature was kept below 25-26 ºC during daytime, and around 20 ºC during nighttime, depending on the external temperature. Air relative humidity was between 55% and 90% (with some records close to 100% at the end of the cycle, with a mean daily value throughout the cycle of *ca*. 70%. Hot greenhouse daytime temperature was kept above 35ºC with daily *Tmax* typically of 42-43ºC, and similar to the cool greenhouse during nighttime. Air relative humidity decreased to around 40% during daytime, with oscillations between 30 and 80%. Photosynthetically active radiation (*PAR*) was similar in both greenhouses, with maximum daily values close to 1500 µmol m^{-2} s^{-1}. Figure 5 illustrates these conditions in both the hot and cool greenhouses for the week from 20th to 27th of July 2015, which matched the moment of FL treatment in the hot greenhouse.

Duration of the vegetative period was recorded in these experiments (reproductive phase duration was not recorded as destructive sampling of ears was not carried out). The effects of the heat stress on the vegetative phase duration was independent of the treatment but for H (Figure 6) in 2015, for which the vegetative period was *ca*. 6 days shorter than for plants under all other treatments. However, duration of the vegetative phase of the H treatment in 2016 was similar to all other treatments.

Number of leaves was recorded in 2016 and it was represented as a function of thermal time accumulation since emergence (Figure 4 c, d). As in the field experiments, a single ligulated leaf appearance rate could be used only when a beta function was applied with the same parameters used in the field experiments (Figure 4d). The R^2 coefficients from the linear regression improved from 0.87 to 0.96 when beta function was fitted to the greenhouse data, and from 0.81 to 0.93 when both field and greenhouse data from 2015 and 2016 were fitted together (Table S1 in suppl. mat, for V-stages).

Crop growth, yield and yield components

Field experiments

Ear leaf area was used as an indicator of total plant leaf area (Table 2). There was a Southwards gradient of decreasing leaf area with mean values of *ca*. 750 cm^2 in the North and of *ca*. 635 cm^2 in the South. These differences between leaf area values registered at each location were significant and they were related to the differences in leaf width, which was *ca*. 8% larger in the North locality as compared to the South one. There were no differences in leaf area and width between sowing dates of the same location (Table 2).

Biomass partitioning between leaves, stems and ears revealed that the ear is the organ accumulating more biomass (between 65-70% of the total biomass) followed by the stem (20-25%) and the leaves (10% of total biomass) (Figure 7). Mean total dry mass per plant ranged between 400 and 500 g/plant across locations, with greater share in the ear, and therefore harvest index, in the North location. Stem biomass represented a higher percentage of the total plant biomass (*ca*. 30%) when conditions were especially hot, as in the second sowing of warmest locations in 2015, than in cooler conditions (e.g. 20% in the North experiment). Differences between sowing dates of the same location were found for leaves biomass in 2014 and 2015 and for stem and ear biomass only in 2015 in the Central location (Figure 7).

Significant differences between locations were also found for tassel size and TAI (Table 3). *TAI* was twice larger in the warmest location in the South (*ca.* 2700) than in the North experiment (*ca.* 1350). Estimated pollen production ranged in an increasing Southwards gradient from less than 3 million of grains in the North to 5 million in the South experiment, accordingly to tassel size, *TAI* and temperature (Table 3).

Number of grains per ear was the most variable yield component ranging between 390 and 600 grains/ear with significant differences between locations and years. A clear spatial pattern could not be identified, but data from the Northern site showed less variation between years and it was the only location not affected by the hot summer of 2015, that reduced kernel number and weight in Central and South locations (Figure 8).

Mean kernel weight showed small variation as expected, ranging between 290 and 350 mg, with the highest values in the North experiments in 2014 and 2016 and in the South in 2015. No differences between sowing dates of the same location were observed for kernel number. Kernel weight was greater for the first sowing date than for the second in both 2014 and 2015.

As a result of both components, crop grain yield in the South location was always lower than in the other locations, with differences up to 500 kg/ha (equivalent to 150 g plant^{-1}, results not shown). Yield was also low in the Central location in the hot summer of 2015 (150 g plant^{-1} lower than in other locations). Yield was greater for the first sowing date than for the second in both 2014 and 2015.

Greenhouse

All measurements refer to the 1st ear as it was the only one hand-pollinated with local or fresh pollen.

Tassels and *TAI* from plants grown during the whole cycle in the hot greenhouse (H treatment) were 40% larger than those of the plants from cool greenhouse (data not shown).

Timing of heat stress affected kernel number and kernel weight differently (Figure 9). The high variability of both yield components within some treatments may have masked some differences. A decrease in the number of kernels per ear respect to C treatment was found for all years for some heat treatments, especially for FL, H and V4c (*ca*. 70-100% of decrease). In 2014, no differences between treatments were found for the mean kernel weight (mean values 380 mg), but in 2015 kernel weight was lower and pollen source made a difference for the H treatment (180 mg for plants pollinated with local-pollen and 250 g for plants pollinated with fresh-pollen). Mean kernel weight for the other treatments was 340 mg in 2015. In 2016, the kernel weight decreased for the same treatments than kernel number (for V4c, FL and H) but in a lesser extent.

In coherence with the effects on kernel number and weight, depending on its timing, heat stress affected crop yield differently (Figure 9). When the heat stress was applied before anthesis (V4 and V9), yield was similar to that of the C treatment (mean yield in

2014 and 2015 was between 165-170 g plant^{-1}). When the heat stress was applied during grain development and filling (LG and GF), a slight decrease of crop yield was found in 2014 and 2016 for LG and for GF in all years, with no effect for the LG treatment in 2015. Yield was especially reduced (50-55 g/plant in 2014 and 2015) when heat stress occurred during anthesis (FL), and maximum losses were found for the H treatment in 2015 (mean yield 35g plant^{-1}) (Figure 9). In 2016, yield followed the same pattern than the yield components, with very low yields for V4c, FL and H treatments.

In the treatments mentioned above, the pollen source (local or fresh) only showed clear influence in plants under LG and H treatments, for which mainly kernel number and weight and consequently yields were dramatically reduced when plants were pollinated with local pollen (Figure 9).

The additional treatments of 2016 allowed evaluating in depth how pollen viability was affected by heat stress (Figure 10). Plants in the cool greenhouse only showed pollen viability problems when pollinated with pollen from the hot greenhouse: kernel number and yield were dramatically reduced, although kernel weight was not. Plants in the hot greenhouse presented an important decrease in kernel number and weight and in final yield whatever the pollen source, and the crop failed (no kernel set) when pollen was local (i.e. from the hot greenhouse) (Figure 10).

Discussion

Crop phenology

The purpose of this study was to assist improving our understanding of the responses of maize crops to heat conditions. Our first concern was the developmental responses. We found one-month difference in crop cycle (emergence-maturity) between our Northern and Southern locations. This is especially significant considering that we worked with a short-season hybrid, and illustrates the adequacy of the sites selected for this study. This also raised a major challenge to correctly calculate thermal time to estimate crop phenology.

The classic maize thermal time model, based on a linear response between a base temperature (*Tb*) of 8 ºC and an optimum temperature (*To*) of 34 ºC, was not able to

436 explain the evolution of the number of leaves for every experiment and year with a
437 single development rate. One of the reasons might be that this type of model does not
438 consider supra optimum temperatures, which according to previous results (Rattalino-
439 Edreira et al., 2011; Ordoñez et al., 2015; Cicchino et al., 2010a) as well as ours
440 strongly influence maize development and growth. Other possibility might be the need
441 to reparametrized *Tb* and/or *To*. Instead, a beta function describes the response to
442 temperature as a smooth curve more realistically than linear or multi-linear models (Yan
443 and Hunt, 1999), and uses biologically meaningful parameters related not only to *To*
444 (and *TTx*), but also to the *Tx* over which crop development would stop. Therefore, the
445 effect of supra optimum temperatures above *To* is considered, with a slower
446 developmental rate between *To* and *Tx* and null development over *Tx* (in agreement
447 with Cicchino et al. 2010a), which makes this parameterization especially useful for
448 assessing adaptation to warming climate conditions and to an increased frequency of
449 extreme *Tmax* events. The improvement of phenological models is not only needed for
450 maize. Traditional phenological models, parameterized for current climate conditions,
451 are being challenged when simulating crop phenology under projected future conditions
452 for many crops as for instance olive (Gabaldón-Leal et al., 2017). Then, extending
453 experimentation to other crops and hotter temperatures more representative of future
454 climate projections is required.
455
456 One main issue in the developmental response to temperature is the uncertainty in *To*
457 and especially in *Tx* parameterization. The reason is that most of the available
458 experimental data are from controlled environments, typically with constant day/night
459 temperatures, and few treatments at supra optimum values and *Tx* determined by
460 extrapolation (e.g. Warrington and Kanemasu, 1983). In other cases, information was
461 originated in field experiments conducted in temperate regions (Kumudini et al., 2014;
462 Bassu et al., 2014) where supra optimum temperatures are not usually reached (notice
463 that both *To* and *Tx* refer to mean daily temperature, not just to instantaneous
464 temperature). Therefore, to reduce the uncertainty in *To* and *Tx* estimation there is a
465 need for field experiments reaching supra optimum daily mean temperatures under non-
466 modified conditions. Our experiments contribute to fill this gap. We included a wide
467 range of conditions, not only by supplementing field experiments with greenhouse data,
468 but also by selecting 3 field locations with contrasting temperature and radiation
469 regimes, two of them with frequent supra optimum summer temperatures. This is a step

forward from former experiments that used polyethylene films to induce warming in the field plots, as these films are reported to reduce incident *PAR* by 12% average (Ordoñez et al., 2015). According to Ordoñez et al. (2015) the reduction in incident PAR could be compensated by the increase in radiation use efficiency due to a larger proportion of diffuse radiation induced by the film. This effect has not been quantified, nor the changes in light quality, red to far-red light ratios (Ballaré and Casal, 2000), and crop evapotranspiration. Part of our data was also collected in a greenhouse, in which *PAR* was reduced by *ca*. 50% compared to outdoors radiation. Yet we complemented this information with field experiments with the same genotype. Also, another step in reducing the mentioned uncertainty would be to check if plant development rate parameterization should be different for the periods before and after anthesis due to intra season acclimation effects (Cicchino et al., 2010a).

The lack of differences between greenhouse treatments in 2016 in the duration of the vegetative phase could have been because temperatures in the greenhouse were slightly hotter in 2016 than in 2015. All treatments in 2016 were exposed to temperatures over the *To* of the vegetative phase (30.04 ºC), including C treatment that registered 21 days with some hours over *To*. This was not the case of C treatment in 2015, with no days over *To*. Finally, it is noticeable that we have not found significant differences between treatments in the *ASI*, unlike previous studies as Ordóñez et al. (2015) and Cicchino et al. (2010a, b). A possible explanation could be again that in our field experiments no cover neither any other heat-forcing modification was introduced, while in the mentioned studies the polyethylene film used could impact the *ASI* through the changes in the crop microenvironment discussed above. According to our results heat stress does not cause silking delay in relation to anthesis, at least in the range of temperatures considered in this study.

Crop growth, yield and yield components

The higher leaf area production in the North experiment compared to the rest of field locations could be explained by the lower radiation and temperature of this site, which would result in a different pattern of assimilate partitioning targeted to maximize radiation interception (Vos et al., 2005). Biomass production was also higher in the North, and it followed an opposite gradient to temperature comparing the 3 locations:

504 the higher the temperature, the lower the biomass and the leaf area, in agreement with
505 Cicchino et al. (2010b). The larger fraction of biomass in stems when the conditions
506 were hotter suggested that the shorter grain filling duration might have limited
507 assimilate mobilization to the sinks. Other possibility might have been that mobilization
508 was restricted by sink size. However, the lack of correlation between the duration of the
509 reproductive phase (anthesis-maturity) and kernel weight, and between stem mass and
510 kernel number, did not allow confirming any of these hypotheses. In addition, it is
511 possible that a high atmospheric VPD-induced water stress might contribute to such
512 impacts in the hottest locations.
513 Tassel size and *TAI*, calculated as the surface covered by male flowers (Fonseca, 2004)
514 from both South and greenhouse experiments was double than in the North. Also H
515 treatments in the greenhouse produced the highest *TAI.* If pollen is produced
516 accordingly, this would imply pollen production being enhanced by higher
517 temperatures. However, previous studies have reported the opposite (Schoper et al.,
518 1987). Fonseca (2004) developed the *TAI* using data from Minnesota (44 °N) and Iowa
519 (42 °N), both sites characterized by mild summer weather, with very rare occurrence of
520 supra optimum temperatures. To the best of our knowledge this is the first time that
521 pollen production per tassel is estimated using *TAI* under hot growing conditions. Our
522 observations question the applicability of the method under heat stress conditions,
523 unless information about pollen production per anther and loss of floral structures is
524 incorporated, as discussed by Fonseca (2004).
525
526 The location with the largest kernel number changed across years, as this is the most
527 responsive yield component to temperature change. In 2015, with warmer temperatures,
528 the largest kernel number site was the North, while in 2014 and 2016 that were closer to
529 average years in terms of temperature, the largest kernel number was found in the
530 Center. However, kernel weight was the most variable factor between sowing dates of
531 the same location, being higher for the first sowing date and resulting in higher yields
532 for this sowing, probably associated to temperatures during grain filling. It is noticeable
533 that at least in Central and South locations crop flowering occurred well over supra
534 optimum temperatures for both sowings; this can contribute to explain the lack of
535 significant differences between kernel number from sowing dates of the same location,
536 as they would be affected similarly by heat stress. A consistent relationship between
537 kernel weight and grain filling duration was not found, when data from all locations and

sowings were compared. Based on these data, we hypothesize that critical temperature for kernel weight would be higher than for kernel number, and it would have been overpassed only by the second sowing. Critical temperature for kernel number would be lower and would have been overpassed by both sowings.

The greenhouse experimental protocol consisted in pollinating exposed silks only once on the third day after silk emergence, with either local or fresh pollen from outside, and preventing any additional exposure to pollen. This allowed evaluating the impact of heat on pollen viability, but not the impact on silk receptivity. According to Bassetti and Westgate (1993) silk receptivity period lasts normally 6 days under non-stress conditions, but some shortening of the receptivity period would be expected under heat stress conditions. From the comparison of kernel number from all treatments pollinated with either pollen source, it became evident that plants exposed to heat around anthesis (FL, H, V4c) were always affected, with an additional impact if pollinated with local (hot) pollen, although due to high plant-to-plant variability, differences were not always significant. Previous reports have shown similar results (Cicchino et al., 2010b; Ordóñez et al., 2015), but always using fresh pollen. Only our experimental protocol examined the effect of fresh *vs.* local pollen. Plants exposed to heat during early grain filling (*ca.* 20 days after anthesis) also exhibited less kernels and grain yield. Since pollination was completed in the cool greenhouse, these reductions in seeds per ear should be the consequence of kernel abortion (Rattalino-Edreira et al., 2011).

We further examined the effects of heat on pollen and pistils by pooling information from additional treatments (CxH, HxC) of our 2016 greenhouse experiment. Same kernel number and yield were observed in plants grown in the cool greenhouse when fertilized with pollen from the cool greenhouse or from outside. This indicated that enough viable pollen and receptive silks were available for maximum kernel set (average C treatments 427.3 ± 50.6 kernels ear^{-1}). When plants were fertilized with pollen from the hot greenhouse, a 75% reduction was observed in kernel number. This reduction was totally ascribed to the loss of pollen viability resulting from the heat applied in the hot greenhouse. On the other hand, the substantial decrease in kernel number observed when fresh pollen or pollen from the cool greenhouse was used to fertilize plants growing in the hot greenhouse cannot be entirely credited to loss of silks receptivity, since under the conditions of the hot greenhouse pollen would desiccate

very rapidly and loss viability (Fonseca and Westgate, 2005). Compared to corresponding kernel numbers in the cool greenhouse, reductions were in the order of 60.7% for plants fertilized with pollen from the cool greenhouse and 83.6% for plants exposed to fresh pollen. Cicchino et al. (2010) reported reductions in kernel number between 52 and 61% for plants exposed to heat and pollinated with fresh pollen. Therefore, our study provides evidence supporting the view that there is a major impact of heat on pollen viability already known (Cicchino et al., 2010). We also established that there is a smaller but significant and additive effect on the female component. The existence of a female component of the impact is also supported by Cicchino et al. (2010b) and Ordoñez et al. (2015), who found both male and female sterility and kernel number reduction mainly related to abortion of fertilized flowers and not to pollination problems when the plants were exposed to fresh pollen (Ratalino-Edreira et al. 2012).

Finally, in this study we have described the main mechanisms driving the impacts of supra optimum temperatures on maize development, growth and yield, in the absence of soil water deficits. Including these mechanisms in a process based crop model will allow assessing more accurately the effect of elevated mean temperatures and single and multiple heat stress events on crop development, yield components, and yield. Further improvements could be possible by examining the combined effects of temperature and limited soil water (Mittler, 2006), and other atmospheric factors, such as VPD and [CO_2], especially relevant under the climate change context which primarily motivated this study. In this sense, a number of recent reports have examined the performance of maize germplasm with limited transpiration under elevated temperature and VPD, while maintaining adequate water supply (Shekoofa et al., 2016; Yang et al., 2012; Gholipoor et al., 2013). Similarly, Perdomo et al. (2016) identified the acclimation capacity of maize photosynthesis to heat, VPD, and drought. Also, the interaction between the atmospheric [CO_2] and temperature can be modified by the water status as shown in FACE experiments. Under no water stress conditions Manderscheid et al. (2015) found that maize responded to high atmospheric [CO_2] by reducing the stomatal conductance and increasing the VPD and the daytime canopy temperature which would worsen the effects of heat stress described in this study. Under water deficit, the reduced transpiration saved soil water displacing the water stress period which might alleviate the effects of heat (Manderscheid et al., 2015). These complex interactions underline the

need to evaluate crop responses to these stresses under field conditions (Cairns et al., 2013).

Conclusions

To and *Tx* are key parameters for estimating plant developmental rate but currently large uncertainty in their estimation under warm conditions affect the quality of the estimations. Thus, additional field experiments in warm conditions, with a wider range of temperatures that assure *To* being overpassed are needed to reduce this uncertainty. The simplified beta function can take this range of temperatures into account and seems to be a more effective approach than classic thermal time accumulation above a *Tb*.

The results obtained in this study indicated that heat stress does not cause silking delay in relation to anthesis, at least in the range of temperatures considered. We conclude that maize yield is reduced under heat stress mainly via pollen viability that in turn determines kernel number, although a smaller but significant additive effect of the female component has also been detected.

Acknowledgements

This work was financially supported by project MULCLIVAR, from the Spanish Ministerio de Economía y Competitividad (MINECO, CGL2012-38923-C02-02); by the Spanish National Institute for Agricultural and Food Research and Technology (INIA, MACSUR01-UPM) through FACCE MACSUR – Modelling European Agriculture with Climate Change for Food Security, a FACCE JPI knowledge hub; and through SUSTAg project (INIA, 652915 ERA-NET Cofund FACCE-SURPLUS). The field and greenhouse support by Roman Zurita was greatly appreciated.

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770 **Table 1**

771 Sowing and emergence dates of the three field experimental sites and greenhouse
772 treatments for 2014, 2015 and 2016. Treatments were identified as follows: plants in
773 the cool greenhouse all crop cycle or control (C), heat at V4 (V4), in cool greenhouse up
774 to V4, then moved to hot greenhouse (V4c), in hot greenhouse up to V4, then moved to
775 the cool greenhouse (V4h), heat at V9 (V9), heat at anthesis (FL), heat at lag phase
776 (LG), heat at early grain filling (GF), heat all crop cycle (H), plants growing in the cool
777 greenhouse pollinated with pollen from the hot greenhouse (CxH), and plants growing
778 in the hot greenhouse pollinated with pollen from the cool greenhouse (HxC).
779

Field experiments				Greenhouse experiments	
Year	Site	Sowing date	Emergence date	Year	Treatment
2014	North	20 May	01-Jun	2014	C
	North	27 May	04-Jun		V4
	Central	20 May	30-May		V9
	Central	06 June	13-Jun		FL
	South	29 May	05-Jun		LG
	South	17 June	24-Jun		GF
2015	North	18 May	24-May	2015	C
	North	25 May	03-Jun		V4
	Central	30 April	07-may		V9
	Central	15 May	28-May		FL
	South	07 May	13-may		LG
	South	21 May	26-May		GF
2016	North	23 May	02-Jun		H
	Central	17 May	25-May	2016	Control

	South	19 May	24-May	V4h
				V4c
				V9
				FL
				LG
				H
				CxH
				HxC

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781

Table 2

Length, width, and area of the ear leaf (as an indicator of total leaf area) for the locations and years for which measurements were done. Significance of the analysis is shown for location and sowing factors. Standard deviation is shown in brackets.

Experiment /factor	Ear leaf length (cm)	Ear leaf width (cm)	Ear leaf area (cm^2)
2015			
North			
1st sowing	89.1 (5)	11.6 (0.2)	775 (50)
2nd sowing	86.9 (4)	11.0 (0.8)	718 (30)
South			
1st sowing	81.1 (4)	10.5 (0.8)	642 (77)
2nd sowing	89.8 (2)	10.3 (0.2)	697 (30)
Location	ns	*	**
Sowing	ns	ns	ns
Location x Sowing	*	ns	*
2016			
North	91.3 (0.4) b	11.0 (0.1) a	753 (3.6) a
Central	96.3 (1.2) a	9.6 (0.3) b	691 (15) b
South	86.9 (1.6) c	8.7 (0.2) c	565 (23) c
Location	***	***	***

*, **, ***, ns: significant at P ≤0.05, 0.01, 0.001, and not significant, respectively. Means within columns followed by different letters differ significantly at P ≤0.05 (Duncan's multiple range test)

Table 3

Tassel size expressed as Tassel Area Index (*TAI*, Fonseca, 2004) and amount of pollen produced (millions of grains), with the corresponding standard deviation, from the 1st sowing of the field experiments. Significance of the analysis is shown for location factor.

Experiment /factor	TAI	SD	Tassel pollen grains (Million grains)	SD
2014				
North	1302 c	246	2.79c	0.46
Central	1719 b	334	3.57b	0.63
South	2526 a	322	5.08a	0.60
Location	***		***	
2015				
North	1396 c	200	2.96c	0.37
Central	1739 b	135	3.60b	0.25
South	2839 a	407	5.66a	0.76
Location	***		***	
2016				
North	NA		NA	
Central	2024	49	4.14	0.09
South	2908	282	5.79	0.53
Location	***		***	

*, **, ***, ns: significant at P ≤0.05, 0.01, 0.001, and not significant, respectively. Means within columns followed by different letters differ significantly at P ≤0.05 (Duncan's multiple range test)

Figure captions:

Fig. 1. Locations and main soil features of the three field experimental sites. (PAWC: Plant available water capacity).

Fig. 2. Observed daily solar radiation (RAD), maximum (Tmax) and minimum (Tmin) temperatures, and cycle length (days after emergence) for North (1st tier), Central (2nd tier) and South (3rd tier) field experiments with the 1st sowing dates and for years 2014, 2015 and 2016. Triangles mark anthesis date. Dashed line indicates 35 ºC threshold.

Fig. 3. Observed anthesis, silking and maturity dates (days after emergence) for North, Central and South field experiments for first (S1) and second (S2) sowing dates in 2014 (14) and 2015 (15), and a single sowing date (S) for 2016 (16). Vertical lines indicate half standard deviation.

Fig. 4. Observed number of leaves (Vstage) as a function of thermal unit accumulation (degree-days) calculated as thermal time over a base temperature (Tb) of 8ºC (a) and by a beta function (b) for North, Central and South field experiments and for first (S1) and second (S2) sowing dates in 2015, and for the greenhouse experiments (c, for thermal time over Tb, d for a beta function). In the greenhouse, lines indicate that the same plants were monitored over time, and V designates V-stage (Ritchie et al., 1993) and T is visible leaf-tip stage.

Fig. 5. Example of conditions in the greenhouse experiments: Registered PAR, temperature and air humidity at the cool and hot greenhouses during the week from 20th to 27th July 2015.

Fig. 6. Observed anthesis and silking dates (days after emergence) in the greenhouse experiment for treatments: plants in the cool greenhouse all crop cycle or control (C), heat at V4 (V4), in cool greenhouse up to V4, then moved to hot greenhouse (V4c), in hot greenhouse up to V4, then moved to the cool greenhouse (V4h), heat at V9 (V9), heat at anthesis (FL), heat at lag phase (LG), heat at early grain filling (GF) and heat all crop cycle (H) in 2014, 2015 and 2016 (see section 2.1.2 for a description of the treatments). Vertical lines indicate half standard deviation.

Fig. 7. Biomass at harvest (g/plant) partitioned in leaves, stems and ears in field experiments. The scales are different for a better view. Vertical lines indicate half standard deviation. Bars with different letters indicate significant differences between locations at $P \leq 0.05$ (Duncan's multiple range test). Bars with asterisk indicate significant differences between sowing dates of the same location at $P \leq 0.05$ (Duncan's multiple range test). No letter or asterisk indicates no significant differences.

Fig. 8. Yield and yield components (kernel number and weight) in the field experiments from both 1st and 2nd sowings at the three studied locations. Vertical lines indicate half standard deviation. Bars with different letters indicate significant differences between locations at $P \leq 0.05$ (Duncan's multiple range test). Bars with asterisk indicate

857 significant differences between sowing dates of the same location at $P \leq 0.05$ (Duncan's
858 multiple range test). No letter or asterisk indicates no significant differences.

859 **Fig. 9.** Yield and yield components (kernel number and weight) of the topmost ear from
860 the greenhouse experiment for the heat treatments: plants in the cool greenhouse all
861 crop cycle or control (C), heat at V4 (V4), in cool greenhouse up to V4, then moved to
862 hot greenhouse (V4c), in hot greenhouse up to V4, then moved to the cool greenhouse
863 (V4h), heat at V9 (V9), heat at anthesis (FL), heat at lag phase (LG), heat at early grain
864 filling (GF), heat all crop cycle (H) and for pollen-source treatments (local and fresh
865 pollen) in 2014, 2015 and 2016 (see section 2.1.2 for a description of the treatments).
866 Vertical lines indicate half standard deviation. Bars with different letters indicate
867 significant differences between heat treatments at $P \leq 0.05$ (Duncan's multiple range
868 test). Bars with asterisk indicate significant differences between plants from the same
869 treatment pollinated with local or fresh pollen at $P \leq 0.05$ (Duncan's multiple range
870 test). No letter or asterisk indicates no significant differences.

871 **Fig. 10.** Grain yield and yield components (kernel weight and number) of the topmost
872 ear from the greenhouse experiment in 2016, for treatments growing all season in the
873 hot greenhouse or in the cool greenhouse and being pollinated with outside pollen
874 (Fresh), pollen from the cool greenhouse (Cool), or pollen from the hot greenhouse
875 (Hot). Vertical lines indicate half standard deviation.

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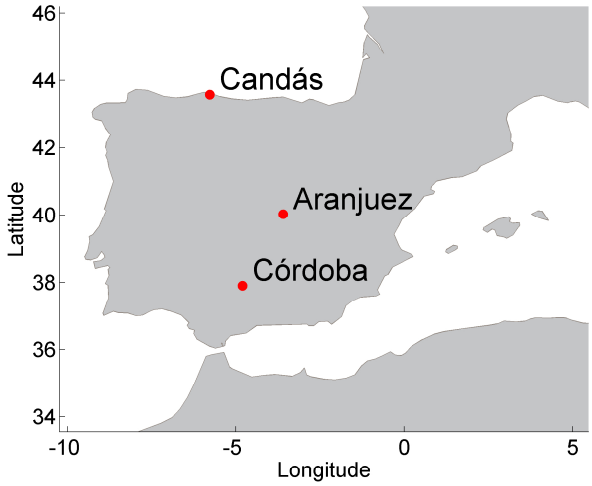
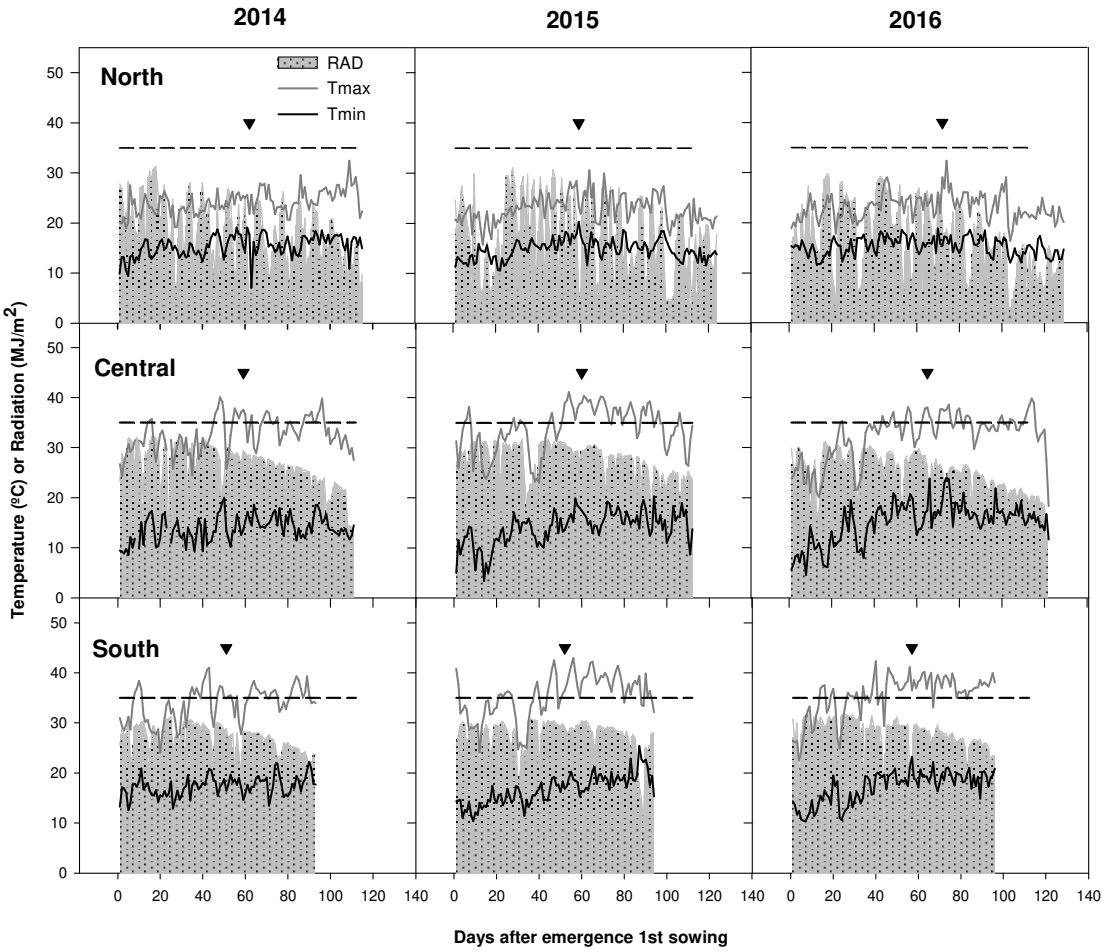


(a)

Soil	North	Centre	South
Localization	Candás 43º 35' N, 5º 47' W	Aranjuez 40º 1' N, 3º 36' W	Córdoba 37º 54' N, 4º 48' W
Elevation (m)	80	525	250
Depth (cm)	100	120	195
Texture	Sandy clay loam	Loam/ silt-loam	Sandy Loam
PAWC (mm)	93	254	284

(b)

Fig. 1. Locations and main soil features



884

885 **Fig. 2.** Observed daily solar radiation (RAD), maximum (Tmax) and minimum (Tmin)
886 temperatures, and cycle length (days after emergence) for North (1st tier), Central (2nd
887 tier) and South (3rd tier) field experiments with the 1st sowing dates and for years 2014,
888 2015 and 2016. Triangles mark anthesis date. Dashed line indicates 35 ºC threshold.

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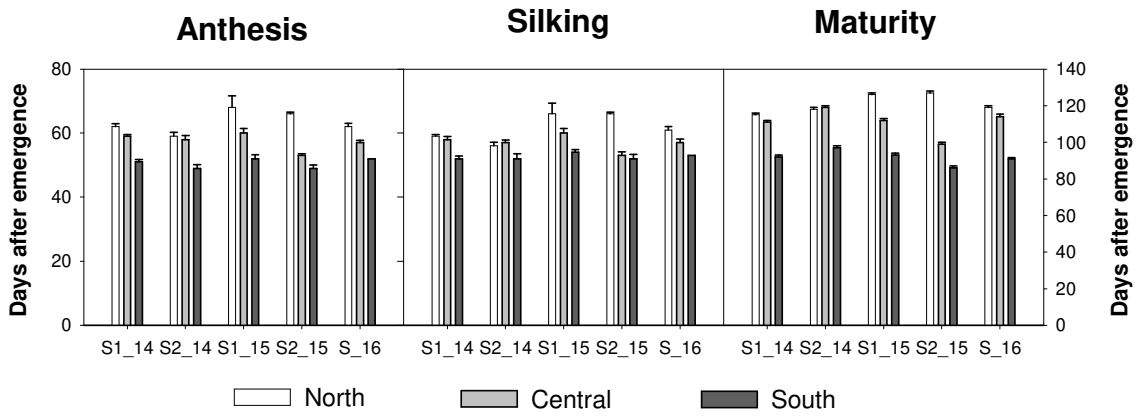

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893 **Fig. 3.** Observed anthesis, silking and maturity dates (days after emergence) for North,
894 Central and South field experiments for first (S1) and second (S2) sowing dates in 2014
895 (14) and 2015 (15), and a single sowing date (S) for 2016 (16). Vertical lines indicate
896 half standard deviation.

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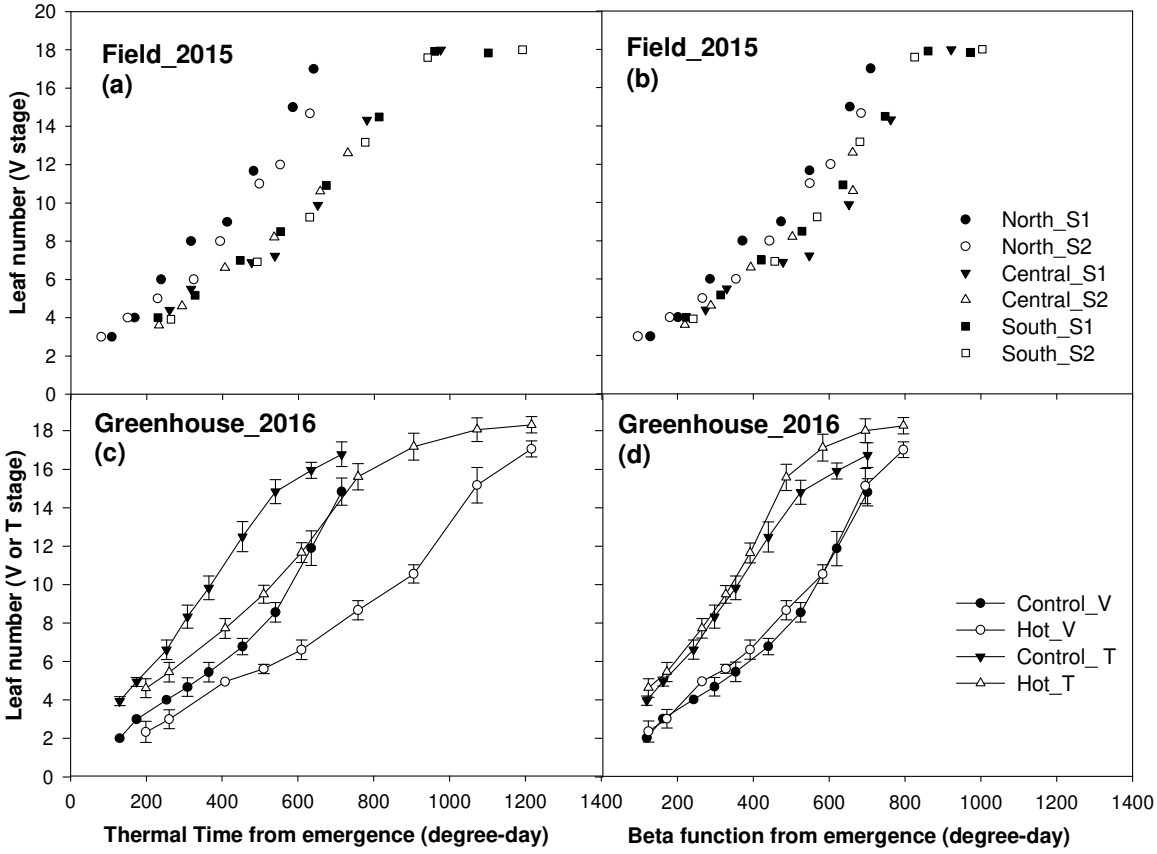


900

901 **Fig. 4.** Observed number of leaves (Vstage) as a function of thermal unit accumulation
902 (degree-days) calculated as thermal time over a base temperature (Tb) of 8ºC (a) and by
903 a beta function (b) for North, Central and South field experiments and for first (S1) and
904 second (S2) sowing dates in 2015, and for the greenhouse experiments (c, for thermal
905 time over Tb, d for a beta function). In the greenhouse, lines indicate that the same
906 plants were monitored over time, and V designates V-stage (Ritchie et al., 1993) and T
907 is visible leaf-tip stage.

908

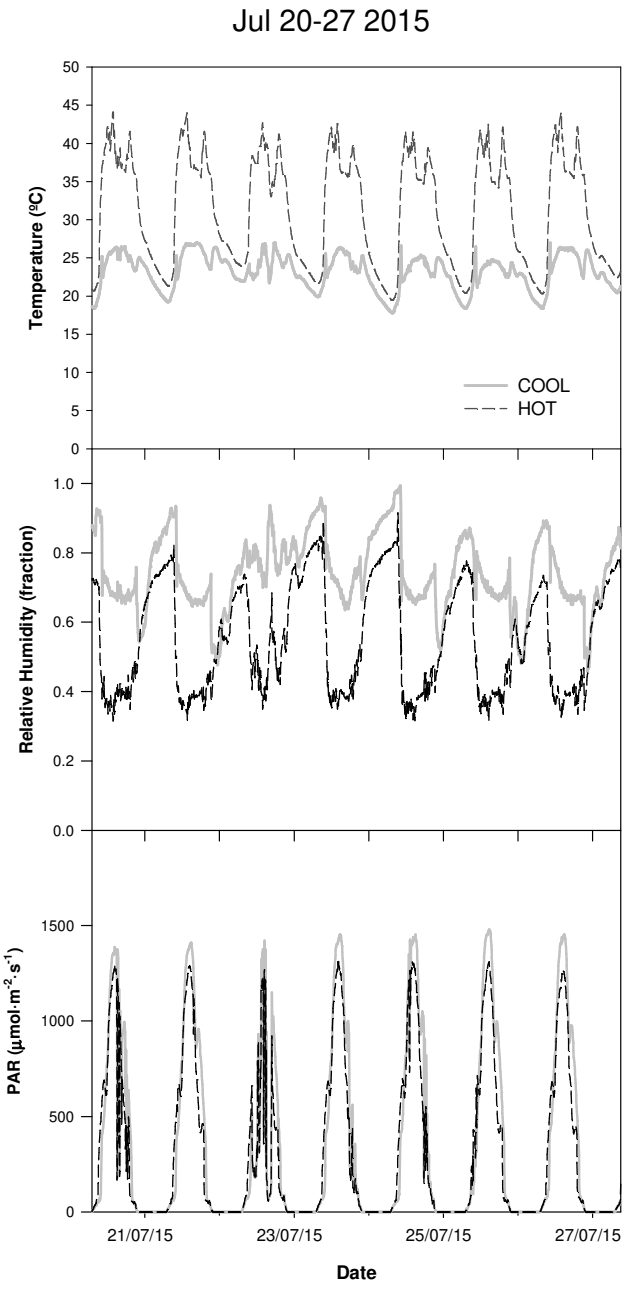
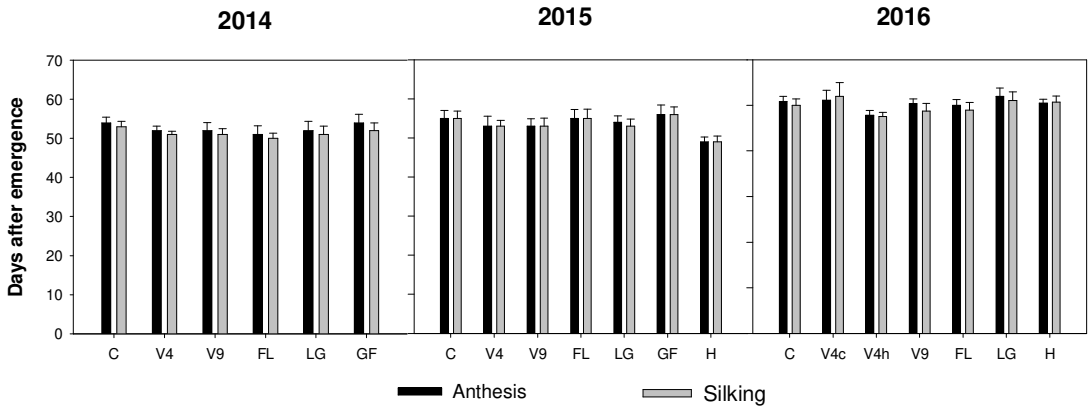


Fig. 5. Example of conditions in the greenhouse experiments: Registered PAR, temperature and air humidity at the cool and hot greenhouses during the week from 20th to 27th July 2015.

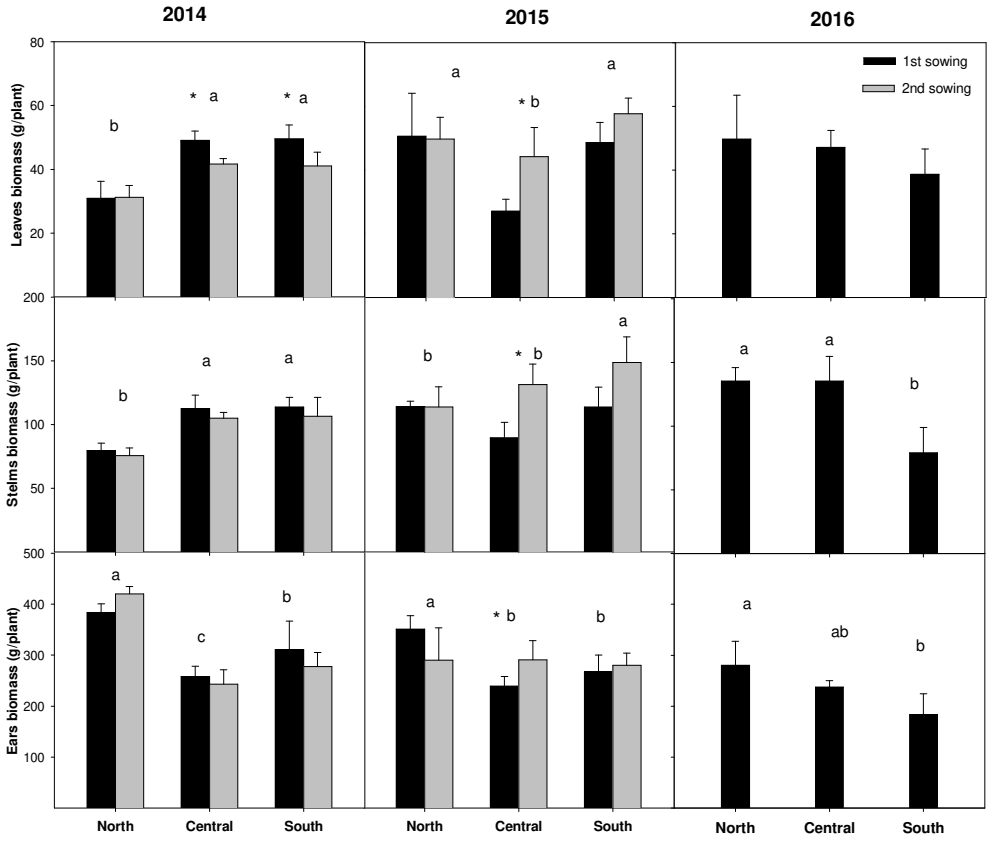


915

916 **Fig. 6.** Observed anthesis and silking dates (days after emergence) in the greenhouse
917 experiment for treatments: plants in the cool greenhouse all crop cycle or control (C),
918 heat at V4 (V4), in cool greenhouse up to V4, then moved to hot greenhouse (V4c), in
919 hot greenhouse up to V4, then moved to the cool greenhouse (V4h), heat at V9 (V9),
920 heat at anthesis (FL), heat at lag phase (LG), heat at early grain filling (GF) and heat all
921 crop cycle (H) in 2014, 2015 and 2016 (see section 2.1.2 for a description of the
922 treatments). Vertical lines indicate half standard deviation.

923

924



925

926 **Fig. 7.** Biomass at harvest (g/plant) partitioned in leaves, stems and ears in field
927 experiments. The scales are different for a better view. Vertical lines indicate half
928 standard deviation. Bars with different letters indicate significant differences between
929 locations at $P \leq 0.05$ (Duncan's multiple range test). Bars with asterisk indicate
930 significant differences between sowing dates of the same location at $P \leq 0.05$ (Duncan's
931 multiple range test). No letter or asterisk indicates no significant differences.

932

933

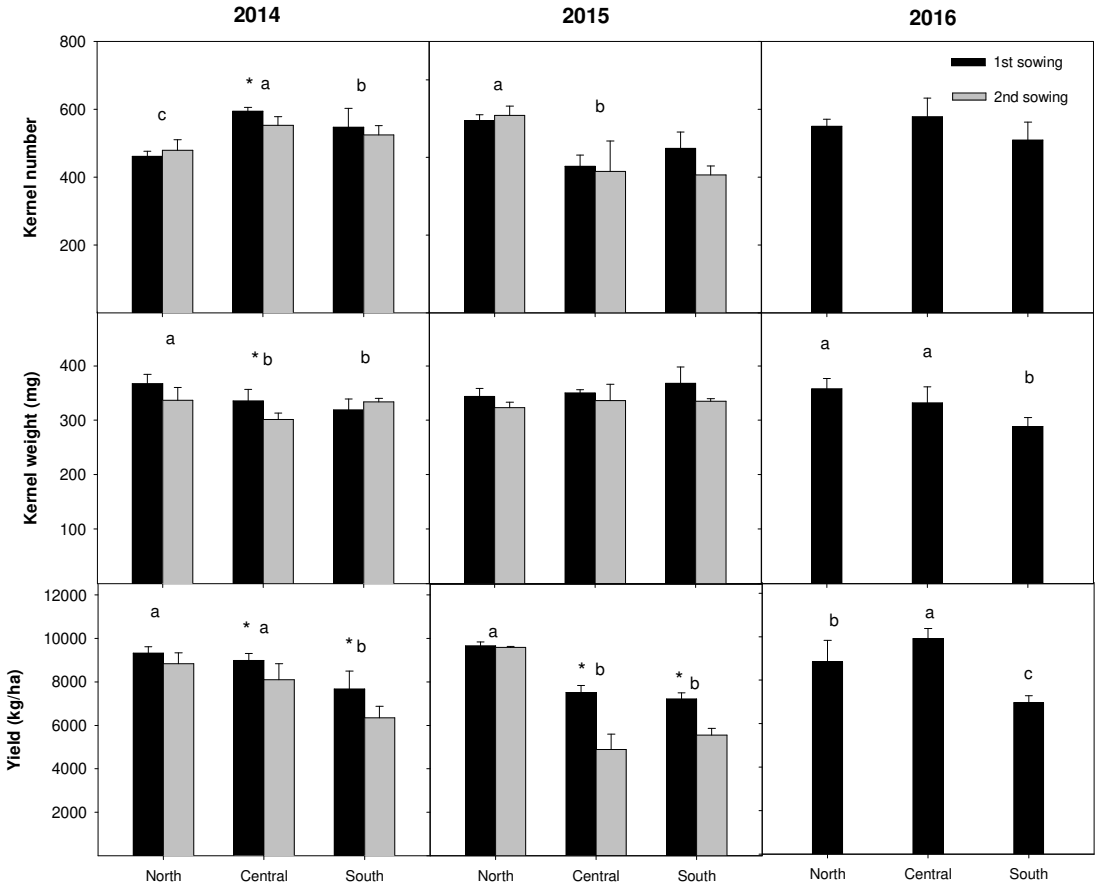
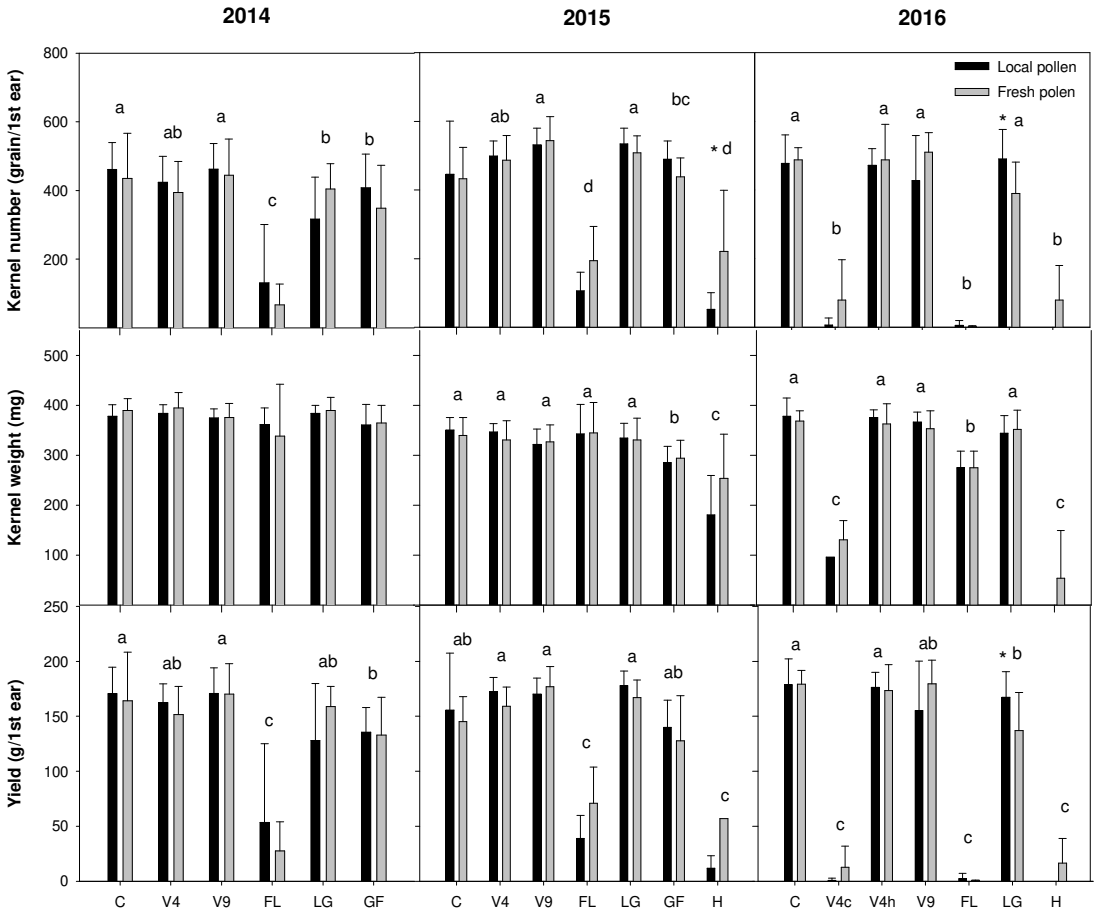


Fig. 8. Yield and yield components (kernel number and weight) in the field experiments from both 1st and 2nd sowings at the three studied locations. Vertical lines indicate half standard deviation. Bars with different letters indicate significant differences between locations at $P \leq 0.05$ (Duncan's multiple range test). Bars with asterisk indicate significant differences between sowing dates of the same location at $P \leq 0.05$ (Duncan's multiple range test). No letter or asterisk indicates no significant differences.



943

944 **Fig. 9.** Yield and yield components (kernel number and weight) of the topmost ear from
945 the greenhouse experiment for the heat treatments: plants in the cool greenhouse all
946 crop cycle or control (C), heat at V4 (V4), in cool greenhouse up to V4, then moved to
947 hot greenhouse (V4c), in hot greenhouse up to V4, then moved to the cool greenhouse
948 (V4h), heat at V9 (V9), heat at anthesis (FL), heat at lag phase (LG), heat at early grain
949 filling (GF), heat all crop cycle (H) and for pollen-source treatments (local and fresh
950 pollen) in 2014, 2015 and 2016 (see section 2.1.2 for a description of the treatments).
951 Vertical lines indicate half standard deviation. Bars with different letters indicate
952 significant differences between heat treatments at $P \leq 0.05$ (Duncan's multiple range
953 test). Bars with asterisk indicate significant differences between plants from the same
954 treatment pollinated with local or fresh pollen at $P \leq 0.05$ (Duncan's multiple range
955 test). No letter or asterisk indicates no significant differences.

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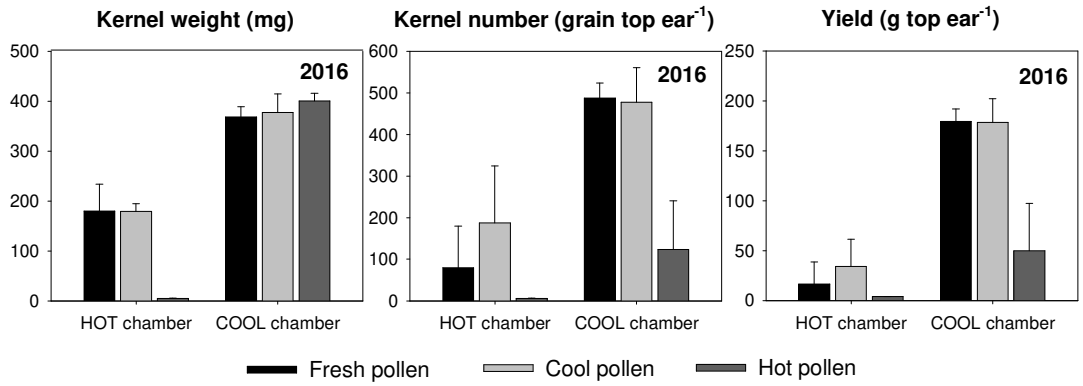

960

961 **Fig. 10.** Grain yield and yield components (kernel weight and number) of the topmost
962 ear from the greenhouse experiment in 2016, for treatments growing all season in the
963 hot greenhouse or in the cool greenhouse and being pollinated with outside pollen
964 (Fresh), pollen from the cool greenhouse (Cool), or pollen from the hot greenhouse
965 (Hot). Vertical lines indicate half standard deviation.

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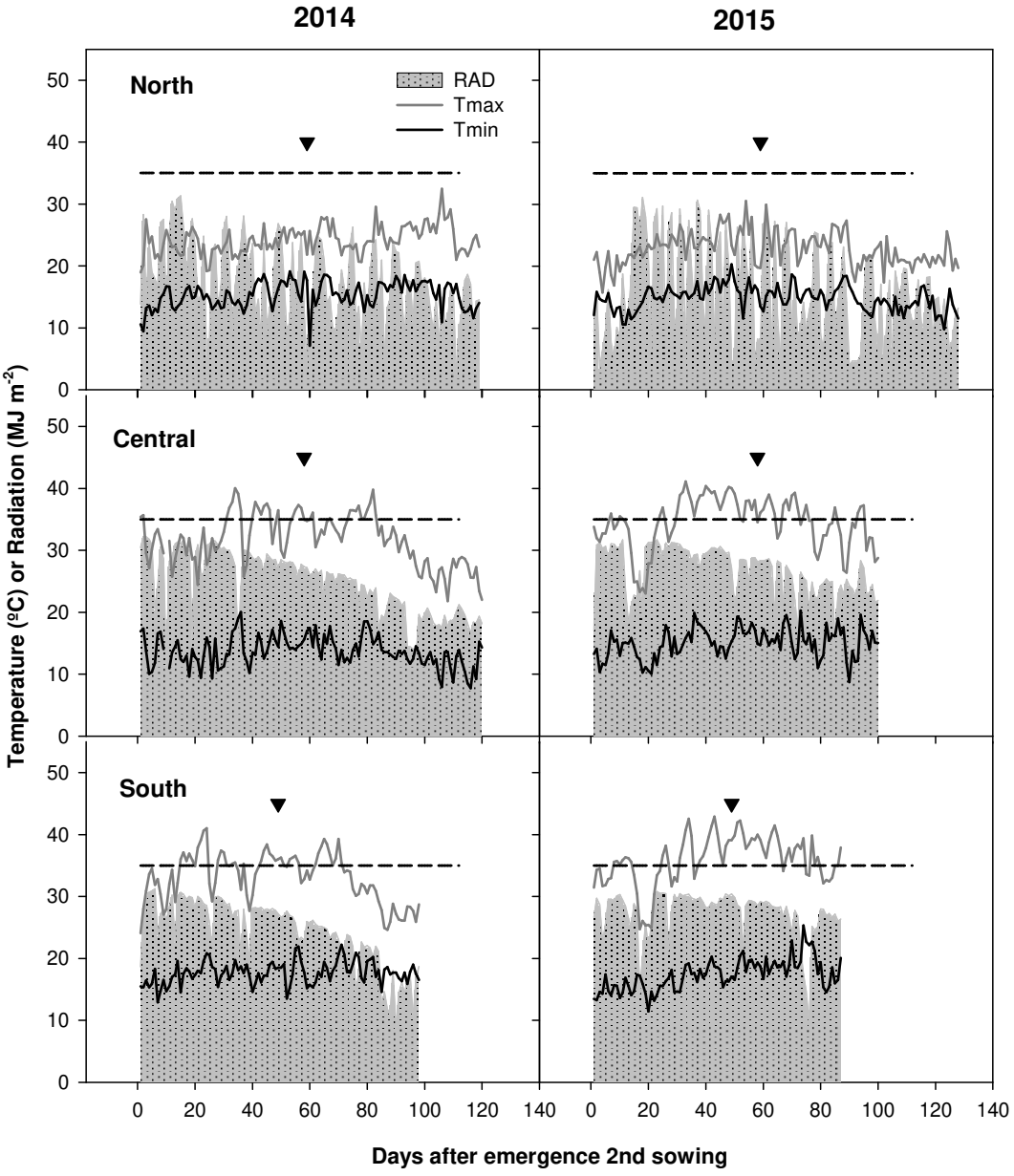
967

968 # Supplemental information

969



970

971 **Figure S1.** Observed daily solar radiation (RAD), maximum (Tmax) and minimum
972 (Tmin) temperatures, and cycle length (days after emergence) for North (1st tier),
973 Central (2nd tier) and South (3rd tier) from field experiments for the 2nd sowing dates
974 in years 2014 and 2015. Triangles mark anthesis date. Dashed line indicates 35 ºC
975 threshold.

976

977 **Table S1**. Coefficients of determination R^2 from the regressions of visible leaf tips (T
978 stages) and ligulate leaves (V stages) versus thermal time (TT) calculated with the linear
979 8 – 34 °C classic method and with the simplified beta function (Yan and Hunt, 1999)
980 using greenhouse and field data.

Experiment	R^2 from classic TT estimations	R2 from beta function estimations
Greenhouse (V stages, 2016)	0.87	0.96
Green house (T stages, 2016)	0.85	0.96
Field (V stages, 2015)	0.85	0.92
Field (T stages, 2015)	0.56	0.67
Field and greenhouse together (V-stages, 2015, 2016)	0.81	0.93
Field and greenhouse together (T-stages, 2015, 2016)	0.77	0.95

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