

# Heat Stress during Late Vegetative Growth of Maize: Effects on Phenology and Assessment of Optimum Temperature

M. Cicchino, J. I. Rattalino Edreira, and M.E. Otegui\*

## ABSTRACT

Prediction of phenology is based on thermal time (TT) computation, which requires the correct definition of base ( $T_B$ ) and optimum ( $T_O$ ) temperatures. Most information on these traits came from controlled environments using a wide range of mean air temperatures ( $T_X$ ), including  $T_X > T_O$  and  $T_X < T_B$ . These conditions are rarely found in field environments. We assessed the effect on development of day-time temperatures above  $T_O$  during late-vegetative growth of maize (*Zea mays* L), and established a model based on TT computation on a per hour ( $TT_h$ , in °C h) rather than per day basis ( $TT_d$ ) for  $T_O$  estimation. Field experiments included two temperature regimes ( $T_C$ : control;  $T_H$ : heated) between  $V_{11}$  and tasseling of  $T_C$ . We registered temperature at ear level, and dates of anthesis and silking. We computed developmental rates (DR),  $TT_h$  above 8°C during treatment period ( $TT_{h1}$ ) and between  $V_{11}$  and silking ( $TT_{h2}$ ), a stress index based on the quotient of differences in  $TT_h$  ( $\Delta TT_h$ ) between  $T_H$  and  $T_C$  ( $SI = \Delta TT_{h2} / \Delta TT_{h1}$ ), and  $T_O$ . Heat stress caused a delay in flowering events, and a decline in DRs. Estimated  $T_B$  was higher (12.7°C) than normally used in computations. Estimated  $T_O$  was within the expected range (36°C >  $T_O$  > 30°C), independently of  $T_B$ . Stressful temperatures promoted a delayed in silking, identified as an increase of at least 2.14°C h in  $TT_h$  for each degree above  $T_O$ . Estimated  $T_O$  differed between growing seasons ( $P = 0.04$ ), suggesting possible variation due to climatic effects.

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**Abbreviations:**  $\Delta TT_n$ , difference between  $T_H$  and  $T_C$  during period  $n$ ; ASI, anthesis-silking interval; DR, developmental rate;  $Exp_n$ , experiment  $n$ ;  $GS_n$ , growth stage  $n$ ; SI, stress index;  $T_B$ , base temperature;  $T_C$ , air temperature of control plots;  $T_H$ , air temperature of heated plots;  $T_M$ , maximum temperature;  $T_O$ , optimum temperature; TT, thermal time;  $TT_d$ , cumulative daily thermal time in °C d;  $TT_h$ , cumulative hourly thermal time in °C h;  $TT_S$ , cumulative stressful temperatures;  $TT_{SC}$ , cumulative stressful temperatures of control plots;  $TT_{SH}$ , cumulative stressful temperatures of heated plots;  $T_X$ , mean ambient temperature;  $T_{Xd}$ , daily mean ambient temperature;  $T_{Xh}$ , hourly mean ambient temperature.

ACCURATE PREDICTION of phenology is essential for the correct application of agronomic decisions. In current crop simulation models (e.g., CERES Maize; Jones and Kiniry, 1986) this prediction is based on TT for a temperature range between  $T_B$  and  $T_O$  values. Within this range, TT represents the inverse of the slope of the relationship between the developmental rate (in  $d^{-1}$ ) and temperature (Monteith, 1984), has units of °C d ( $TT_d$ ) and is usually computed as cumulative daily values of the difference between  $T_X$  and  $T_B$ . The DR represents the relationship between crop development (i.e., achievement of a given stage) and time requirement for reaching a stage (Trudgill et al., 2005). It is maximum at  $T_O$ , and tends to zero when  $T_X$  approaches  $T_B$  or a maximum temperature ( $T_M$ ). These are known as cardinal temperatures ( $T_M > T_O > T_B$ ),

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and their values vary among species, genotypes, growth processes, and developmental stages (Ritchie and NeSmith, 1991; Ellis et al., 1992; Porter and Gawith, 1999). For a given genotype, response to photoperiod may affect the DR and consequently  $TT_d$  requirements, but not cardinal temperatures. Contrarily, there are evidences of variation in cardinal temperatures depending on previous temperature regime, that is, an acclimation process (Fowler et al., 1999; Crafts-Brandner and Salvucci, 2002).

Several studies have been conducted in maize for the determination of cardinal temperatures. Most of them included ambient temperature regime in field experiments (Olsen et al., 1993) or constant settings in cabinets or glass-houses (Tollenaar et al., 1979; Warrington and Kanemasu, 1983; Ellis et al., 1992). In most studies, however, one or more weaknesses can be pointed out. For instance (i) the explored temperature range in the field did not include temperatures high enough for the determination of  $T_O$ , which cannot be obtained by extrapolation like  $T_B$ , (ii) fixed temperature regimes did not allow for the expression and evaluation of acclimation in many growth-cabinet studies, (iii) the evaluated period was too long (e.g., sowing to flowering or to maturity), introducing a confounded effect if cardinal temperatures vary along the cycle, (iv) use of sowing dates for exploring a wide range of temperatures, introducing the confounded effect of source-sink ratio on cycle duration when the grain-filling period is included in the analysis, and (v) analysis of temperature effects on DR based on daily  $T_x$  ( $T_{xd}$ ), which prevents the correct representation of critical thresholds for temperature-related stress because it is extremely rare for the mean daily temperature to exceed  $T_O$  or be less than  $T_B$  in maize growing areas. Research in cabinets performed by Ellis et al. (1992) took into account many of these constraints for early vegetative growth (i.e., up to tassel initiation), but no information exists for the late vegetative period before flowering, when reproductive structures have started active growth (Otegui, 1997; Otegui and Bonhomme, 1998).

The objectives of present research were to assess the developmental responses of field-grown maize plants to contrasting temperature regimes applied during late vegetative growth, and to determine the threshold temperature for plants to undergo heat stress at this stage (i.e.,  $T_O$ ). To avoid the limitations of previous studies, our protocol included (i) a heating system based on solar radiation, which allowed for a gradual temperature increase during daytime hours up to values between 35 and 48°C for a few hours a day, (ii) heating during a period of <3 wk immediately before 50% tasseling, (iii) heating during a variable number of days along this period depending on the fluctuation of sunny and cloudy days, which mimics many natural conditions (e.g., subtropical monsoonal climates), and (iv) TT computation based on hourly temperature records ( $TT_h$ ) obtained at the uppermost ear level for all treatments.

## MATERIALS AND METHODS

### Crop Husbandry and Treatment Description

Field experiments were conducted during 2006–2007 (Exp. 1) and 2007–2008 (Exp. 2) on a silty clay loam soil (Typic Argiudoll; USDA soil survey system) at the experimental unit of the National Institute of Agricultural Technology (INTA) located in Pergamino (33°56' S, 60°34' W), Argentina. Single-cross, temperate maize hybrid Nidera AX 842 CL MG (semident, 119 RM, and approximately 800°C d between emergence and silking for  $T_B = 8^\circ\text{C}$ ) was sown late in both experiments (12 December), at a stand density of 9 plants  $\text{m}^{-2}$ . Delayed sowing was selected for starting differential temperature treatments after the period of highest irradiance and temperature, which takes place between late December and the first half of January (Otegui et al., 1996). This was necessary to avoid overheating of heat-stressed plots. Experiments were hand-planted at three seeds per hill, and thinned to the desired plant population at the three-ligulated leaf stage ( $V_3$ ; Ritchie and Hanway, 1982). Total area covered by Exp. 1 was 870  $\text{m}^2$ , and by Exp. 2 was 1400  $\text{m}^2$ . Soil analysis (0–40 cm) indicated a pH (water) of 7.1, an organic matter content of 23  $\text{g kg}^{-1}$ , and a mineral P content of 35  $\text{mg kg}^{-1}$ . The experimental site was fertilized with 200  $\text{kg N ha}^{-1}$  at  $V_6$ . Weeds were controlled with 4  $\text{L ha}^{-1}$  atrazine [6-chloro-*N*-ethyl-*N'*-(1-methylethyl)-1,3,5-triazine-2,4-diamine] (half-strength) at sowing, and by hand weeding after the crop was established. Pests and diseases were adequately controlled. Water availability of the uppermost 1 m of soil was kept near field capacity throughout the growing season by means of sprinkler irrigation.

Treatments included (i) two temperature conditions ( $T_H$  and  $T_C$ ) between  $V_{11}$  and approximately 17 d after  $V_{11}$  (visible tassels among the whorl of leaves in  $T_C$ ) in Exp. 1, and (ii) a factorial combination of two temperature conditions ( $T_H$  and  $T_C$ ) and two growth stages ( $GS_1$ : between  $V_{11}$  and  $V_{11}+17$  d;  $GS_2$ : between tasseling and silking + 15 d of  $T_C$  plots) in Exp. 2. Only  $GS_1$  data were included in the present analysis because heating had no effect on flowering dates of  $GS_2$  plots. Treatments were distributed in (i) a completely randomized block design in Exp. 1 (i.e., six plots) and (ii) a split plot design in Exp. 2, with  $GS_n$  in the main plot and temperature regime in the subplot (i.e., 12 plots). There were always three replicates. Main plots were nine rows; rows were 0.7 m apart and 10 m in length. Temperature treatments covered 1.43 m (Exp. 1) or 2.86 m (Exp. 2) along two adjacent rows, and were always separated by at least one row of untreated plants and 1 m from border plants. These treatment areas were enclosed with polyethylene film (100- $\mu\text{m}$  thickness) fixed to wood stilks (laterals and roof), yielding rigid shelters of 2.3 m height. One shelter was for the  $T_H$  treatment and had the film reaching the soil surface on all sides, except one side that had a 10-cm opening at the bottom for allowing adequate gas exchange. The other shelter was for the  $T_C$  treatment and had laterals open up to 1.4 m above soil surface, and the south side completely open. Heating of  $T_H$  treatments depended exclusively on temperature rise promoted by the greenhouse effect of polyethylene enclosure, a system that allowed reproducing the expected natural variation in temperature associated with varying solar radiation in the region under study. Open shelters were used for avoiding differences in light due to the polyethylene film. All roofs were slightly pierced to avoid water accumulation (all shelters) and facilitate gas exchange at the top of the canopy

( $T_H$  treatments). These considerations in the design usually grant adequate gas exchange even in actively growing plants (i.e., not affected by abiotic stress), as documented in Cirilo and Andrade (1996). All shelters were removed at the end of each heating period.

## Measurements

Air temperature at the experimental site was monitored by means of a CR10X (Campbell Scientific Inc., Logan, UT). Air temperature of each shelter ( $T_H$  and  $T_C$ ) was registered hourly throughout the treatment period by means of sensors (TC1047, Microchip Technologies, Chandler, AZ) connected to dataloggers (Temp-Logger, Cavadevices, Buenos Aires, Argentina). Sensors were sheltered in white double-walled plastic cylinders with open ends, which were fixed to a stick and positioned in the center of each plot at the uppermost ear level. Cylinders had an internal diameter of 4.5 cm (innermost) or 10.5 cm (outermost), a total length of 18 cm, and were separated by a 2.75 cm gap by means of four adjustable screws. Data registered in each sheltered area were used for computing cumulative hourly thermal time ( $TT_h$ ; in  $^{\circ}\text{C h}$ ) above a  $T_B$  of  $8^{\circ}\text{C}$  (Jones and Kiniry, 1986; Ritchie and NeSmith, 1991). Difference in  $TT_h$  between  $T_C$  and  $T_H$  was determined for (i) the treatment period, and (ii) the period between the start of heating and silking of each treatment. Leaf temperature at the uppermost ear level (i.e., between leaves 12 and 15 for the tested hybrid) was also surveyed on specific dates during the treatment period of Exp. 1. Measurements were performed on well-illuminated leaves by means of an infrared thermometer (OS 541, Omegaette, European Community) held at approximately 1 cm from the leaf blade. At least two measurements were done in each sheltered area at each observation time.

Nine plants were tagged within each shelter at  $V_{11}$  in both experiments. Additionally, nine plants were tagged outside the shelters in all plots. The dates of anthesis (i.e., at least one extruded anther visible) and silking (i.e., at least one extruded silk visible) were recorded on all tagged plants. For each group of plants, median dates of anthesis and silking corresponded to the date when 50% of the plants reached the stage. The anthesis-silking interval (ASI; Bolaños and Edmeades, 1993) was computed as the difference in days between median silking date and median anthesis date.

The developmental rate (in  $\text{d}^{-1}$ ) of the late vegetative phase (i.e., between  $V_{11}$  and silking) was calculated for each group of plants (Eq. [1])

$$\text{DR} = (\text{Silking date} - V_{11} \text{ date})^{-1} \quad [1]$$

## Optimum Temperature Estimation and Statistical Analysis

The algorithm for computing  $T_O$  combined (i) differences in  $TT_h$  between  $T_H$  and  $T_C$  related exclusively to differential temperature regimes (i.e., the treatment period), identified as  $\Delta TT_{h1}$ , (ii) differences in  $TT_h$  between  $T_H$  and  $T_C$  related to a clear developmental event (i.e., between the start of heating and 50% silking), identified as  $\Delta TT_{h2}$ , and (iii) the quotient between these records (Eq. [2]), which quantified the effect of heat stress on  $TT_h$  requirements for development and was identified as a stress index (SI).

$$\text{SI} = \Delta TT_{h2} / \Delta TT_{h1} \quad [2]$$

Values of  $\text{SI} > 1$  are indicative of increased  $TT_h$  requirement because of reduced developmental rate when hourly mean air

temperature records ( $T_{Xh}$ ) were larger than  $T_O$ . Silking was selected as reference developmental stage because air temperature was always monitored near ear height.

The computation of  $TT_h$  was based on Eq. [3], using  $T_B = 8^{\circ}\text{C}$  (Jones and Kiniry, 1986; Ritchie and NeSmith, 1991).

$$TT_h = \sum_1^n (T_{Xh} - T_B) \quad [3]$$

Temperature accumulation spanned from the first to the last ( $n$ ) hour of the treatment period at VT (Ritchie and Hanway, 1982) of  $T_C$  plots.

Optimum temperature was obtained by iteration as the value that solved the equality in Eq. [4].

$$\Delta TT_{h1} = \Delta TT_S \times \text{SI} \quad [4]$$

$$TT_S = \sum_1^n (T_{Xh} - T_O), \text{ for } T_{Xh} > T_O \quad [5]$$

$$\Delta TT_S = TT_{SH} - TT_{SC} \quad [6]$$

where  $TT_S$  (Eq. [5]) represents cumulative stressful temperatures on a per hour basis between the start of treatment period and silking of each plot ( $n$ ), for heated ( $TT_{SH}$ ) and control plots ( $TT_{SC}$ ). A numeric example of SI computation is given in the Appendix.

All data were analyzed by ANOVA to evaluate the effects of experimental year, temperature treatments and their interactions. A  $t$  test was used to determine significant differences ( $P < 0.05$ ) between means. Regression analysis was applied to the relationship between DR and daily mean air temperature ( $T_{Xd}$ ). Eight data pairs were used for the model fitted to  $T_C$  conditions, including the average from all plants tagged outside the shelters in each experiment. For these plants, mean daily air temperature was obtained from the weather station records. Only six data pairs (from individual replicates in each experiment) were used in the model fitted to  $T_H$  conditions.

## RESULTS

### Temperature Regimes and Phenology

La Niña phase of the El Niño southern oscillation occurred during the period between July 2007 and June 2008 (National Oceanic and Atmospheric Administration, 2008) and caused an increase in summer air temperature for the region under study. For the pretreatment period (i.e., sowing to  $V_{11}$ ),  $T_{Xd}$  averaged  $22.8^{\circ}\text{C}$  for Exp.1 and  $23.1^{\circ}\text{C}$  for Exp. 2, and maximum daily temperature averaged  $28.8^{\circ}\text{C}$  for Exp. 1 and  $30.9^{\circ}\text{C}$  for Exp. 2. Similarly,  $T_{Xd}$  averaged across treatments for the period between  $V_{11}$  and silking were larger ( $P = 0.02$ ; Table 1) for Exp. 2 ( $24.5^{\circ}\text{C}$ ) than for Exp. 1 ( $22.8^{\circ}\text{C}$ ). A similar trend ( $P = 0.10$ ) was detected for the treatment period (i.e., between  $V_{11}$  and tasseling of  $T_C$ ), for which  $T_{Xd}$  based on 24-h records never exceeded  $26.6^{\circ}\text{C}$  (Table 1). Hourly temperatures ( $T_{Xh}$ ) differed markedly between temperature regimes (Fig. 1), mostly during day-time hours. Difference between treatments was also noticeable for leaf temperature records (Fig. 1b). Leaf temperature obtained by infrared thermometry in Exp. 1 reproduced air temperature trends very closely, which confirmed the effect of treatments on plant temperature at ear level.

**Table 1.** Mean air temperature, developmental rate (DR), and cumulative hourly thermal time ( $TT_h$ ; base temperature of 8°C) for the treatment period between  $V_{11}$  and tasseling of control plots ( $V_{11}$ - $VT_C$ ), and for the period between  $V_{11}$  and silking of each plot ( $V_{11}$ -S) in two experiments (Exp. n).

Experiments and temperature regime	Air temperature		DR $V_{11}$ -S $d^{-1}$	$TT_h$	
	$V_{11}$ - $VT_C$	$V_{11}$ -S		$V_{11}$ - $VT_C$	$V_{11}$ -S
	°C			°C h	
Exp. 1					
Control	22.9	22.4	0.0412	5778	8114
Heated	25.5	23.1	0.0326	6803	11060
Exp. 2					
Control	24.2	23.9	0.0517	7374	8150
Heated	26.6	25.1	0.0411	8475	10848
Year effect	0.100 <sup>†</sup>	0.02	0.007	0.003	ns
Temperature effect	0.003	0.04	0.0001	0.004	0.0004
Year × Temperature	ns <sup>‡</sup>	ns	ns	ns	ns

<sup>†</sup>P values of main and interaction effects.

<sup>‡</sup>ns: not significant ( $P > 0.10$ ).

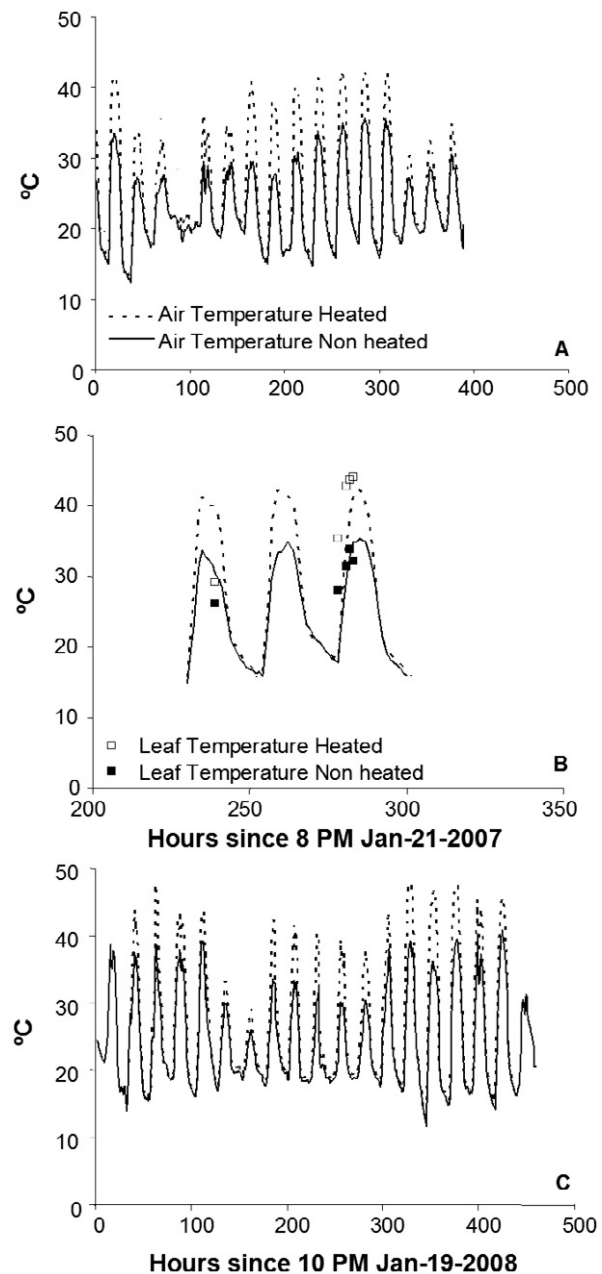
All flowering events were delayed by heating (Table 2), but the effect was larger on silking date than on anthesis date, and resulted in an increase of the ASI in heated plots (Table 2). This response was larger in Exp. 2 (4-d increase in ASI) than in Exp. 1 (1 d increase in ASI). All plants in  $T_H$  plots reached complete tassel extrusion before starting anthesis. Treatments had no significant effect on the final proportion of plants that reached anthesis or silking (Table 2), but male and female sterile individuals were detected in heated plots.

Year and temperature regime effects on phenology promoted differences in DR between  $V_{11}$  and silking (Table 1). The DR was always larger for  $T_C$  than for  $T_H$ , and for Exp.2 than for Exp.1. The response of the DR to  $T_{Xd}$  was established (Fig. 2), including data from plants tagged outside the shelters in the model fitted to  $T_C$  plots. Estimated  $TT_d$  (inverse of the slope) was larger for  $T_H$  (323°C d) than for  $T_C$  (233°C d). The extrapolation of fitted models indicated a  $T_B$  of ~12.7°C for both temperature regimes.

The heating system established between  $V_{11}$  and tasseling of  $T_C$  caused an increase in  $TT_h$  values of  $T_H$  plots (Table 1). The difference between  $T_H$  and  $T_C$  in mean hourly cumulative  $TT_h$  values above 8°C ( $\Delta TT_{h1}$ ) was  $1024 \pm 41^\circ\text{C h}$  in Exp.1 and  $1102 \pm 514^\circ\text{C h}$  in Exp. 2. This difference increased to  $2946 \pm 549^\circ\text{C h}$  in Exp.1 and  $2698 \pm 487^\circ\text{C h}$  in Exp.2 for the period between  $V_{11}$  and silking ( $\Delta TT_{h2}$ ; Table 1), because of the significant delay in silking registered for heated plants (Table 2). When  $TT_h$  was computed using a  $T_B$  of 12.7°C, estimated values held for the treatment period but declined for the period between  $V_{11}$  and silking ( $2199 \pm 331$  for Exp.1 and  $2190 \pm 426$  for Exp. 2).

### Assessment of Optimum Temperature

Contrasting temperature regimes started at the same phenological stage for all plots, but the developmental condition of plants differed between regimes at the end of the treatment period because shelters were removed on a fixed



**Fig. 1.** Hourly air temperature evolution along treatment period obtained at ear height. In (A) and (B) data correspond to Exp. 1, and in (C) to Exp. 2. Lines in figure (B) represent hourly temperature evolution along 3 d during the treatment period, and symbols correspond to leaf temperature obtained by infrared thermometry.

date (at tasseling of  $T_C$  plants). Therefore, differences in cumulative  $TT_h$  at the end of treatment could not be associated directly with a clear phenological stage for  $T_H$  plots. Additionally, differences between  $T_H$  and  $T_C$  in cumulative  $TT_h$  during the treatment period increased when a subsequent phenological stage was surveyed (e.g.,  $TT_h$  to silking; Table 1). Differences in temperature related exclusively to the treatment period ( $\Delta TT_{h1}$ ;  $T_B = 8^\circ\text{C}$ ) expanded ( $SI = \Delta TT_{h1} / \Delta TT_{h2}$ ) by  $2.87 \pm 0.42$  (Exp.1) or  $2.86 \pm 0.96$  (Exp.2) for the period between the start of heating and 50% silking. Based on these findings, and the iterative algorithm for

$T_O$  computation, a threshold temperature was established beyond which plants experienced heat stress. This temperature was higher ( $P < 0.04$ ) for Exp. 2 ( $35.5 \pm 1.25^\circ\text{C}$ ) than for Exp.1 ( $32.2 \pm 1.11^\circ\text{C}$ ).

The SI decreased ( $2.14 \pm 0.26$  in Exp.1 and  $2.32 \pm 0.79$  in Exp.2) when computations were based on a higher  $T_B$  ( $12.7^\circ\text{C}$ ), because differences in  $TT_h$  did not change for the treatment period (same  $\Delta TT_{h1}$ ) but decreased for the period between  $V_{11}$  and silking (reduced  $\Delta TT_{h2}$ ). The consequence of this change was a decline in estimated  $T_O$ , to  $30.0 \pm 1.03^\circ\text{C}$  in Exp.1 and  $33.5 \pm 1.09^\circ\text{C}$  in Exp.2.

## DISCUSSION

An important feature of the current research was the quantification of the effect of temperatures above  $T_O$  on maize reproductive development during the late vegetative period, especially of a dominated structure like the ear (Otegui, 1997; Westgate et al., 2004). Heat stress delayed silking and caused a decline in the DR, in agreement with previous evidences in maize summarized by Yan and Hunt (1999). Interestingly, heating also delayed anthesis and increased the occurrence of male-sterile plants, a condition rarely reported in this species. The relative impact of stressful temperatures was captured by the stress index (SI). Computed SIs indicated that each degree above  $T_O$  was amplified in terms of  $TT_h$  and translated into a delay of at least  $2.14^\circ\text{C h}$  to silking (on a  $T_B$  of  $12.7^\circ\text{C}$ ), a response that has been never determined previously in field conditions. This amplification effect of heat on development was larger for a  $T_B$  of  $8^\circ\text{C}$ , highlighting the importance of accurate estimation of all cardinal temperatures for correct evaluation of heat effects. The response of ear development could not be attributed entirely to enhanced apical dominance under stress (Jacobs and Pearson, 1991; Bolaños and Edmeades, 1993; Otegui, 1997), because (i) the effect of heat was very similar on the developmental rate to anthesis, especially in Exp.1, and (ii) most tassels were severely damage, exhibiting little or no pollen production.

A high temperature regime applied to  $T_H$  plots in the present research resulted in hourly  $T_x$  values clearly beyond the range of suboptimal temperatures, because estimated  $TT_h$  requirements for the period between  $V_{11}$  and silking were always larger for  $T_H$  plots than for  $T_C$  plots. These requirements are expected to be almost constant within the suboptimal temperature range if the photoperiod regime does not change (Hodges, 1991; Ellis et al., 1992; Bonhomme et al., 1994). Similarly, increased  $TT_d$  of  $T_H$  plots was indicative of conditions that reduced the rate of progress toward silking during the period of analysis, but also of the inaccuracy of daily mean ambient temperature ( $T_{xd}$ ) for representing stressful temperatures in the field (i.e.,  $T_{xd} > T_O$ ). Explored  $T_{xd}$  values of  $T_H$  plots were very similar to those of  $T_C$  plots (Fig. 2), and never reached figures larger than expected for  $T_O$  ( $\sim 30^\circ\text{C}$ ; Warrington and Kanemasu,

**Table 2. Median date of flowering events, final proportion of plants that reached the stage, and anthesis-silking interval (ASI) based on median dates of anthesis and silking in two experiments (Exp. n).**

Experiments and temperature regimes	Anthesis		Silking		ASI
	Date	Proportion	Date	Proportion	
Exp. 1					d
Control	13 Feb.	1.00	13 Feb.	1.00	0.33
Heated	18 Feb.	$0.83 \pm 0.17^\dagger$	20 Feb.	$0.96 \pm 0.05$	1.33
Exp. 2					
Control	9 Feb.	1.00	9 Feb.	1.00	0.00
Heated	10 Feb.	1.00	14 Feb.	$0.89 \pm 0.09$	4.00
Year effect	0.015 <sup>‡</sup>	ns <sup>§</sup>	0.026	ns	0.057
Temperature effect	0.023	ns	0.001	ns	0.001
Year × temperature	0.066	ns	ns	ns	0.007

<sup>†</sup>Mean value  $\pm$  SD for treatments that exhibited sterile plants.

<sup>‡</sup>P values of main and interaction effects.

<sup>§</sup>ns: not significant ( $P > 0.10$ ).

1983; Olsen et al., 1993). This restriction of  $T_{xd}$  is independent of the system for its computation, because 24-h averages do not differ markedly from the average of daily maximum and daily minimum records (Purcell, 2003).

On one hand, limitations of  $T_{xd}$  for precise estimation of  $TT_d$  can be overcome by means of optimization techniques (Olsen et al., 1993). On the other hand, these mathematical corrections are of value for agronomic purposes but do not contribute to our understanding of temperature effects on development within the conceptual model of  $TT$  (Ritchie and NeSmith, 1991). Our approach solved this restriction, and allowed for the estimation of  $T_O$  and its variation across environments (i.e., years in our research) and replications using field-grown plants. Estimated  $T_O$  was within the expected range for the period under analysis (Warrington and Kanemasu, 1983; Jones and Kiniry, 1986; Olsen et al., 1993), independently of the value of  $T_B$  used for computations. Estimated  $T_B$  ( $12.7^\circ\text{C}$ ) was higher than expected for the evaluated period (Warrington and Kanemasu, 1983; Jones and Kiniry, 1986), and for temperate Argentine hybrids (Padilla and Otegui, 2005), and above the value of  $10^\circ\text{C}$  used in pioneering work on this topic (Gilmore and Rogers, 1958; Mederski et al., 1973). This difference may acknowledge two origins: (i) a simple artifact of the approach when the model is based on a narrow range of temperatures that additionally did not include figures close to  $T_B$  (Ritchie and NeSmith, 1991; Padilla and Otegui, 2005), or (ii) the expected increase of  $T_B$  from early to late vegetative stages (Slafer and Rawson, 1995), for which there is no definitive reference in maize because most studies focused on early vegetative growth (Ellis et al., 1992) or the whole period between sowing and flowering (Olsen et al., 1993). The only evidence close to our analysis corresponds to the period between tassel initiation and anthesis in cabinet studies (Warrington and Kanemasu, 1983),

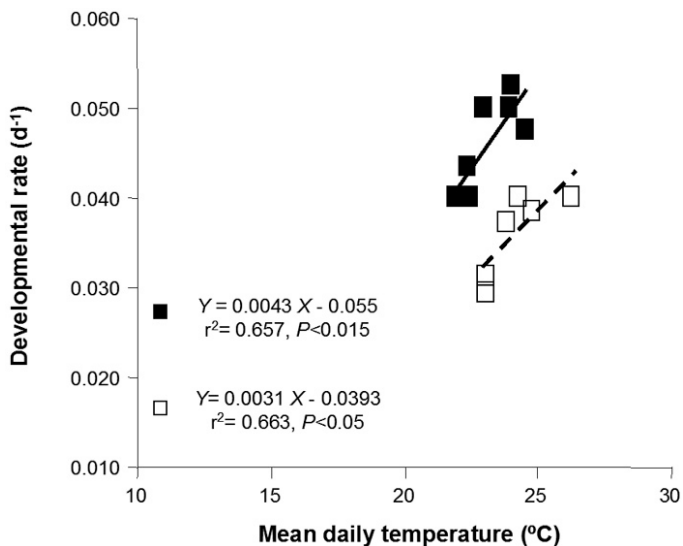


Fig. 2. Relationship between developmental rate and mean air temperature for the period between  $V_{11}$  and silking. Black squares are for control plots ( $n = 8$ ) and white squares for heated plots ( $n = 6$ ).

which suggests a decline in cardinal temperatures along the vegetative stages rather than the expected increase. Reanalysis of responses to temperature regime based on the new estimated  $T_B$  changed the estimation of  $T_O$  by  $2^\circ\text{C}$  for both experiments, an indication of the need for more research with maize in the determination of precise cardinal temperatures, ideally including field grown plants subjected to the natural temperature fluctuations of the environment.

Finally, estimated  $T_O$  differed between experiments, a response that may be attributed to acclimation processes because ambient temperatures along the pretreatment period were higher during Exp. 2 than during Exp.1. Acclimation to cold temperatures has been analyzed in detail (Thomashow, 2001), and predictive response models were established that include estimation of hardening and dehardening in field conditions (Fowler et al., 1999). The state of the art is less developed for acquired thermotolerance, with some evidences for maize photosynthesis (Crafts-Brandner and Salvucci, 2002) but only a peripheral analysis for development (Ellis et al., 1992). The conceptual

model for physiological responses that are involved with thermotolerance includes the involvement of heat shock proteins, but, as yet, there is no evidence of their function in acclimation to heat stress (Wahid et al., 2007).

## CONCLUSIONS

Our research on the effect of heat stress on maize development during the late-vegetative period allowed for the determination of a threshold  $T_O$  based on hourly records of field measured temperatures ( $T_{Xh}$ ), which has been impossible to estimate by means of daily mean records ( $T_{Xd}$ ). Estimated  $T_O$  differed between growing seasons, but was always within the range of values obtained in controlled environments, independently of the  $T_B$  used for computations. Estimated  $T_B$  from current research was higher than expected for present temperate maize hybrids. This trend in  $T_B$  suggests an apparent variation in cardinal temperatures due to the growth stage included in the analysis, an aspect still poorly understood in maize. Differences in  $T_O$  across years may be indicative of climatic processes affecting it, usually described as *acclimation*. Field experiments seem crucial for the correct elucidation of these processes in future research.

## APPENDIX

This section includes a numeric example of the computation of SI and associated variables. Data in Table 3 correspond to the mean of all replicates for control and heated plots during Exp.1, from which SI in Eq. [2] can be computed as  $SI = (11,060^\circ\text{C h} - 8114^\circ\text{C h}) / (6802^\circ\text{C h} - 5778^\circ\text{C h}) = 2946^\circ\text{C h} / 1024^\circ\text{C h} = 2.88$ . This value of SI indicates that each degree above  $T_O$  represents an increase in thermal time of  $2.88^\circ\text{C h}$ .

Optimum temperature ( $T_O$ ) is unknown, but it is known that cumulative stressful temperatures ( $TT_S$ ) up to silking can be computed from the difference between  $T_{Xh}$  and  $T_O$  for  $T_{Xh} > T_O$  (Eq. [5]), and the effect of treatments described as the difference in  $TT_S$  ( $\Delta TT_S$ ) between heated ( $TT_{SH}$ ) and control ( $TT_{SC}$ ) plots (Eq. [6]). Because each degree above  $T_O$  causes an increase of 2.88 in thermal time, it can be assumed that  $\Delta TT_{h1} = \Delta TT_S \times 2.88$  (Eq. [4]). Estimated  $T_O$  is the value

Table 3. Example for the computation of temperature accumulation during treatment period ( $TT_{h1}$ ) and up to silking ( $TT_{h2}$ ).

Date	Hour	$T_{Xh} (^\circ\text{C})$		$TT_{h1} (^\circ\text{C h}) = \sum (T_{Xh} - T_B)^\dagger$		$TT_{h2} (^\circ\text{C h}) = \sum (T_{Xh} - T_B)$	
		Heated	Control	Heated	Control	Heated	Control
20 Jan.	2000 h	33.97	26.82	25.97	18.82	25.97	18.82
20 Jan.	2100 h	29.86	25.56	47.83	36.38	47.83	36.38
.....	.....						
6 Feb. <sup>‡</sup>	2300 h	17.13	17.13	6802	5778	6,802	5778
.....	.....						
13 Feb. <sup>§</sup>	2300 h	21.27	21.27			9,138	8114
.....	.....						
18 Feb. <sup>¶</sup>	2300 h	20.20	12.20			11,060	

<sup>†</sup> $T_B = 8^\circ\text{C}$ .

<sup>‡</sup>End of differential temperature treatments (VT of  $T_C$  plots) and of temperature accumulation for computation of  $TT_{h1}$  in all plots.

<sup>§</sup>Mean silking date of  $T_C$  plots. End of temperature accumulation for computation of  $TT_{h2}$  of  $T_C$  plots.

<sup>¶</sup>Mean silking date of  $T_H$  plots. End of temperature accumulation for computation of  $TT_{h2}$  of  $T_H$  plots.

that solves this equality, for which  $\Delta T T_h$  is known (Table 3) and  $\Delta T T_s$  is obtained by iteration across hourly records of heated ( $T T_{SH}$ ) and control ( $T T_{SC}$ ) plots.

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