



Heat stress in temperate and tropical maize hybrids: Differences in crop growth, biomass partitioning and reserves use

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ABSTRACT

Maize (*Zea mays* L.) hybrids with tropical genetic background are a promising source of heat stress tolerance, but their performance in high yielding environments remains poorly understood. Our objective was to assess (i) genotypic differences in the ecophysiological determinants of grain yield; i.e., fraction of light intercepted by crop (fIPAR), radiation use efficiency for biomass production (RUE), and harvest index (HI), and (ii) the responses of mentioned traits to brief episodes of high temperature. The contribution of stored reserves to grain yield was also analyzed. Field experiments included three contrasting maize hybrids (Te: temperate; Tr: tropical; TeTr: Te × Tr) grown under two temperature regimes (control and heated) during daytime hours. We tested heating (ca. 33–40 °C at ear level) along three 15-d periods (GS₁: pre-silking; GS₂: from silking onwards; GS₃: active grain filling). Heat stress had no effect on leaf area and fIPAR, but heating during grain filling affected light capture through reduced cycle duration, especially for the Te hybrid (average of –16.5 d). Heating caused a large reduction in RUE, but this trait had a rapid recovery after heat removal and final shoot biomass was not much affected (between –3% and –33%). HI was markedly reduced by heating and its variation was associated with changes in reserves use ($r^2 = 0.61$). Grain yield in heated plots was better explained ($r^2 \geq 0.92$) by the variation in HI than by the variation in final shoot biomass ($r^2 \geq 0.59$). Heat effects on grain yield were larger (i) when they occurred around flowering (–527 g m⁻² for GS₁ and –545 g m⁻² for GS₂) than during grain filling (–352 g m⁻² for GS₃), and (ii) for the Te hybrid (–599 g m⁻²) than for the TeTr (–440 g m⁻²) and the Tr hybrids (–384 g m⁻²). Heating around silking (GS₁ and GS₂) caused apparent accumulation of reserves during the effective grain-filling period. The opposite trend was detected among plots heated during active grain formation (GS₃). The tropical genetic background did not penalize yield potential and conferred an enhanced capacity for enduring heat effects.

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1. Introduction

In field conditions, crops are usually exposed to episodes of abiotic stress (e.g. water deficit, nutrient deficiency, extreme temperatures) during the cycle, which affect their productivity

depending upon the opportunity, intensity and duration of the stress (Loomis and Connor, 1992). The magnitude of the response can be analyzed in terms of the physiological determinants of grain yield; i.e., amount of resource captured by the crop, efficiency for converting a resource into biomass, and biomass partitioning to reproductive organs (Sinclair and de Wit, 1975; Gifford et al., 1984; Passioura, 1996). In general, negative effects of water and nitrogen deficiencies are associated with reductions in tissue expansion and photosynthetic capacity (Boyer, 1970; Sadras and Milroy, 1996; Lemaire and Gastal, 2009). In determinate annual crops like maize, the former prevails during the pre-anthesis period when canopy size is defined (NeSmith and Ritchie, 1992; Uhart and Andrade, 1995). The latter is the predominant response during grain filling, and is usually accompanied by accelerated leaf senescence (Uhart and Andrade, 1995; Earl and Davis, 2003). Under heat stress, loss in productivity is chiefly related to decreased assimilatory capacity (Berry and Bjorkman, 1980; Sinsawat et al., 2004). This response is caused by reduced photosynthesis due to negative effects of

Abbreviations: CG, crop growth; CGR, crop growth rate; CGR_{EGF}, CGR effective grain-filling period; CGR_{POST}, CGR post-silking; CGR_{PRE}, CGR pre-silking; Exp_n, experiment n; fIPAR, fraction of incident photosynthetically active radiation intercepted by canopy; fIPAR_{EGF}, fIPAR effective grain-filling period; fIPAR_{POST}, fIPAR post-silking; fIPAR_{PRE}, fIPAR pre-silking; GS_n, growth stage n; GY, grain yield; H, hybrid; HI, harvest index; IPAR, incident photosynthetically active radiation; IPAR_i, intercepted IPAR; LAI, leaf area index; RUE, radiation use efficiency; RUE_{EGF}, RUE effective grain-filling period; RUE_{POST}, RUE post-silking; RUE_{PRE}, RUE pre-silking; T_c, non-heated control plot; Te, temperate hybrid; TeTr, Te × Tr hybrid; T_h, heated plot; Tr, tropical hybrid; TR, thermal regime.

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above-optimum temperatures on membrane stability (Barnabás et al., 2008) and enhanced maintenance respiration costs (Loomis and Connor, 1992; Hay and Porter, 2006). At the crop level, the consequence of these trends is a reduction in radiation use efficiency (RUE, biomass production per unit of light intercepted by the canopy), as has been reported for wheat (Reynolds et al., 2007) and maize (Cicchino et al., 2010b).

Heat stress can also reduce grain yield due to a decline in harvest index (Ferris et al., 1998; Craufurd et al., 2002). This response usually takes place when above-optimum temperatures occur around flowering, and is linked to their negative effects on kernel set (Vara Prasad et al., 1999). In maize, these effects were primarily attributed to reduced pollen shed (Schoper et al., 1987) and pollen viability (Herrero and Johnson, 1980; Mitchell and Petolino, 1988). Recent research, however, demonstrated that poor grain yield and low harvest index of a temperate maize hybrid did not improve when fresh pollen was added to ears of plants heated around silking (Cicchino et al., 2010b).

The development of germplasm adapted to environments prone to high temperature stress has been a key strategy for reducing the associated loss in grain yield. In maize, this germplasm derives mostly from lowland tropical populations. In spite of the existence of this promising source of heat stress tolerance, tropical maize genotypes sometimes show undesirable agronomic traits as tall plants, excessive foliage, very long cycle and poor harvest index (Fischer and Palmer, 1984). A new breeding era started in the 1980s, when programs shifted to a new plant type. The novel phenotype included shortening of the vegetative stage and plant height through reduction in total leaf number, with the concomitant enhanced allocation of assimilates to the ear that reduced plant barrenness and increased harvest index (Johnson et al., 1986). These changes led to an increase in optimum stand density (Fischer and Palmer, 1984; Johnson et al., 1986). Most breeding effort in tropical maize, however, continued oriented to improve reproductive performance under drought (Bolaños and Edmeades, 1993; Edmeades et al., 1999), and to a lesser extent to reduced nitrogen availability (Lafitte and Edmeades, 1994a; Lafitte and Edmeades, 1994b). Yield gains in these low yielding environments were mainly associated with increases in the numbers of grained ears per plant (prolificacy) and grains per ear, delayed leaf senescence, and reduced anthesis-silking interval and kernel abortion (Monneveux et al., 2005, 2006).

In spite of mentioned breeding efforts, reduced potential grain yield continued to be the main restriction of tropical hybrids. This trend was chiefly attributed to poor interest and research of seed companies (Duvick, 2005), largely focused to germplasm aimed to high yielding temperate environments. The superior productivity of temperate genotypes respect to the tropical ones seemed to be related to differences in harvest index (Fischer and Palmer, 1984) and not to variations in light capture or radiation use efficiency (Lafitte and Edmeades, 1997), but the actual physiological bases of their differences remained poorly understood. Nevertheless, some breeders developed commercial hybrids by crossing inbreds of temperate origin with those of tropical background, eager to combine features responsible of high yield potential among the former with those responsible of improved adaptation to tropical environments among the latter (Griffing and Lindstrom, 1954; Abadassi and Hervé, 2000; Vasic et al., 2006; Whitehead et al., 2006).

Independently of the benefits and drawbacks of the 'top-down' approach used by breeders (Jackson et al., 1993), comparisons between temperate and tropical maize hybrids are scarce or of limited breadth. First, because temperate hybrids cannot express their yield potential in most tropical conditions. Second, because many temperate environments do not allow a fair comparison when hybrids are cropped in a common sowing date, for which the occurrence of the critical period for kernel set (Fischer and Palmer, 1984; Kiniry and Ritchie, 1985) varies markedly among hybrids due to

differences in cycle duration (Capristo et al., 2007). Based on these restrictions, we compared maize hybrids of temperate and tropical background using variable sowing dates. Our objectives were (i) to assess genotypic differences in the ecophysiological determinants of grain yield (i.e. intercepted radiation, radiation use efficiency and biomass partitioning to grains), and (ii) to evaluate the responses of mentioned traits to brief episodes of high temperature during three different growth stages.

2. Materials and methods

2.1. Crop husbandry and experimental design

Field experiments were conducted during 2008–2009 (Exp₁) and 2009–2010 (Exp₂) at the experimental field of the University of Buenos Aires, Argentina (34°25'S, 58°25'W) on a silty clay loam soil (Vertic Argiudoll; USDA soil survey system). Treatments included a factorial combination of (i) three F1 hybrids (H) of contrasting genetic background (Te: temperate, Tr: tropical, and TeTr: temperate × tropical), (ii) two temperature regimes (T_C: control with no heating, T_H: heated) applied during daytime hours (ca. 33–40 °C at ear level), and (iii) three different growth stages (GS). These stages covered the first half of the critical period for kernel set (GS₁: 15 days immediately before anthesis), the second half of this same period (GS₂: 15 days from start of silking onwards), and the first part of the active grain-filling period (GS₃: 15 days from the end of GS₂ onwards). Hybrids were 2M545 HX (Te), 2B710 HX (Tr), and 2A120 HX (TeTr), all currently produced by Dow Agrosience Argentina for different regions of this country (Rattalino Edreira et al., 2011). The relative maturities of tested hybrids were 124 for Te, 136 for Tr, and 128 for TeTr hybrid. In both experiments, a single stand density of 9 plants m⁻² was used. Crops were fertilized with urea at a rate of 200 kg N ha⁻¹ at V₆ (Ritchie and Hanway, 1982). Water availability of the uppermost 1 m of the soil profile was kept near field capacity throughout the growing season by means of drip irrigation. Weeds, diseases and insects were adequately controlled. More details about crop husbandry can be found in Rattalino Edreira et al. (2011).

Treatments were distributed in a split split-plot design, with growth stages, hybrids and thermal regimes (TR) in the main plot, subplot and sub-subplot (hereafter termed plots), respectively. Three replicates were always used. Main plots were 10 m length, with six rows separated at 0.5 m between rows. Temperature regimes covered an area of 6 m² of the four central rows of each subplot. These treatment areas were enclosed with polyethylene film (100 μm thickness) mounted on wood structures (Cicchino et al., 2010a). For T_C shelters, the lateral films were open up to 1.4 m above soil surface. This was done to avoid differences in light offer due to the polyethylene film. For T_H shelters, the film reached the soil surface on all sides, except one side that had a 10 cm opening at the bottom. Additionally, roofs of all shelters were pierced for avoiding excessive heating at the top of the canopy and for helping with adequate gas exchange. Heating of T_H treatments depended mainly on temperature rise promoted by the greenhouse effect of the polyethylene enclosure (Cicchino et al., 2010a). Nonetheless, shelters for the T_H condition were supplemented with an electric fan heater monitored by an automated control unit (Cavadevices, Buenos Aires, Argentina).

Heating of GS₁ started when 50% of the plants in T_C plots of each hybrid reached ca. V₁₅–V₁₇ (Ritchie and Hanway, 1982), and finished when 10% of these plants reached anthesis. Heating of GS₂ started when 10% of plants in T_C plots reached R₁ and finished 15 days later. Finally, the GS₃ heating period spanned between 15 days after R₁ of T_C plots and ca. 15 days later. All shelters were removed at the end of each heating period. Different sowing dates

Table 1
 Treatments effect on (i) duration of the period between V_{15} and R_6 , (ii) incident photosynthetically active radiation (IPAR) experienced by the crop during different growth periods, (iii) maximum (R_1) and final (R_6) leaf area index (LAI), and (iv) fraction of IPAR intercepted by canopy (fIPAR) during different growth periods. Heat effect represents the difference between heated (T_H) and control (T_C) plots. ANOVA results are presented at the bottom of the table.

Exp ^a	GS	H	TR	Sowing date	Duration				IPAR				LAI				fIPAR						
					V_{15} – R_6	Heat effect V_{15} – R_6	PRE	POST	EGF	Heat effect V_{15} – R_6	R_1	R_6	PRE	POST	EGF	Heat effect V_{15} – R_6	R_1	R_6	PRE	POST	EGF	Heat effect V_{15} – R_6	
																				MJ m ⁻²			
Exp ₁	GS ₁	Te	T_C	22-12-08	70	141	128	255	9.3	2.9	0.97	0.99	0.98	0.00	0.00	0.97	0.99	0.98	0.00	0.00			
			T_H	22-12-08	69	144	118	241	9.1	2.8	0.97	0.98	0.97	0.98	0.00	0.00	0.97	0.98	0.97	0.00	0.00		
		TeTr	T_C	22-12-08	71	144	128	269	8.7	2.0	0.97	0.99	0.97	0.97	0.00	0.00	0.97	0.99	0.97	0.00	0.00		
			T_H	22-12-08	70	138	118	232	8.4	1.6	0.96	0.99	0.97	0.97	0.00	0.00	0.96	0.99	0.97	0.00	0.00		
		Tr	T_C	16-12-08	72	140	121	274	10.6	2.9	0.98	0.99	0.99	0.99	0.00	0.00	0.98	0.99	0.99	0.00	0.00		
			T_H	16-12-08	77	144	115	283	10.6	3.2	0.98	0.99	0.99	0.99	0.00	0.00	0.97	0.98	0.99	0.00	0.00		
	GS ₂	Te	T_C	09-12-08	70	156	145	298	8.7	5.2	0.97	0.98	0.97	0.00	0.00	0.97	0.98	0.97	0.00	0.00			
			T_H	09-12-08	69	153	145	246	8.6	7.1	0.99	0.99	0.97	0.97	0.00	0.00	0.99	0.99	0.97	0.00	0.00		
		TeTr	T_C	09-12-08	67	156	145	289	8.8	4.6	0.98	0.98	0.97	0.97	0.00	0.00	0.98	0.98	0.97	0.00	0.00		
			T_H	09-12-08	69	156	145	297	8.8	5.1	0.98	0.98	0.97	0.97	0.00	0.00	0.98	0.98	0.97	0.00	0.00		
		Tr	T_C	02-12-08	70	142	133	302	10.3	5.8	0.98	0.98	0.97	0.97	0.00	0.00	0.98	0.98	0.97	0.00	0.00		
			T_H	02-12-08	69	143	134	305	10.4	4.9	0.97	0.97	0.97	0.97	0.00	0.00	0.97	0.97	0.97	0.00	0.00		
GS ₃	Te	T_C	20-11-08	66	180	152	318	8.7	5.1	0.98	0.98	0.96	0.00	0.00	0.98	0.98	0.96	0.00	0.00				
		T_H	20-11-08	44	179	158	143	8.5	7.9	0.98	0.97	0.95	0.95	0.00	0.00	0.98	0.97	0.95	0.00	0.00			
	TeTr	T_C	20-11-08	69	181	158	333	8.7	5.1	0.97	0.96	0.95	0.95	0.00	0.00	0.97	0.96	0.95	0.00	0.00			
		T_H	20-11-08	69	181	158	319	8.8	5.1	0.97	0.95	0.94	0.94	0.00	0.00	0.97	0.95	0.94	0.00	0.00			
	Tr	T_C	14-11-08	71	175	140	342	10.4	6.0	0.97	0.97	0.96	0.96	0.00	0.00	0.97	0.97	0.96	0.00	0.00			
		T_H	14-11-08	66	170	140	292	10.0	4.7	0.98	0.96	0.94	0.94	0.00	0.00	0.98	0.96	0.94	0.00	0.00			
Exp ₂	GS ₁	Te	T_C	18-12-09	70	107	114	225	8.4	5.5	0.98	0.98	0.98	0.00	0.00	0.98	0.98	0.98	0.00	0.00			
			T_H	18-12-09	64	110	104	184	7.8	5.0	0.98	0.98	0.98	0.98	0.00	0.00	0.98	0.98	0.98	0.00	0.00		
		TeTr	T_C	18-12-09	66	106	113	213	6.7	5.0	0.98	0.98	0.98	0.98	0.00	0.00	0.98	0.98	0.98	0.00	0.00		
			T_H	18-12-09	68	112	97	201	8.4	4.9	0.98	0.98	0.98	0.96	0.00	0.00	0.98	0.98	0.96	0.00	0.00		
		Tr	T_C	11-12-09	72	105	110	229	8.5	5.4	0.97	0.97	0.97	0.97	0.00	0.00	0.97	0.97	0.97	0.00	0.00		
			T_H	11-12-09	76	109	100	219	7.7	4.8	0.98	0.98	0.97	0.97	0.00	0.00	0.98	0.98	0.97	0.00	0.00		
	GS ₂	Te	T_C	02-12-09	67	163	103	244	8.0	5.1	0.95	0.96	0.96	0.00	0.00	0.95	0.96	0.96	0.00	0.00			
			T_H	02-12-09	64	158	104	215	7.6	5.4	0.97	0.97	0.96	0.96	0.00	0.00	0.97	0.97	0.96	0.00	0.00		
		TeTr	T_C	02-12-09	69	163	103	256	8.8	4.9	0.96	0.96	0.96	0.96	0.00	0.00	0.96	0.96	0.96	0.00	0.00		
			T_H	02-12-09	66	164	108	232	9.0	4.7	0.97	0.97	0.97	0.97	0.00	0.00	0.97	0.97	0.97	0.00	0.00		
		Tr	T_C	20-11-09	66	154	104	228	8.1	6.2	0.97	0.97	0.96	0.96	0.00	0.00	0.97	0.97	0.96	0.00	0.00		
			T_H	20-11-09	66	151	108	246	8.4	5.2	0.96	0.96	0.97	0.97	0.00	0.00	0.96	0.97	0.97	0.00	0.00		
GS ₃	Te	T_C	16-11-09	62	164	139	229	7.8	4.5	0.97	0.98	0.97	0.97	0.00	0.00	0.97	0.98	0.97	0.00	0.00			
		T_H	16-11-09	50	164	137	156	7.4	4.1	0.97	0.98	0.97	0.97	0.00	0.00	0.97	0.98	0.97	0.00	0.00			
	TeTr	T_C	16-11-09	65	162	132	240	8.5	4.2	0.97	0.97	0.96	0.96	0.00	0.00	0.97	0.97	0.96	0.00	0.00			
		T_H	16-11-09	60	162	135	216	7.1	3.3	0.98	0.97	0.96	0.96	0.00	0.00	0.98	0.97	0.96	0.00	0.00			
	Tr	T_C	02-11-09	63	161	119	233	7.6	6.0	0.95	0.97	0.96	0.96	0.00	0.00	0.95	0.97	0.96	0.00	0.00			
		T_H	02-11-09	62	163	131	226	8.9	6.3	0.95	0.97	0.95	0.95	0.00	0.00	0.95	0.97	0.95	0.00	0.00			

^a Exp, experiment; GS, growth stage; H, hybrid; TR, temperature regime; Te, temperate; Tr, tropical; TeTr, Te × Tr; PRE, pre-silking period of the critical period; POST, post-silking period of the critical period; EGF, effective grain-filling period.
^b P values of main and interaction effects for which at least one variable was detected as significant; ns, not significant (P > 0.05).

were used for each GS × H combination (Table 1) in order to start all heating treatments at a same calendar date. This was done to achieve similar stress intensities for avoiding the confounded effect of the environment on treatments evaluation. More details about the heating system and heat stress characteristics can be found in Rattalino Edreira et al. (2011).

2.2. Measurements and computations

Daily incident photosynthetically active radiation (IPAR, in MJ m⁻² d⁻¹) and mean air temperature were registered at the experimental site (Weather Monitor II, Davis Instruments, USA). Additionally, air temperature of each shelter (T_H and T_C) was recorded hourly throughout the treatment period by means of a sensor connected to a datalogger (Temp-Logger, Cavadevices, Buenos Aires, Argentina). These sensors were positioned in the center of each plot at the uppermost ear level. The temperature of different organs (tassel, ear, leaves and basal internodes) was measured between 1100 and 1300 h once along the heating periods (on a clear day) by means of an infrared thermometer (OS 541, Omegaette, European Community). Organ temperature was surveyed on three plants per plot in all treatment combinations (except for tassel, ear and uppermost leaf of GS₁ plots because they were not present during heating).

Nine plants per plot were tagged at V₁₁ in both experiments. The stages of V₁₅, R₁, R₂ and R₆ were registered in all tagged plants. All measurements performed on the crop were referred to three periods: (i) the pre-silking period (PRE) of the critical period (CP) for kernel set, i.e. between V₁₅ and R₁, (ii) the post-silking period (POST) of the CP, i.e. between R₁ and R₂, and (iii) the effective grain-filling period (EGF), i.e. between R₂ and R₆.

Plant leaf area was surveyed at silking of each tagged plant and expressed as the sum of the area of all green leaves (visual assessment). Individual leaf area was computed as lamina length × maximum width × 0.75 (Montgomery, 1911). Leaf area index (LAI) was calculated at R₁ (LAI_{R1}) and R₆ (LAI_{R6}) as the product of plant leaf area and stand density.

Fraction of IPAR intercepted by canopy (fIPAR) was measured weekly between V₁₅ and R₆. Records were obtained at midday, between 1130 and 1430 h, using a line quantum-sensor (Cavabar, Cavadevices, Argentina). In each plot, one meter of the sensor was placed (i) once at the top of the canopy and outside the shelters to determine incident photosynthetically active radiation (IPAR₀; in μmol m⁻² s⁻¹), and (ii) four times diagonally across the interrow space of the two central rows of the sheltered area, immediately below the lowermost green leaves of the canopy (IPAR_{SB}, average of four records). Values of IPAR₀ were corrected for the attenuation produced by the polyethylene film (–15%) for obtaining incident photosynthetically active radiation at the top of the canopy and inside the shelters (IPAR_{ST}). At each observation date, fIPAR of each plot was calculated using Eq. (1).

$$fIPAR = 1 - \left(\frac{IPAR_{SB}}{IPAR_{ST}} \right) \quad (1)$$

Daily values of fIPAR were estimated by linear interpolation between observed values. fIPAR was calculated for the pre-silking period (fIPAR_{PRE}), the post-silking period (fIPAR_{POST}), and the effective grain-filling period (fIPAR_{EGF}) as the average of daily fIPAR in each period.

Daily IPAR was corrected for the attenuation caused by the polyethylene film. Cumulative IPAR was calculated for the pre-silking period (IPAR_{PRE}), the post-silking period (IPAR_{POST}), and the effective grain-filling period (IPAR_{EGF}) as the sum of daily IPAR in each period. Cumulative IPAR intercepted by the canopy (IPAR_i) was also estimated for these periods as the daily product of IPAR and fIPAR in each period.

Shoot biomass of all tagged plants was estimated at V₁₅, R₁ and R₂ by means of allometric models based on the relationship between plant biomass and morphometric variables (Vega et al., 2000; Maddonni and Otegui, 2004; Pagano et al., 2007). For all treatment combinations, 12–15 plants of variable size (i.e. plant height, stalk diameter) were harvested at V₁₅, R₁ and R₂ to obtain the parameters of mentioned models. Estimates for heated plants were obtained from models built with heated individuals and those for control plants from models built with non-heated material. Morphometric measurements included stem diameter at the base of the stalk, plant height from ground level to the collar of the last fully expanded leaf, and maximum ear diameter (only at R₁ and R₂). All tagged plants were harvested when 50% of the grains from the mid portion of the ears showed black layer formation, and final shoot biomass was registered for each plant. The sum of individual plant biomass of all tagged plants (i.e. estimated at V₁₅, R₁ and R₂, and observed at physiological maturity) was used to calculate crop growth rate (CGR; in g m⁻² d⁻¹) during the pre-silking period (CGR_{PRE}), the post-silking period (CGR_{POST}), and the effective grain-filling period (CGR_{EGF}). Radiation use efficiency (RUE, in g MJ⁻¹) was computed as the quotient between cumulative shoot biomass and cumulative IPAR_i during the pre-silking period (RUE_{PRE}), the post-silking period (RUE_{POST}), and the effective grain-filling period (RUE_{EGF}).

For granting adequate pollination of all tagged plants, fresh pollen was added manually at silking of each plot, as described in Rattalino Edreira et al. (2011). At final harvest, ears of each tagged plant were hand shelled, and kernels were weighed to determine plant (PGY, in g plant⁻¹) and crop grain yield (GY, in g m⁻²). Harvest index (HI) was calculated as the ratio between PGY and plant shoot biomass at physiological maturity. Grain yield was analyzed in terms of the apparent contribution of stored reserves and actual crop growth during the effective grain-filling period (CG_{EGF} = shoot biomass at R₆ – shoot biomass at R₂), assuming that (i) biomass accumulation in grains is negligible until R₂, (ii) reserve use = GY – CG_{EGF}, and (iii) no actual crop growth takes place when shoot biomass at R₆ ≤ shoot biomass at R₂ (i.e. CG_{EGF} = 0). This approach is similar to that used for the analysis of individual kernel weight response to crop growth per set kernel based on biomass accumulation during effective grain filling (Cirilo and Andrade, 1996; Borrás and Otegui, 2001). There is (i) apparent reserve use when GY > CG_{EGF} (i.e. reserve use > 0), (ii) a trade-off between reserve use and crop growth when GY = CG_{EGF} (i.e. apparent reserve use = 0), and (iii) apparent accumulation of reserves during active grain filling when GY < CG_{EGF} (i.e. apparent reserve use < 0).

2.3. Statistical analysis

Heat effect on grain yield and each studied variable was estimated as the difference between values computed for heated and control plots. The effect of treatments and their interactions was analyzed for all described traits by ANOVA performed across experimental years. A *t*-test was used to determine significant differences ($P < 0.05$) among means. Linear regression was used to test the relationship between variables.

3. Results

3.1. Weather conditions and cycle duration

A detailed information on meteorological conditions during experiments can be found in Rattalino Edreira et al. (2011). Briefly, different experimental years and sowing dates caused variations in the photothermal regime during the critical period for kernel

set (Table 1), both before and after silking. Mean air temperatures around flowering (ca. 30 d centered at silking of T_C plots) were similar between experimental years (25.5 °C for Exp₁ and 25.8 °C for Exp₂) but differed among sowing dates. The most delayed sowing (i.e. GS₁) exposed the crop to slightly lower temperatures (24.6 °C) than the intermediate (26.1 °C) and early (26.3 °C) sowings. A similar trend was registered for this variable during the grain-filling period, with mean records of 23.5 °C for plots of GS₁, 25.2 °C for plots of GS₂ and 25.4 °C for plots of GS₃.

Heating increased air temperature at ear level during the treatment period, especially around midday (see Fig. 1 in Rattalino Edreira et al., 2011). Mean daily absolute maximum air temperature at ear height was 35.2 ± 3.5 °C for T_H plots and 30.2 ± 3.3 °C for T_C plots across all treatment combinations. Within each experiment, the intensity of heat stress was similar for each GS × H combination, but it was larger for Exp₁ than for Exp₂. Heating caused a gradual increase in organs temperature across the canopy (Fig. 1). This trait was larger for the uppermost organs (i.e. tassel, uppermost leaves) than for the lowermost ones (i.e. basal internodes). There were no differences among sowing dates or hybrids in the temperature experienced by each organ during the heating period.

In Exp₁, characterized by low rainfall and high heliophany, crops experienced higher cumulative IPAR values around flowering (157 MJ m⁻² for IPAR_{PRE} and 138 MJ m⁻² for IPAR_{POST}; averaged across H × TR treatments) than in Exp₂ (143 MJ m⁻² for IPAR_{PRE} and 114 MJ m⁻² for IPAR_{POST}). Delayed sowing date (GS₁ > GS₂ > GS₃) caused reductions in cumulative IPAR values during this period. Averaged across Year × H × TR, the level of cumulative IPAR during the 15-d period before silking (PRE in Table 1) was 125 MJ m⁻² for plots of GS₁, 155 MJ m⁻² for plots of GS₂, and 170 MJ m⁻² for plots of GS₃. For the 15-d period after silking (POST in Table 1) this level was 114 MJ m⁻², 123 MJ m⁻², and 141 MJ m⁻², respectively. A similar trend was registered for this variable during the grain-filling period (EGF in Table 1), especially in Exp₁ (266, 296 and 331 MJ m⁻² for GS₁, GS₂, and GS₃, respectively; average of T_C plots across hybrids for avoiding the bias of reduced cycle duration due to heating). Differences among sowing dates during this stage were less pronounced in Exp₂ (223, 234 and 243 MJ m⁻² for GS₁, GS₂, and GS₃, respectively). Cumulative IPAR during grain filling was mainly associated with the duration of this period in heated plots. Heating caused a shortening in cycle duration, but significant ($P \leq 0.001$) interactions detected a stronger effect of above-optimum temperatures (i) during grain filling than before this stage, and (ii) for the Te hybrid (reduction of 21 d for Exp₁ and 12 d for Exp₂; Table 1) than for the other hybrids. Consequently, plots of the Te hybrid heated during GS₃ had the largest reduction in cumulative IPAR_{EGF} values as compared to its non-heated counterparts (−170 MJ m⁻² in Exp₁ and −76 MJ m⁻² in Exp₂).

3.2. Leaf area and light interception

Leaf area index at silking (LAI_{R1}) differed significantly ($P < 0.001$) between years and among hybrids. Thermal regimes, however, had no effect on this trait (Table 1). Largest LAI_{R1} values always corresponded to the Tr hybrid (10.4 in Exp₁ and 8.2 in Exp₂; averaged across studied periods and temperature regimes), and exceeded those registered for the Te (8.8 in Exp₁ and 7.8 in Exp₂) and the TeTr (8.7 in Exp₁ and 8.1 in Exp₂) hybrids. LAI_{R1} values were always above 6 (between 6.7 and 10.6), which granted high fIPAR during the critical period for kernel set (i.e. fIPAR_{PRE} and fIPAR_{POST} ≥ 0.95; Table 1). Thermal regimes and hybrids had no effect on fIPAR at this period.

As for LAI_{R1}, there was no effect of heating on LAI at physiological maturity (LAI_{R6}) and fIPAR during effective grain filling (Table 1). Most of the variation in these traits was linked to variations in sowing date. In general, post-flowering leaf senescence was largest for

plots of the last sowing date (GS₁ treatment), but the magnitude of this effect varied between experiments (significant Exp × GS interaction; Table 1). LAI_{R6} was smaller for GS₁ than for GS₂ and GS₃ (2.6, 5.4 and 5.6, respectively) in Exp₁, but no difference was found among studied periods in Exp₂. Despite these differences, fIPAR during grain filling remained close to the high values mentioned for the critical period (fIPAR_{EGF} ranged between 0.94 and 0.99; Table 1).

3.3. Biomass production and RUE

Crop growth rate (CGR) during the critical period for kernel set (CGR_{PRE} and CGR_{POST}) and the effective grain-filling period (CGR_{EGF}) differed significantly ($P < 0.003$) between years for non-heated plots, but hybrids and sowing dates had no effect on these traits (Table 2). Heat stress reduced CGR in each studied period (i.e. CGR_{PRE} for GS₁, CGR_{POST} for GS₂, and CGR_{EGF} for GS₃; Table 2, $P < 0.001$). In spite of similar heating intensity for all GS × H combinations (Rattalino Edreira et al., 2011), CGR was more affected by heating during GS₂ (−31.7 g m⁻² d⁻¹ respect to T_C plots; averaged across experiments and hybrids) than during GS₁ and GS₃ (−21.7 and −13.6 g m⁻² d⁻¹, respectively). Additionally, hybrids differed in the response to above-optimum temperatures for this trait. The Te hybrid tended to show the largest reduction in CGR (Te ≥ Tr ≥ TeTr), but the magnitude of this effect varied among periods. Heating during GS₁ caused a larger effect in CGR during the pre-silking period (CGR_{PRE}) of the Te and Tr hybrids (−25.6 and −24.6 g m⁻² d⁻¹ respect to T_C plots, respectively; averaged across experiments) than of the TeTr hybrid (−15 g m⁻² d⁻¹). Similarly, heating during GS₂ had a larger negative effect on CGR during silks exposure (CGR_{POST}) of the Te and Tr hybrids (−37.3 and −31.1 g m⁻² d⁻¹) than of the TeTr hybrid (−26.8 g m⁻² d⁻¹). Heating during GS₃, however, affected CGR during effective grain filling (CGR_{EGF}) in a larger extent for the Te hybrid (−25.4 g m⁻² d⁻¹) than for the Tr (−11 g m⁻² d⁻¹) and the TeTr (−4.5 g m⁻² d⁻¹) hybrids.

After heat stress removal, plants subjected to heat stress around flowering (i.e. GS₁ and GS₂) exhibited a recovery in growth, and reached CGR values similar to those registered among non-heated plants. This means that $T_C = T_H$ for (i) CGR_{POST} of plots heated during GS₁, and for (ii) CGR_{EGF} of plots heated during GS₂ (Table 2). This trend, however, did not compensate for plant biomass reductions caused by heating (Table 2 and Fig. 2). Consequently, shoot biomass at physiological maturity was significantly reduced ($P < 0.01$) in all heated plots as compared to non-heated plots. The only exception was the TeTr hybrid heated during GS₃, which did not differ markedly from its non-heated counterpart, particularly during Exp₁. The significant ($P < 0.05$) H × TR interaction detected for this variable indicated that (i) final shoot biomass in non-heated plots was slightly larger for the Tr hybrid (2564 g m⁻², averaged across experiments and growth stages) than for the Te and TeTr hybrids (2388 and 2372 g m⁻², respectively), and (ii) negative effects of heat stress on final shoot biomass were larger for the Te hybrid (−603 g m⁻² respect to T_C plots, averaged across experiments and studied periods) than for the other two hybrids (−373 g m⁻² for TeTr and −489 g m⁻² for Tr hybrid). There were no differences among GSs in the response to heating for this variable.

Negative effects of heating on biomass production could not be linked to reductions in leaf area and fIPAR (Section 3.2). This trend was supported by the scarce difference in cumulative IPAR between T_C and T_H plots (Table 1 and Fig. 2). The only clear exception was for the Te hybrid during grain filling (Table 1 and Fig. 2g), for which heating caused accelerated maturity in both experiments. For all H × GS combinations, the main effect of above-optimum temperatures was a marked decline in biomass production per unit of cumulated IPAR_i (i.e. RUE; Table 2). Heat stress always reduced RUE in each studied period (i.e. RUE_{PRE} for GS₁, RUE_{POST} for GS₂, and RUE_{EGF} for GS₃; Table 2). The negative effect of heating was larger

Table 2

Treatments effect on crop growth rate (CGR), radiation use efficiency (RUE), final shoot biomass, grain yield (GY), and harvest index (HI). Heat effect represents the difference between heated (T_H) and control (T_C) plots. ANOVA results are presented at the bottom of the Table.

Exp ^a	GS		H		TR		CGR		RUE		POST		EGF		Heat effect (heating period)		Final shoot biomass		Heat effect		HI		Heat effect																													
	GS ₁	GS ₂	GS ₃	Te	TeTr	T _C	T _H	PRE	POST	EGF	g m ⁻² d ⁻¹	PRE	POST	EGF	g MJ ⁻¹	Heat effect V _{15-R₆}	g m ⁻²	Heat effect	GY	Heat effect	HI	Heat effect																														
Exp ₁	GS ₁	Te	T _C	49.0	53.7	7.5	5.4	6.4	1.1	2371	967	0.41	2371	967	0.41	2371	967	0.41	2371	967	0.41	2371	967	0.41	2371	967																										
				21.7	39.4	16.0	2.4	5.1	2.4	2.4	1.0	2047	379	0.18	2047	379	0.18	2047	379	0.18	2047	379	0.18	2047	379	0.18	2047	379																								
				31.9	43.5	19.2	3.5	5.2	3.0	3.5	3.0	2496	980	0.39	2496	980	0.39	2496	980	0.39	2496	980	0.39	2496	980	0.39	2496	980																								
				22.8	22.4	19.8	2.6	2.9	3.1	2.6	3.1	1985	357	0.18	1985	357	0.18	1985	357	0.18	1985	357	0.18	1985	357	0.18	1985	357																								
	GS ₂	Tr	T _C	43.7	36.0	12.8	4.8	4.5	2.0	2.0	2490	884	0.35	2490	884	0.35	2490	884	0.35	2490	884	0.35	2490	884	0.35	2490	884																									
				16.6	24.9	19.2	1.8	3.3	3.1	1.8	3.1	2370	120	0.02	2370	120	0.02	2370	120	0.02	2370	120	0.02	2370	120	0.02	2370	120																								
				44.4	54.8	3.0	4.4	5.8	0.4	4.4	0.4	2344	847	0.36	2344	847	0.36	2344	847	0.36	2344	847	0.36	2344	847	0.36	2344	847																								
				40.9	9.2	4.5	4.1	1.0	0.6	4.1	0.6	1566	778	0.03	1566	778	0.03	1566	778	0.03	1566	778	0.03	1566	778	0.03	1566	778																								
	GS ₃	Te	T _C	56.7	33.9	5.0	5.6	3.6	0.7	2355	893	0.38	2355	893	0.38	2355	893	0.38	2355	893	0.38	2355	893	0.38	2355	893																										
				55.1	0.1	11.4	5.4	0.0	1.5	5.4	1.5	2060	295	0.15	2060	295	0.15	2060	295	0.15	2060	295	0.15	2060	295	0.15	2060	295																								
				46.7	18.5	12.4	5.0	2.1	1.7	5.0	1.7	2554	883	0.35	2554	883	0.35	2554	883	0.35	2554	883	0.35	2554	883	0.35	2554	883																								
				47.4	-13.9	10.1	5.1	-1.6	1.5	5.1	1.5	2089	465	0.19	2089	465	0.19	2089	465	0.19	2089	465	0.19	2089	465	0.19	2089	465																								
GS ₁	TeTr	T _C	43.1	31.9	22.8	3.7	3.2	2.6	2.6	2629	830	0.25	2629	830	0.25	2629	830	0.25	2629	830	0.25	2629	830	0.25	2629	830																										
			45.5	34.7	-8.2	4.2	4.0	2.4	4.2	2.4	2804	73	0.05	2804	73	0.05	2804	73	0.05	2804	73	0.05	2804	73	0.05	2804	73																									
			49.2	40.7	18.9	4.3	3.9	2.0	4.3	2.0	2731	1009	0.36	2731	1009	0.36	2731	1009	0.36	2731	1009	0.36	2731	1009	0.36	2731	1009																									
			50.4	38.9	15.6	3.5	2.2	1.9	3.5	1.9	2782	656	0.22	2782	656	0.22	2782	656	0.22	2782	656	0.22	2782	656	0.22	2782	656																									
Exp ₂	GS ₁	Te	T _C	42.1	26.2	20.7	6.0	4.0	3.7	2568	998	0.39	2568	998	0.39	2568	998	0.39	2568	998	0.39	2568	998	0.39	2568	998																										
				18.0	28.0	22.6	2.5	4.2	3.8	2.5	3.8	1960	607	0.12	1960	607	0.12	1960	607	0.12	1960	607	0.12	1960	607	0.12	1960	607																								
				31.5	32.9	25.4	4.5	4.5	4.4	4.5	4.4	2401	886	0.37	2401	886	0.37	2401	886	0.37	2401	886	0.37	2401	886	0.37	2401	886																								
				10.5	33.3	17.6	1.4	5.3	3.0	1.4	3.0	1822	579	0.17	1822	579	0.17	1822	579	0.17	1822	579	0.17	1822	579	0.17	1822	579																								
	GS ₂	Tr	T _C	35.2	27.4	27.0	5.2	4.2	5.0	5.0	2816	1075	0.38	2816	1075	0.38	2816	1075	0.38	2816	1075	0.38	2816	1075	0.38	2816	1075																									
				13.2	22.2	22.0	1.9	3.4	4.0	1.9	4.0	2044	772	0.22	2044	772	0.22	2044	772	0.22	2044	772	0.22	2044	772	0.22	2044	772																								
				33.9	30.7	12.4	3.3	4.7	2.0	3.3	2.0	2085	506	0.24	2085	506	0.24	2085	506	0.24	2085	506	0.24	2085	506	0.24	2085	506																								
				31.6	1.6	13.4	3.1	0.3	2.1	3.1	2.1	1616	469	0.04	1616	469	0.04	1616	469	0.04	1616	469	0.04	1616	469	0.04	1616	469																								
	GS ₃	TeTr	T _C	35.9	25.8	15.0	3.4	3.9	2.4	2.4	2112	613	0.28	2112	613	0.28	2112	613	0.28	2112	613	0.28	2112	613	0.28	2112	613																									
				34.6	6.0	10.4	3.3	0.9	1.6	3.3	1.6	1601	510	0.02	1601	510	0.02	1601	510	0.02	1601	510	0.02	1601	510	0.02	1601	510																								
				31.7	15.7	20.8	3.2	2.3	3.3	3.2	3.3	2299	634	0.26	2299	634	0.26	2299	634	0.26	2299	634	0.26	2299	634	0.26	2299	634																								
				32.5	-14.2	19.2	3.4	-2.0	3.0	3.4	3.0	1821	478	0.11	1821	478	0.11	1821	478	0.11	1821	478	0.11	1821	478	0.11	1821	478																								
GS ₁	Tr	T _C	33.4	29.7	20.2	3.1	3.3	3.0	3.0	2332	923	0.40	2332	923	0.40	2332	923	0.40	2332	923	0.40	2332	923	0.40	2332	923																										
			35.4	33.2	0.4	3.3	3.7	0.0	3.3	0.0	1724	608	0.35	1724	608	0.35	1724	608	0.35	1724	608	0.35	1724	608	0.35	1724	608																									
			39.5	24.1	15.5	3.8	2.8	2.3	3.8	2.3	2063	792	0.38	2063	792	0.38	2063	792	0.38	2063	792	0.38	2063	792	0.38	2063	792																									
			40.6	20.9	9.8	3.8	2.4	1.4	3.8	1.4	1796	267	0.02	1796	267	0.02	1796	267	0.02	1796	267	0.02	1796	267	0.02	1796	267																									
GS ₂	Tr	T _C	27.7	20.7	20.4	2.7	2.7	3.1	3.1	2443	876	0.36	2443	876	0.36	2443	876	0.36	2443	876	0.36	2443	876	0.36	2443	876																										
			32.5	20.7	12.9	3.2	2.4	1.9	3.2	1.9	1998	445	0.04	1998	445	0.04	1998	445	0.04	1998	445	0.04	1998	445	0.04	1998	445																									
			0.003 ^b	0.003	0.001	0.003	0.047	<0.001	ns	<0.001	ns	0.013	ns	ns	0.016	ns	ns	ns	0.016	ns	ns	ns	ns	ns	ns	ns	ns																									
			<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.015	ns	<0.001	<0.001	<0.001	ns	ns	0.030	0.004	0.003	<0.001	<0.001	<0.001	<0.001	<0.001																										
Exp × GS	Exp × H	Exp × TR	GS × H	GS × TR	H × TR	Exp × GS × H	Exp × GS × TR	GS × H × TR	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns																										
																											0.002	ns	ns	<0.001	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns			
																											ns	0.010	ns	ns	0.032	ns	ns	ns	ns	0.001	ns	ns	0.029	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	
																											ns	<0.001	ns	ns	0.015	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Exp × GS × TR	GS × H × TR	H × TR	Exp × GS × H	Exp × GS × TR	GS × H × TR	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns																										
																											ns	0.001	ns	ns	0.001	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns		
																											ns	0.001	ns	ns	0.001	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
																											0.005	0.015	0.004	ns	0.046	0.009	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

^a Exp, experiment; GS, growth stage; H, hybrid; TR, temperature regime; Te, temperature; Tr, tropical; TeTr, Te × Tr; PRE, pre-silking period of the critical period; POST, post-silking period of the critical period; EGF, effective grain-filling period.

^b P values of main and interaction effects for which at least one variable was detected as significant; ns, not significant (P > 0.05).

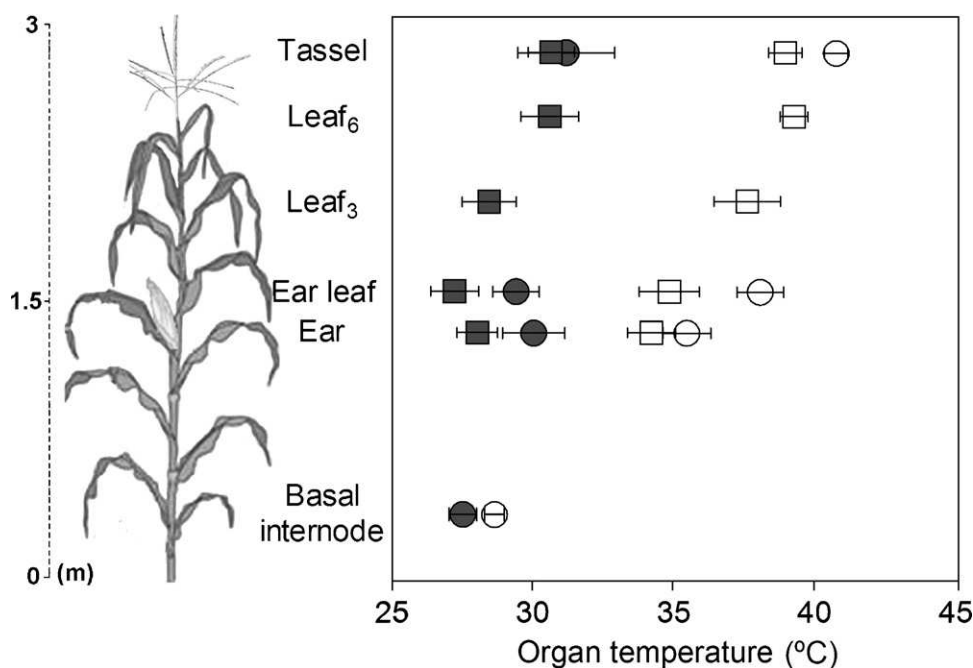


Fig. 1. Organs temperature across the canopy of plants exposed to two temperature regimes (black: control; white: heated during daytime hours) during two experimental years (Exp₁: circles; Exp₂: squares). Each symbol represents the average of 27 data (three growth stages, three hybrids, and three replicates). Error bars represent the 95% confidence interval. Air temperature at ear level averaged 39.1 °C for Exp₁ and 36.6 °C for Exp₂ at the time of measurement. Plant height represents the average of all treatment combinations.

when it was performed during GS₂ (-4 g MJ^{-1} respect to T_C plots, averaged across experiments and hybrids) than during GS₁ and GS₃ (-2.8 and -1.8 g MJ^{-1} , respectively). For this trait, hybrids differed in their response to stress among evaluated periods, and followed the trend previously described for CGR. Heating around flowering caused a larger effect on RUE of the Te and Tr hybrids than of the TeTr hybrid. However, when heating occurred during the grain-filling period, the negative effect of above-optimum temperatures on RUE was largest for the Te hybrid.

Estimated RUE values in non-heated plots were similar among hybrids across all evaluated periods (i.e. PRE, POST and EGF period; Table 2) and appeared to be larger during the critical period for kernel set (4.2 g MJ^{-1} for RUE_{PRE} and 3.8 g MJ^{-1} for RUE_{POST}, averaged across all non-heated treatment combinations) than during the effective grain-filling period (2.5 g MJ^{-1} for RUE_{EGF}). A wide range of variation in RUE values was observed around flowering (between 2.1 and 6.4 g MJ^{-1} across all non-heated treatment combinations; Table 2) when this trait was computed as the quotient between biomass increase and IPARI increase during the pre- and the post-silking periods. Variation in RUE values was smaller (ranged between 2.6 and 5.7 g MJ^{-1} ; data not shown) when this trait was estimated as the slope of the relationship between cumulative shoot biomass and cumulative IPARI for the whole period around flowering.

3.4. Grain yield and harvest index

Grain yield in non-heated plots was higher during Exp₁ (971 g m^{-2} , averaged of T_C plots across growth stages and hybrids) than during Exp₂ (811 g m^{-2}). The significant ($P=0.003$) Exp \times GS \times TR interaction detected for this trait indicated that grain yield in T_C plots was similar among studied periods during Exp₁, but not during Exp₂. For the latter, grain yield registered in control plots of GS₂ (584 g m^{-2} , averaged of T_C plots across hybrids) was lower than that registered in plots of GS₁ and GS₃ (864 and 986 g m^{-2} , respectively). Grain yield of non-heated plots never differed among hybrids.

Heat stress reduced grain yield ($P<0.001$, Table 2), and this negative effect was similar between experiments but not among evaluated growth stages and hybrids. The interaction effects detected for this trait in the response to above-optimum temperatures indicated that (i) grain yield reduction was stronger when heating occurred during the period around flowering (-527 for GS₁ and -545 g m^{-2} for GS₂ respect to T_C plots; averaged across experiments and hybrids) than during the grain-filling period (-352 g m^{-2} for GS₃), (ii) the Te hybrid was the most sensitive to heating (-599 g m^{-2} , averaged across experiments and studied periods), followed by the TeTr (-440 g m^{-2}) and the Tr hybrids (-384 g m^{-2}), and (iii) the largest reduction in grain yield across experimental years corresponded to the Te hybrid heated during GS₂ (-797 g m^{-2}).

The observed variation in grain yield was well explained by the variation registered in total biomass at maturity ($r^2=0.81$), but heat stress affected the relationship between these traits (Fig. 3a). Grain yield variation of T_H plots was better explained ($r^2=0.92$) by the variation registered in harvest index, than by the variation registered in final shoot biomass ($r^2=0.6$, Fig. 3b). Harvest index was markedly reduced by heating ($P<0.001$; Table 2), but this negative effect varied among growth stages and hybrids. It was severely reduced when heating occurred around flowering, particularly for the Te (-0.25 in GS₁ and -0.25 in GS₂, averaged across experiments) and TeTr (-0.21 in GS₁ and -0.24 in GS₂) hybrids. Harvest index of the Tr hybrid was less affected by heating (-0.07 in GS₁ and -0.15 in GS₂). Heating during the grain-filling period had a lower negative effect on harvest index than heating around flowering, and the magnitude of this reduction was larger for the Te (-0.11) and Tr (-0.11) hybrids than for the TeTr hybrid (-0.02).

Observed variations in harvest index were analyzed in terms of the apparent contribution of reserves to grain yield (i.e. GY-CG_{EGF}, Fig. 4a). This variable was always negative for plots heated around flowering (-266 g m^{-2} for GS₁ and -241 g m^{-2} for GS₂), and hence these treatments were usually distributed below the 1:1 relationship. Reserves contribution to grain yield increased for plots heated during the grain-filling period (423 g m^{-2}). Moreover, we detected

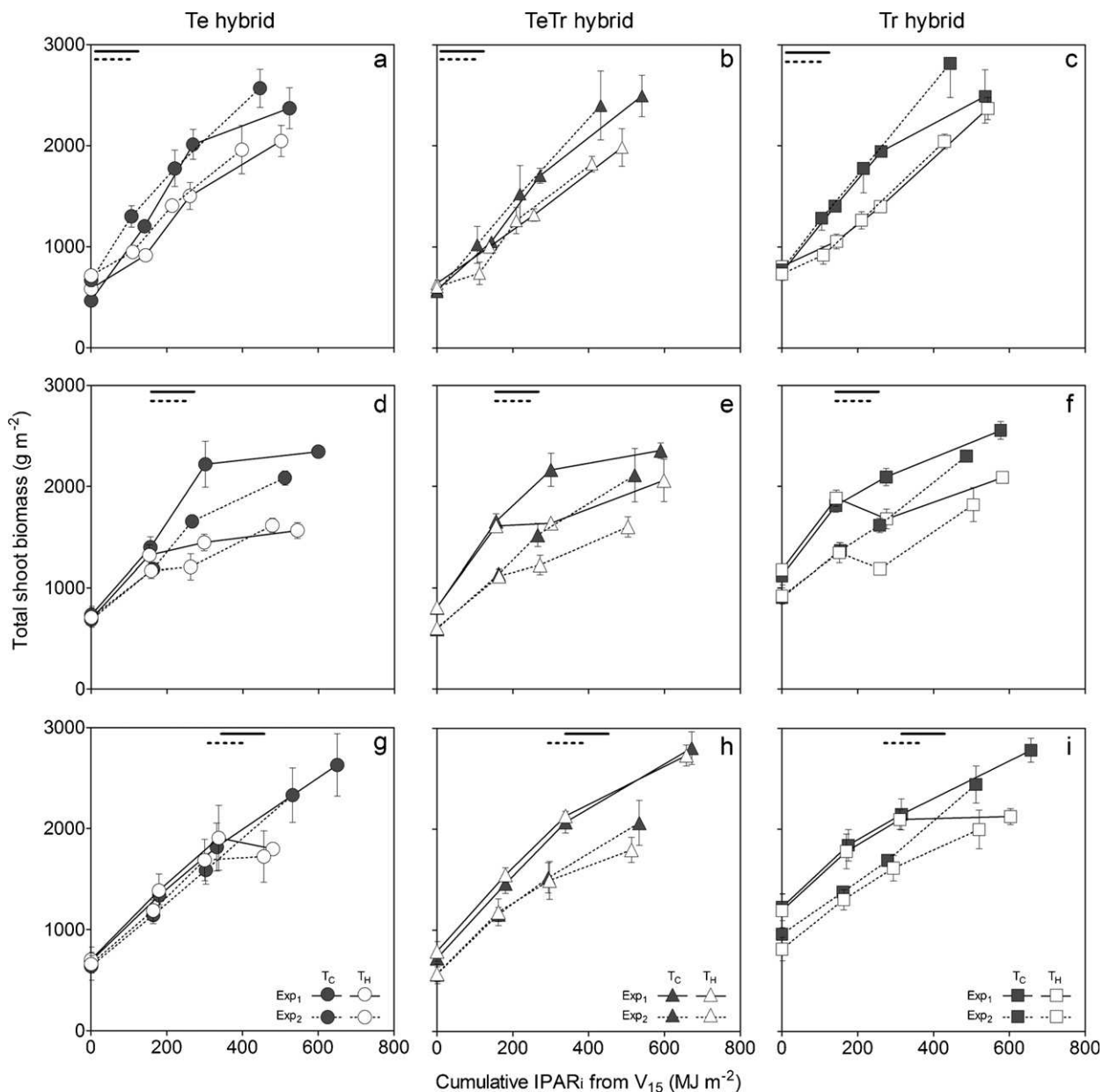


Fig. 2. Relationship between total shoot biomass and cumulative incident photosynthetically active radiation intercepted by the crop (IPARi) from V₁₅ to physiological maturity of three maize hybrids of contrasting origin (Te: temperate; Tr: tropical; TeTr: Te × Tr) grown under two temperature regimes (T_C : control; T_H : heated during daytime hours) during three growth stages (GS₁: a–c; GS₂: d–f; GS₃: g–i) in two experimental years (Exp_n). Each GS covered a 15-d period (GS₁: immediately before anthesis; GS₂: from the start of silking onwards; GS₃: from the end of GS₂ onwards). Horizontal bars indicate the heating period for Exp₁ (filled) and Exp₂ (dotted). Points along each curve correspond to the ontogenetic stages of V₁₅, R₁, R₂ and R₆.

that biomass accumulated in grains could be attributed exclusively to apparent reserves use in plots of the Te and Tr hybrids heated during grain filling in Exp₁. Variations in HI were well explained by estimated variations in reserves use ($r^2 = 0.59$, Fig. 4b).

4. Discussion

In the current research we studied biomass production and grain yield determination of three maize hybrids of contrasting genetic background, which were grown in the same temperate environment and were exposed to above-optimum temperatures during three different growth periods along the cycle. For the analysis we used the general ecophysiological framework proposed by Passioura (1996), based on resource capture, resource use efficiency for biomass production and biomass allocation to grains. Because

soil resources (i.e. water and nutrients) were non-limiting, analysis was based on IPAR. In our experiments, all treatment combinations reached the critical LAI (i.e. LAI that grants $fIPAR \sim 0.95$; Brougham, 1957) during the critical period for kernel set and the grain-filling period, with the concomitant maximization of light interception. The tropical hybrid had the largest maximum LAI (i.e. LAI_{R1}), as expected for genotypes with this genetic background (Fischer and Palmer, 1984). The lack of effect of brief episodes of heat stress on LAI_{R1} and LAI_{R6} may account for the low influence of above-optimum temperatures on tissue expansion (Cicchino et al., 2010b) and leaf senescence (never reported in maize), a major aspect that distinguishes this constraint from water (Boyer, 1970; Chenu et al., 2008) or nitrogen stress (Uhart and Andrade, 1995). Post-flowering leaf senescence differed only in response to contrasting light offer (i.e. IPAR) during grain filling, and variations in this resource

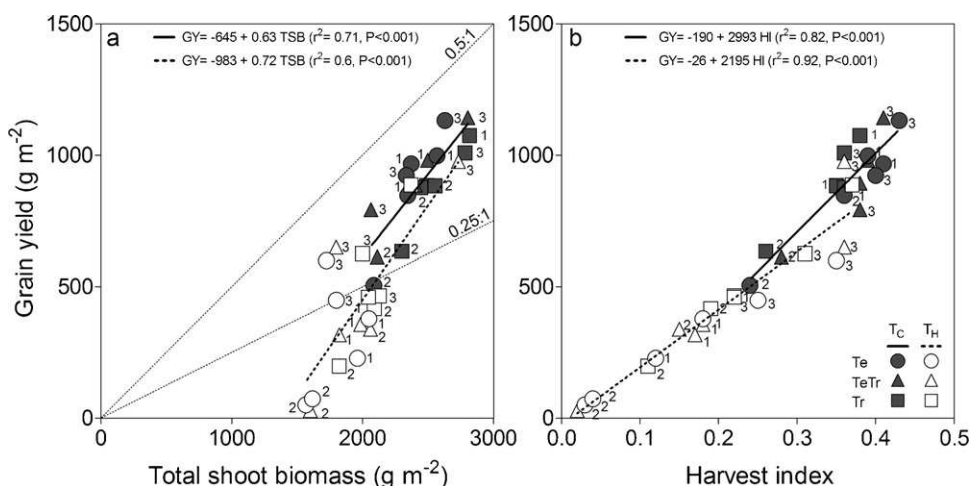


Fig. 3. Relationship between grain yield (GY) and (a) total shoot biomass (TSB), or (b) harvest index (HI). Data correspond to three maize hybrids (Te: temperate; Tr: tropical; TeTr: Te × Tr) of contrasting origin grown under two temperature regimes (T_C : control; T_H : heated during daytime hours) during three different growth stages (GS₁: 1; GS₂: 2; GS₃: 3) and two experimental years. Each GS covered a 15-d period (GS₁: immediately before anthesis; GS₂: from the start of silking onwards; GS₃: from the end of GS₂ onwards). Dotted lines in figure (a) represent the 0.25:1 and 0.5:1 ratios between grain yield and total shoot biomass (i.e. harvest index). Equations represent models fitted to each thermal regime.

could be attributed exclusively to natural variations linked to experiments (i.e. year effect) and sowing dates. Contrasts observed in the senescence patterns, therefore, support previous evidence of a strong control exerted by the post-flowering source–sink ratio on leaf area duration in this species (Borrás et al., 2003).

Although fIPAR was always very high (≥ 0.94), heat stress had a large effect on the amount of light captured by the canopy after flowering due to shortening of the grain-filling period. Variations in grain-filling duration have been documented for maize cropped under contrasting growing conditions (Allison and Daynard, 1979; Cirilo and Andrade, 1996). In this species, the effective duration of grain filling is regulated by the availability of assimilates per kernel during this phase (Gambín et al., 2006), independently of the limiting factor; i.e. similar response for defoliation (Tollenaar and Daynard, 1978; Jones and Simmons, 1983), shading (Andrade and Ferreiro, 1996), drought (Ouattar et al., 1987; Westgate, 1994), or above-optimum temperature (Wilhelm et al., 1999; Commuri and Jones, 2001). In our experiments, the shortening of this phase was particularly large for the Te hybrid heated during GS₃. Observed genotypic differences in grain-filling duration of field grown maize crops exposed to heat stress during their effective grain-filling period have been empirically attributed to an enhanced persistence

of the photosynthetic tissues (i.e. leaf greenness or stay-green) supposedly conferred by the tropical genetic background. These observations, however, were never quantified for contrasting genotypes grown under contrasting thermal regimes in the same field environment. Interestingly, our results did not fully support this contention. No clear trend was detected among hybrids for physiological indicators of stay-green (LAI, fIPAR) across treatment periods. Additionally, premature maturity observed in the Te hybrid heated during GS₃ could not be clearly linked to severe cellular injury that leads to a catastrophic collapse in cellular organization (Pennell and Lamb, 1997; Fan and Xing, 2004), because no case of sudden plant death ever occurred during heating (i.e. complete plant senescence always took place after heat stress removal).

Crop growth rate was severely reduced by heat stress in all treatment combinations. In our experiments, the magnitude of this reduction was even larger than that reported for water (Çakir, 2004; Echarte and Tollenaar, 2006) or nitrogen deficiencies (Lemcoff and Loomis, 1986; Uhart and Andrade, 1995) in field conditions. Moreover, we detected negative CGRs values caused by heating. This response was related exclusively to variations in radiation use efficiency (Fig. 2), because heating had no sizeable effect on fIPAR. These results are supported by previous findings based on

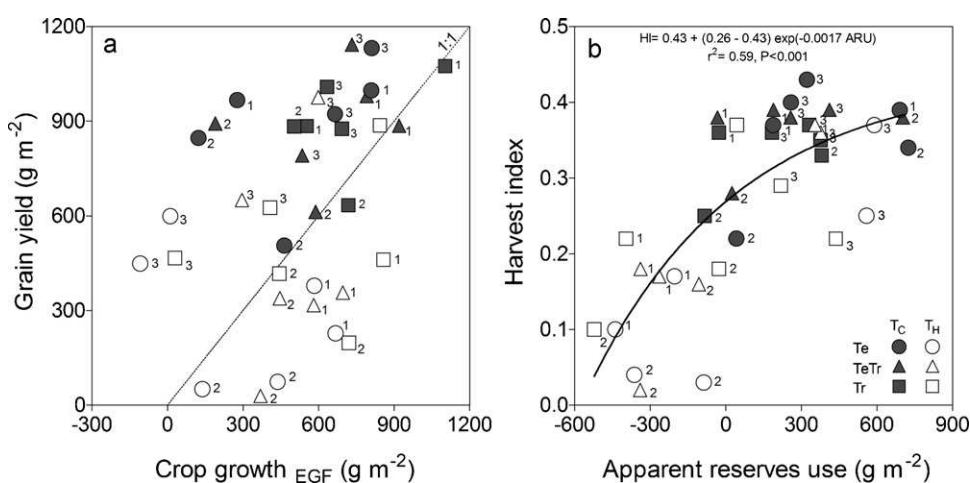


Fig. 4. Relationship between (a) grain yield and crop growth during effective grain-filling period, or (b) harvest index (HI) and apparent reserves use (ARU). Symbols as in Fig. 3. The dotted line in figure (a) represents the 1:1 relationship. Equation and filled line in (b) represent the model fitted to the whole data set.

a temperate maize hybrid (Cicchino et al., 2010b), where authors reported larger effects of heating on RUE than on light interception. Collectively, all evidence suggests that heat stress may affect RUE not only by damaging the photosynthetic system (Berry and Bjorkman, 1980; Crafts-Brandner and Salvucci, 2002), but also by increasing maintenance respiration (Penning de Vries et al., 1979). In spite of the large reduction in RUE registered during the heating period (between –15 and –187% across all treatment combinations), the negative effect of stress on final shoot biomass was less variable (between –3 and –33%). This may be attributable to the relatively brief duration of stress (it accounted for ca. 12–18% of the total crop cycle), and to rapid plant growth recovery after stress removal (Karim et al., 1999; Kreslavski et al., 2008). Our results clearly demonstrated the existence of genotypic differences in the sensitivity of RUE to stress ($T_e > T_r > T_{eTr}$), which had been never reported previously for field grown maize and could not be predicted from previous research using genotypes of diverse origin grown in non-stressed conditions (Dwyer and Tollenaar, 1989; Lafitte and Edmeades, 1997). Estimated RUE values in non-heated plots were similar among hybrids and greater than those usually reported for maize (Sinclair and Muchow, 1999; Lindquist et al., 2005). The latter may be related to the beneficial greenhouse effect on crop growth promoted by the polyethylene enclosures, due to an increased proportion of diffuse over direct solar radiation inside them (Cabrera et al., 2009). This trend enhances light distribution within the canopy with the concomitant benefit in photosynthetic activity (Sinclair et al., 1992). However, caution must be exercised in making direct comparisons between current RUE values and those obtained in most field conditions. First, because of mentioned effects of polyethylene film on the light environment (diffuse vs. direct solar radiation). Second, because the computation method may introduce important differences in the final result (Demetriades-Shah et al., 1992), and most research on RUE used a linear regression approach (Sinclair and Muchow, 1999) rather than a direct quotient (current research). Finally, RUE comparisons must take into account ambient temperature, which in control plots of our study was within the range for maximum RUE (Kiniry et al., 1989; Andrade et al., 1993).

Grain yield reductions caused by heat stress were mainly related to variations in harvest index, and to a lesser extent to variations in final shoot biomass. This response deserves two considerations, one referred to the rate of development of the stress and the other to its opportunity. On one hand, the observed response to heat stress differed from those usually reported under moderate water or nitrogen deficiencies along the cycle (Sinclair et al., 1990; Muchow, 1994; D'Andrea et al., 2006). It must be pointed out that mentioned deficiencies cause a gradual limitation to growth and allow plants to adjust their size to resource availability, whereas heat shock takes place more abruptly, particularly during the summer season in temperate environments of Argentina (Maddoni, 2012). On the other hand, the negative effect on final maize grain yield of all studied abiotic stresses was larger when they took place around flowering (Fischer and Palmer, 1984; Andrade et al., 2002), and heat stress did not escape from this general pattern. In this situation, the magnitude of harvest index decline observed in plots heated before (GS_1) or after (GS_2) silking did not depend upon the intensity of stress, because all heated plots experienced a similar heat intensity (Rattalino Edreira et al., 2011). Similarly, the decline was independent of negative heat effects on pollen viability (Herrero and Johnson, 1980), because fresh pollen was added daily to newly exposed silks in heated plots. Therefore, marked harvest index decline observed in these plots can be attributed exclusively to the large reduction registered in kernel set, as proposed by Echarte and Andrade (2003) for stand density effects. The associated response to this reduction is a decrease in reproductive sink strength for assimilates, which in current research matched high

crop growth rate during grain filling of both treatments (Fig. 2 a–f). This source-sink imbalance may explain the apparent accumulation of reserves registered during the effective grain-filling period of plots heated around silking (Uhart and Andrade, 1991; Borrás and Otegui, 2001). The opposite trend was detected among most plots heated during the effective grain-filling period (GS_3). These plots experienced no severe growth restriction for kernel set, a condition that enhanced sink demand for assimilates during grain filling (Borrás and Otegui, 2001; Uribelarrea et al., 2008). This demand was strongly supported by stored reserves, which seemed the only source of assimilates for some plots heated during GS_3 . Described variations in the source-sink ratio during grain filling had a clear effect on harvest index. This effect could be summarized in a simple exponential relationship (Fig. 4b).

Contrary to previous evidence (Fischer and Palmer, 1984; Duvick, 2005), the temperate background did not confer enhanced grain yield and harvest index in non-stressed conditions, but genotypes did have an important effect on these traits under heat stress. The temperate hybrid was the most sensitive across all studied periods. These differences could not be related to a differential capacity among hybrids to store assimilates in the stem and/or remobilize them to grains. Storage capacity is strongly linked to plant height, which has been markedly reduced in species with a large breeding history like maize (Hay and Gilbert, 2001; Lemaire and Gastal, 2009). Hybrids used in current research, however, did not differ markedly in this trait (data not shown). Similarly, no clear trend was detected among hybrids in (i) apparent reserves use, seemingly more affected by previously described source-sink ratio effects than by the genetic background, or (ii) stay-green, already analyzed. Differences, therefore, seemed chiefly caused by their contrasting sensitivity to above-optimum temperatures in (i) photosynthesis rather than in stay-green, evidenced in the large variation registered in RUE but not in LAI or fIPAR, and (ii) kernel set, analyzed in a previous paper (Rattalino Edreira et al., 2011).

5. Conclusions

Heat stress had a negative effect on biomass production, which was linked to variations in radiation use efficiency and the amount of light captured by the crop. The latter, however, was not associated with the effects of above-optimum temperatures on the fraction of IPAR intercepted by the canopy (i.e. tissue expansion effects on canopy size). It was due to negative effects of heat on cycle duration. Grain yield reductions caused by heat stress were more related to variations in harvest index than to variations in final shoot biomass, because hybrids were more sensitive to heating around flowering when kernel set is defined than to heating during the effective grain-filling period. Our most important findings were (i) the detection of genotypic differences among hybrids of contrasting origin in the response to heating for many evaluated traits, and (ii) the assessment of the relative contribution of current biomass production and apparent reserves remobilization during the grain-filling period to grain yield. The hybrid of full temperate genetic background was identified as the most sensitive to heating. This trend was not biased by the way heat effect was computed (absolute difference between T_H and T_C), because no genotypic differences were detected among hybrids in grain yield and its physiological determinants when non-heated plots were compared. We demonstrated the importance of reserves contribution to grain yield when heating was performed during the effective grain-filling period.

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