

Oil Yield Components of Maize Crops Exposed to Heat Stress during Early and Late Grain-Filling Stages

Luis I. Mayer,^{*} Juan I. Rattalino Edreira, Gustavo A. Maddonni

ABSTRACT

Heat stress frequently limits grain yield of summer crops. Most research on maize (*Zea mays* L.) has analyzed heat stress effects on crop physiology and kernel set but little attention has been given to kernel weight and its composition. The objectives of this work were (i) to assess the response of maize oil yield components (kernel number and weight, embryo oil concentration, and embryo/kernel ratio) to post-flowering heat stress and (ii) to explore changes in the sensitivity to this constraint across developmental stages and genotypes. Hybrids with different kernel types (flint, popcorn, and semident) were exposed to contrasting temperature regimes (nonheated and heated: air temperature at ear level > 35°C) during early or late stages of the effective grain filling. Oil yield was affected by early (up to -60%), and to a lesser extent, late (up to -40%) heat-stress episodes. These effects were mediated by lighter kernels ($r^2 = 0.94$; $P < 0.001$) and lower kernel oil concentrations ($r^2 = 0.80$; $P < 0.001$). Reductions of kernel weight were related to shorter grain-filling periods ($r^2 = 0.48$; $P < 0.01$), and those of kernel oil concentration were mainly associated with lower embryo oil concentrations ($r^2 = 0.64$; $P < 0.001$). Kernels of semident hybrids were the most prone to alter their weights and oil concentrations in the face of a wide range of heat stress intensities. Variations in kernel oil concentration due to high temperature became notorious when kernel weight reductions were larger than 20%.

L.I. Mayer, J.I. Rattalino Edreira, and G.A. Maddonni, Departamento de Producción Vegetal, Facultad de Agronomía, Universidad de Buenos Aires and Instituto de Fisiología y Ecología Vinculado a la Agricultura, Consejo Nacional de Investigaciones Científicas y Técnicas (IFEVA-CONICET), Av. San Martín 4453, C1417DSE, Ciudad de Buenos Aires, Argentina. Received: 28 Nov. 2013 ^{*}Corresponding author (mayer@agro.uba.ar).

Abbreviations: CST, cumulative stressful temperatures; Exp_n, experiment *n*; Flint, orange-flint hybrid; GS, growth stage; H, hybrid; HSE, heat stress episodes; KW, kernel weight; Pop, popcorn hybrid; SD, sowing date; Te, temperate semident hybrid; TeTr, temperate and tropical semident hybrid; T_{MAX}, average of daily maximum air temperatures; TR, temperature regime; T_T, threshold temperature (35°C) used in the quantification of the CST.

MAIZE (*Zea mays* L.) production plays a major role in human diet. Approximately 50% of worldwide production (ca. 442 million tons) is annually used as feedstock for dairy cattle, beef cattle, and poultry (FAO, 2009). Maize kernels with high oil concentration are desirables in feed rations of mentioned animal productions due to their large caloric content (Perry, 1988) and as substitutes for animal fats in these rations (Thomison et al., 2003). On the other hand, many maize-based foods (e.g., snacks, sweet corn, and porridge and bread) and industrial subproducts (e.g., starch, glucose, dextrose, fructose, syrup, refined oil and alcohol) also contribute to human diet. Specifically, refined maize oil is a high-quality subproduct (based on its physical and nutritional characteristics) obtained from embryo tissues in the milling industry (Watson, 1987).

At the crop level, maize oil yield is the product of kernel number per unit land area, kernel weight, and kernel oil concentration.

Published in Crop Sci. 54:2236–2250 (2014).

doi: 10.2135/cropsci2013.11.0795

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The latter trait is determined by the embryo/kernel ratio and the embryo oil concentration (Ingle et al., 1965; Tanaka and Maddonni, 2008). Accordingly, the analysis of the impact of cultural practices (e.g., genotype selection) and abiotic stresses (e.g., episodes of extremely high temperatures) on maize oil yield not only would have to consider their effects on grain yield components (i.e., kernel number and kernel weight) but also on the chemical composition of kernel tissues (i.e., embryo oil concentration).

Severe yield penalties in maize crops, likely associated to declines in net photosynthesis rates, increases in crop development rates, canopy respiration, reproductive processes failures, and water stress (Crafts-Brandner and Salvucci, 2002; Wahid et al., 2007; Stone, 2001), have been ascribed to global seasonal warming (Lobell and Asner, 2003; Lobell and Field, 2007; Tao et al., 2008; Sakurai et al., 2011; Schlenker and Lobell, 2010). Further research regarded the incidence of extremely high temperatures (e.g., days with air temperatures above 30–35°C), which has gradually increased during the last decades (Alexander et al., 2006), as the main factor responsible behind those productivity losses (Lobell et al., 2011; Schlenker and Roberts, 2009). Recent studies (Maddonni, 2012; Rattalino Edreira et al., 2011b) based on climatic records of the last 28 to 40 yr have quantified the occurrence of days with air temperatures above 35°C for different maize production areas of Argentina. Several days with high temperatures occur during the period when maize kernel weight and kernel oil concentration are defined (i.e., the effective grain-filling period). This scenario will be more drastic for the end of the current century because a positive trend in the frequency of hot days is expected over most of the world land areas (Field et al., 2012).

Previous evidence in maize crops revealed that temperatures higher than approximately 30 to 35°C caused detrimental effects on reproductive events (Cicchino et al., 2010a, 2010b; Rattalino Edreira et al., 2011a; Schoper et al., 1986), crop development (Cicchino et al., 2010a; Olsen et al., 1993), and crop growth (Cicchino et al., 2010b; Rattalino Edreira and Otegui, 2012). Considering grain yield components, kernel number and kernel weight were drastically reduced by high temperatures imposed around female flowering (i.e., silking) and immediately after silking, respectively (Cicchino et al., 2010b; Commuri and Jones, 1999; Cheikh and Jones, 1994; Jones et al., 1984; Rattalino Edreira and Otegui, 2012). In these studies, lack of fertile pollen and the abortion of fertilized flowers were the driving forces of kernel number reductions. With respect to kernel weight, heat stress imposed around silking affected kernel growth rate probably by its impact on potential kernel size (Gambín et al., 2006). When high temperatures were imposed during the effective grain-filling period, kernel set was not affected but kernel weight was reduced by the shortening of the kernel growth period

due to the limited source of assimilates to sustain kernels demand, that is, due to a lower postflowering source/sink ratio (Badu-Apraku et al., 1983; Rattalino Edreira, 2013).

The premature cessation of the effective grain filling by heat stress could reduce kernel oil concentration through changes of the embryo/kernel ratio and/or the embryo oil concentration. This hypothesis is supported by (i) the dynamics of both kernel oil components (Tanaka and Maddonni, 2008), (ii) results of sunflower (*Helianthus annuus* L.) and maize crops exposed to severe shadings during the effective grain-filling period (Izquierdo et al., 2008; Tanaka and Maddonni, 2009), and (iii) results of sunflower crops subjected to brief intervals of extremely high temperatures during the same period (Rondanini et al., 2003). Based on Tanaka and Maddonni (2008), the impact of heat stress on maize kernel oil concentration would depend on the time of occurrence of the stress. During the first half of the effective grain-filling period, kernel oil concentration sharply increases because both embryo/kernel ratio and embryo oil concentration augment. During the second half of this period, kernel oil concentration is almost stabilized because the increment of embryo/kernel ratio is counterbalanced by the reduction of embryo oil concentration. Hence, an interruption of kernel growth by heat stress at early stages of the grain-filling period would lead to a sharper decline of kernel oil concentration with respect to that imposed at late stages of this period.

Recent studies documented the different tolerance of maize hybrids to heat stress (Rattalino Edreira et al., 2011a; Rattalino Edreira and Otegui, 2012; Rattalino Edreira, 2013). Grain yield components of a tropical hybrid were less affected by heat stress imposed around silking and during the first half of the effective grain-filling period than those with a temperate background. However, neither the effect of heat stress on kernel composition nor heat stress effects at late stages of the grain-filling period were explored.

The objectives of the current research were (i) to assess the effects of brief episodes of extremely high temperatures during the postflowering period on maize oil yield and its principal components (i.e., kernel number, kernel weight, embryo/kernel ratio, and embryo oil concentration) and (ii) to explore changes in the sensitivity to heat stress across different grain-filling stages and genotypes through a quantitative approach. For these purposes, field-grown hybrids with different kernel types, such as those of the flint (ssp. *indurata*), popcorn (ssp. *everta*), and semident (ssp. *indentata*) genotypes, were exposed to contrasting temperature regimes at early or late stages of the effective grain-filling period.

MATERIALS AND METHODS

Crop Husbandry and Treatment Descriptions

Two field experiments (Exp₁: 2009–2010 and Exp₂: 2010–2011) were conducted at the Department of Plant Production,

Table 1. Detail of sowing, silking, and temperature regimes (TR) initiation dates, and characterization of heat stress imposed during early growth stages (GS₁) and late growth stages (GS₂) of the effective grain-filling period, according to air temperature measured at ear height: mean daily maximum air temperatures (T_{MAX}), number of days with hourly air temperatures above 35°C (heat stress episodes [HSE]), and cumulative hourly air temperatures above 35°C (cumulative stressful temperatures [CSE]). Values are the means ± the standard error (SE).[†]

Exp	GS	H	Sowing	Silking	TR initiation	T _{MAX} (°C)	HSE (d)	CST (°C h)
Exp ₁	GS ₁	Flint	13 Nov. 2009	19 Jan. 2010	09 Feb. 2010	36.3 ± 0.5	10.3 ± 0.3	145.9 ± 22
		Pop	15 Nov. 2009	22 Jan. 2010	10 Feb. 2010	35.1 ± 0.1	10.0 ± 0.0	61.2 ± 6
		Te	16 Nov. 2009	21 Jan. 2010	10 Feb. 2010	33.3 ± 0.6	7.0 ± 0.6	25.0 ± 7
		TeTr	16 Nov. 2009	22 Jan. 2010	10 Feb. 2010	33.3 ± 0.8	7.3 ± 0.3	32.9 ± 5
	GS ₂	Flint	15 Oct. 2009	26 Dec. 2009	31 Jan. 2010	34.6 ± 1.5	4.0 ± 0.6	55.4 ± 31
		Pop	19 Oct. 2009	01 Jan. 2010	07 Feb. 2010	40.3 ± 2.6	3.7 ± 0.3	99.1 ± 15
		Te	14 Oct. 2009	—	—	—	—	—
		TeTr	13 Oct. 2009	27 Dec. 2009	01 Feb. 2010	33.0 ± 0.5	2.0 ± 0.6	15.5 ± 5
Exp ₂	GS ₁	Flint	06 Dec. 2010	31 Jan. 2011	20 Feb. 2011	37.2 ± 0.8	13.3 ± 0.3	108.1 ± 30
		Pop	20 Nov. 2010	22 Jan. 2011	11 Feb. 2011	38.8 ± 1.5	11.7 ± 0.9	291.3 ± 68
		Te	01 Dec. 2010	28 Jan. 2011	18 Feb. 2011	38.8 ± 2.4	13.0 ± 0.6	335.3 ± 66
		TeTr	01 Dec. 2010	29 Jan. 2011	18 Feb. 2011	41.0 ± 0.7	13.7 ± 0.3	365.1 ± 24
	GS ₂	Flint	15 Nov. 2010	16 Jan. 2011	18 Feb. 2011	36.8 ± 1.1	10.7 ± 0.3	119.5 ± 29
		Pop	12 Nov. 2010	16 Jan. 2011	14 Feb. 2011	37.7 ± 0.4	11.7 ± 0.7	209.2 ± 12
		Te	11 Nov. 2010	15 Jan. 2011	17 Feb. 2011	37.7 ± 3.2	3.7 ± 0.9	64.8 ± 11
		TeTr	11 Nov. 2010	15 Jan. 2011	20 Feb. 2011	35.5 ± 1.8	6.7 ± 0.9	102.7 ± 38
Exp						***	***	***
GS						NS	***	**
H						**	***	NS
Exp × GS						**	NS	**
Exp × H						**	NS	*
GS × H						**	***	NS
Exp × GS × H						**	***	*

[†] Exp, experiment; GS, growth stage; H, hybrid; Flint, orange-flint; Pop, popcorn; Te, temperate semident; TeTr, temperate and tropical semident; NS, not significant.

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

University of Buenos Aires, Argentina (34°35' S, 58°29' W) on a silty clay loam soil (Vertic Argiudoll; Soil Survey Staff, 2010). Treatments included a combination of four maize hybrids (H) with distinctive kernel type (orange-flint [Flint]; popcorn [Pop]; temperate semident [Te]; and temperate and tropical semident [TeTr]) and two temperature regimes applied at day-time hours (nonheated control and heated) during two different growth stages (GS) of the effective grain-filling period (early and late) of 15-d duration. Tested hybrids were Mill 522 (Flint), 2M545 HX (Te), and 2A120 HX (TeTr), all currently produced by Dow Agrosiences Argentina, and P802 (Pop) produced by Agricultural Alumni Seed Improvement Association Inc.

Different sowing dates (SD) were used for each growth stage and hybrid combination (Table 1) to start temperature regime at a same calendar date. This was done to apply heat treatments with similar environmental conditions (mainly incident solar radiation). All experiments were sown with the same stand density of 9 plants m⁻². Treatments were distributed in a split split-plot design, with growth stage in the main plot with three replicates, hybrid in the subplot, and temperature regime in the sub-subplot. The subplots involved six rows of 15-m length, 0.5 m apart, and the sub-subplots were comprised by three lineal meters of the four central rows of each subplot (ca. 6 m²). Crops were fertilized with urea (200 kg N ha⁻¹)

at V₆ (Ritchie and Hanway, 1982). Water availability of the uppermost meter 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.

At the beginning of each heating period (see below), sub-subplots of the heated treatment were enclosed with transparent polyethylene film (100 µm thickness) mounted on wood structures to increase air temperature (Cicchino et al., 2010a). Lateral films reached the soil surface on all sides, except one side that had a 10-cm opening at the bottom for helping adequate gas exchange. Additionally, roofs of shelters were pierced to avoid excessive heating and gas blockage at the top of the canopy. Similar shelters were built in sub-subplots of the nonheated treatment but with the lateral films opened up to 1.4 m above soil surface. This was done to avoid differences in incident solar radiation between temperature regimes due to the polyethylene film. Although heat treatment was mainly promoted by the greenhouse effect of the polyethylene enclosure (Cicchino et al., 2010a), shelters were supplemented with an electric fan heater monitored by an automated control unit (Cavadevices, Buenos Aires, Argentina) to increase air temperature during cloudy days. More details about the heating system can be found in Rattalino Edreira et al. (2011a).

Heat treatment during early stages of the effective grain filling started approximately 18 to 20 d after 50% of plants at each subplot reached R_1 , and finished 15 d later. Heat treatment during late stages started approximately 32 to 35 d after 50% of plants at each subplot reached R_1 , and finished 15 d later. All shelters were set up and dismantled at the start and at the end of their corresponding heating period, respectively.

Although the experimental technique used in this research for promoting heat stress can modify relative humidity and maximum air temperature within the shelters, it does not affect plant water status. This fact was proved by means of oxygen and carbon isotopes signatures analyzed in mature kernels of crops at the end of the experiments (data not shown; Araus et al., 2010; Barbour, 2007; Cabrera-Bosquet et al., 2009a, 2009b; Farquhar et al., 2007; Scheidegger et al., 2000).

Measurements and Computations

Characterization of the Temperature Regime

Hourly air temperature of nonheated and heated shelters was measured along the entire temperature regime period by means of sensors (independent of the one from the automated control unit) connected to data loggers (Temp-Logger, Cavadevices, Buenos Aires, Argentina). These sensors, shielded in double-walled plastic cylinders with open ends, were positioned in the center of each sub-subplot at the apical ear height.

For each sub-subplot, temperature regime intensity was characterized through computing (i) the mean maximum temperature (T_{MAX} ; average of daily maximum air temperatures), (ii) the number of heat stress episodes (HSE; record of days in which hourly temperature rose above 35°C), and (iii) the cumulative stressful temperatures (CST [in °C h]; Eq. [1]). The CST was calculated as:

$$CST = \sum_{i=1}^n (T_x - T_T) \quad [1]$$

where n is the duration of the temperature regime (in hours), T_x is the hourly air temperature (in °C), and T_T is the threshold temperature set at 35°C (Badu-Apraku et al., 1983; Berry and Bjorkman, 1980; Commuri and Jones, 2001; Cheikh and Jones, 1994; Rattalino Edreira et al., 2011a).

Mentioned descriptors were eventually quantified until the cessation of the grain-filling period (see *Dynamic of Kernel Growth*, below) whenever it occurred before the end of the heating period.

Plant Biomass, Grain Yield, and Grain Yield Components

A total of 46 plants per sub-subplot were tagged at V_6 . Silking date (i.e., first silk visible) of the apical ear was registered for all tagged plants. Thirty-seven plants were used to evaluate the dynamic of kernel growth during the grain-filling period and kernel oil concentration at R_6 (see *Dynamic of Kernel Growth* and *Kernel and Embryo Oil Concentration and Embryo/Kernel Ratio*, below). The remaining nine tagged plants were used to (i) estimate plant biomass at $R_1 - 15$ d (ca. 15 d before R_1), R_1 and $R_1 + 15$ d (ca. 15 d after R_1), and (ii) determine actual plant biomass at R_6 . Biomass estimations were performed by means of

allometric models broadly used in this species (D'Andrea et al., 2008; Echarte and Tollenaar, 2006; Laserna et al., 2012; Maddonni and Otegui, 2004; Pagano and Maddonni, 2007). Briefly, destructive plant samplings (15–30 plants per GS by H combination) were performed at $R_1 - 15$ d, R_1 and $R_1 + 15$ d to derive relationships between morphometric plant (height from ground level to the ligule of the last leaf and basal stem diameter) and apical ear (maximum diameter size; not included at $R_1 - 15$ d) traits and their actual biomass. Each harvested plant was oven dried at 70°C until constant weight to determine plant and ear biomass. Model parameters fitted for the current study are listed in Supplemental Table 1. Morphometric variables were also registered on the nine tagged plants, and the established allometric models were used to estimate total plant biomass (i.e., shoot vegetative biomass + ear biomass) and ear biomass. At R_6 , the nine tagged plants were harvested and oven dried at 70°C until constant weight to determine actual plant biomass. The apical ear of each plant (no fertile subapical ears were present) was hand shelled for counting kernel number per plant, and kernels were weighed to determine plant grain yield. Individual kernel weight was calculated as the quotient between plant grain yield and kernel number per plant. Kernel number per unit land area was computed as kernel number per plant multiplied by stand density.

Plant growth rate around flowering was estimated from the slope of the linear regression fitted to the response of total plant biomass to time (in d). This function was fitted to data obtained at $R_1 - 15$ d, R_1 and $R_1 + 15$ d. Source/sink ratio around flowering was computed as the ratio between plant growth rate and kernel number per plant. On the other hand, plant growth during the effective grain-filling period was calculated as the difference between observed individual plant biomass at R_6 and estimated plant biomass at $R_1 + 15$ d. Postflowering source/sink ratio was computed as the quotient between plant growth during the effective grain-filling period and kernel number per plant. Plants with less than 20 kernels were excluded from these computations.

Dynamic of Kernel Growth

Kernel growth was evaluated during the grain-filling period by means of periodic samplings of kernels from tagged plants. Two plants per sub-subplot were sampled weekly from 6–8 d after silking to 10–15 d after R_6 . At each sampling date, 10–15 kernels were taken from spikelet positions 10 to 15 from the bottom of the apical ear to avoid possible variation of kernel weight due to floret position (Borrás and Otegui, 2001; Tanaka and Maddonni, 2009; Tollenaar and Daynard, 1978). Kernel weight (KW) was registered after drying the kernels in an air-forced oven at 65°C for at least 96 h. Kernel growth rate and duration of the effective grain-filling period were estimated by fitting the response of kernel weight to time after silking by means of a trilinear with plateau model (Eq. [2–4]):

$$KW = aDAS, \text{ for } DAS \leq b \quad [2]$$

$$KW = ab + c(DAS - b), \text{ for } d \geq DAS > b \quad [3]$$

$$KW = ab + c(d - b), \text{ for } DAS > d \quad [4]$$

where DAS is days after silking, a is the kernel growth rate during the *lag* phase (in mg kernel⁻¹ d⁻¹), b is the duration of the *lag* phase (in days), c is the kernel growth rate during the effective grain-filling period (in mg kernel⁻¹ d⁻¹), and d is the duration of the grain-filling period (in days). The duration of the effective grain-filling period (in days) was calculated as the difference between grain-filling duration and *lag* phase duration (i.e., $d - b$).

Kernel and Embryo Oil Concentration and Embryo/Kernel Ratio

At each sub-subplot, kernels from approximately 8 to 10 tagged plants were collected 10 to 15 d after R₆ and analyzed for oil concentration by near-infrared transmittance (Infratec 1227, Tecator, Sweden). Kernel oil concentration (in g kg⁻¹) was expressed on a dry weight basis. Oil yield per unit land area was calculated as kernel oil concentration multiplied by plant grain yield and stand density.

After near-infrared transmittance analysis, at least a 50-g bulk of kernels from each sample was used to determine individual embryo weight and embryo oil concentration. Kernels were counted and soaked in water overnight at room temperature to facilitate the dissection of kernel components. Embryos were dissected from kernels by scalpel, frozen, lyophilized for 48 h and then weighed. Individual embryo weight was computed as the quotient between total embryos weight and the number of embryos. Embryo/kernel ratio was quantified as individual embryo weight divided by kernel weight.

Dissected embryos were ground to powder with an automatic miller (IKA Labortechnik, Staufen, Germany) and relyophilized to dry samples. Oil content of powder sample was determined by the Soxhlet extraction method with hexane for 12 h. Embryo oil concentration was calculated as the quotient of oil content and dry weight of powder sample.

Data Normalization and Statistical Analyses

To make comparable data from different experiments, sowing dates (i.e., GS), and hybrids, the response of each trait to temperature regime was normalized as percentage variation from the control nonheated treatment (Eq. [5]):

$$NV = 100(AV_H - AV_C) / AV_C \quad [5]$$

where NV is the normalized value of a given trait, AV_H and AV_C are the respective absolute values of the trait for the heated and the nonheated control temperature regime, respectively.

Absolute and normalized values of the traits were analyzed by means of a fixed effects ANOVA model in InfoStat Profesional 2012 (Di Rienzo et al., 2012) testing for the effect of treatments and their interactions; each treatment based on the corresponding source of error of a split split-plot design. In Exp₁, there was no record of the Te hybrid at early sowing date (i.e., temperature regimes could not be tested at late grain-filling stages) due to crop establishment failures (excessive wetness of the soil affected emergence of seedlings). This particular treatment combination was treated as missing cell (Shaw and Mitchell-Olds, 1993) for all surveyed traits. The least significant difference (LSD) test was used to detect significant differences ($P < 0.05$) among mean values of the different traits.

To account for relationships among traits, linear regressions were performed with GraphPad Prism 4.0 software (Graphpad Software, San Diego, USA). Model parameters (i.e., slope and y -intercept) were compared using the extra sum-of-squares F -test ($P < 0.05$) of the same software. Models (Eq. [2–4]) were fitted by means of TBLCURVE (Jandel-Scientific, 1991).

RESULTS

Traits under Nonheated Conditions

For the nonheated controls, plant growth rate around flowering differed ($P < 0.001$) among genotypes. The TeTr hybrid attained the highest plant growth rate (ca. 4.2 g d⁻¹), the Flint and Te hybrids attained intermediate rates (ca. 3.5 and 3.3 g d⁻¹, respectively), and the Pop hybrid attained the lowest rate (ca. 3.1 g d⁻¹; averaged across Exp and SD). Plant growth rates were higher ($P < 0.01$) (i) in Exp₁ than in Exp₂ (ca. 3.7 and 3.3 g d⁻¹, respectively; averaged across SD and H), and (ii) at early than at late sowing date (ca. 3.8 and 3.3 g d⁻¹, respectively; averaged across Exp and H; Supplemental Table 2).

At the early sowing date, hybrids set more kernels (ca. +80 kernels per plant) than at the late one, except for the Flint hybrid, which did not show changes in this trait between sowing dates (ca. 475 k; averaged across Exp and SD; $P < 0.05$ for SD $\times$ H interaction). In Exp₁, the Pop hybrid attained the highest kernel number per plant (ca. 490 k for the Pop and 400 k for the other hybrids; averaged between SD). On the other hand, in Exp₂, kernel number per plant of all hybrids tended to be greater than in Exp₁, particularly that of the Te hybrid ($P < 0.01$ for Exp $\times$ H interaction; Supplemental Table 2).

The source/sink ratio around flowering was not modified by sowing date (ca. 7 mg d⁻¹ k⁻¹; averaged across Exp, SD, and H), with the exception of that of the TeTr hybrid in Exp₁ (ca. 10.4 and 12.5 mg d⁻¹ k⁻¹ at early and late sowing date, respectively). Source/sink ratio around flowering was higher in Exp₁ (ca. 9.6 mg d⁻¹ k⁻¹) than in Exp₂ (ca. 6.3 mg d⁻¹ k⁻¹; averaged across SD and H). Only the Pop hybrid, sown early, showed no differences in this variable between experiments (ca. 6 mg d⁻¹ k⁻¹; averaged across Exp and SD). Among genotypes, the TeTr hybrid presented the highest value of this trait (ca. 8.4 mg d⁻¹ k⁻¹ for the TeTr hybrid and 6.2 mg d⁻¹ k⁻¹ for the other genotypes; averaged across Exp and SD) but this trend was only significant at the late sowing date in Exp₁ (ca. 12.6 mg d⁻¹ k⁻¹ for the TeTr hybrid and 6.2 mg d⁻¹ k⁻¹ for the other genotypes; $P < 0.05$ for Exp $\times$ SD $\times$ H interaction; Supplemental Table 2).

Differences in kernel weight across experiments, sowing dates, and hybrids were similar than those described for source-sink ratio around flowering: (i) similar kernel weights between sowing dates (ca. 225 mg k⁻¹; averaged across Exp, SD, and H), (ii) higher kernel weights in Exp₁ (ca. 226 mg k⁻¹) than in Exp₂ (ca. 180 mg k⁻¹; averaged across SD and H), and (iii) the TeTr hybrid

Table 2. Effect of heat stress (quantified as percentage variation from nonheated treatment; in %) imposed during early growth stages (GS₁) and late growth stages (GS₂) of the effective grain-filling period on oil yield, kernel oil concentration, embryo oil concentration, embryo/kernel ratio, kernel number, kernel weight, kernel growth rate, kernel growth duration and postflowering source/sink ratio (SSR_{PF}).[†]

Exp	GS	H	Oil yield	Kernel oil concentration	Embryo oil concentration	Embryo/kernel ratio	Kernel number	Kernel weight	Kernel growth rate	Kernel growth duration	SSR _{PF}
Exp ₁	GS ₁	Flint	-46.8	-9.4	-3.9	-2.3	-11.0	-33.9	-9.0	-8.6	-43.8
		Pop	-4.2	-2.3	-0.1	-3.6	0.4	-2.5	1.8	-6.2	-20.9
		Te	-41.8	-10.9	-7.3	-1.3	-2.3	-30.9	7.0	-35.8	-96.8
		TeTr	-28.2	-13.1	-1.2	-6.6	0.1	-15.5	-1.3	-10.8	-44.0
	GS ₂	Flint	-22.4	-3.7	-3.3	4.3	0.6	-19.7	-3.1	-8.8	-23.5
		Pop	-15.1	-1.8	-1.0	2.4	-3.1	-8.4	13.5	-18.0	-23.2
		Te	—	—	—	—	—	—	—	—	—
		TeTr	-18.6	-2.9	-4.2	0.4	3.8	-19.5	12.7	-24.9	-38.6
Exp ₂	GS ₁	Flint	-28.5	-4.2	-4.2	-1.4	-0.7	-24.7	-8.3	-8.7	-36.4
		Pop	-21.1	-3.9	-7.3	4.1	1.8	-19.4	-7.7	-12.0	-39.0
		Te	-58.7	-20.1	-12.8	-10.5	-0.5	-47.4	-16.3	-35.1	-74.7
		TeTr	-57.4	-21.6	-16.4	-4.4	4.1	-48.2	1.2	-45.9	-107.7
	GS ₂	Flint	-19.8	-6.4	-4.9	-1.9	6.7	-18.7	-11.0	-2.1	-33.5
		Pop	-23.5	-7.4	-1.8	-3.4	0.5	-17.9	11.3	-20.9	-49.7
		Te	-38.0	-11.1	-9.1	2.2	-1.4	-29.5	10.0	-43.3	-52.7
		TeTr	-44.2	-15.7	-9.4	3.1	6.0	-36.3	11.7	-33.8	-99.6
Exp			*	**	*	NS	NS	*	NS	*	*
GS			*	*	NS	*	NS	*	*	NS	NS
H			***	***	*	NS	NS	***	**	***	***
Exp × GS			NS	NS	NS	NS	NS	NS	NS	NS	NS
Exp × H			***	**	NS	NS	NS	***	NS	NS	***
GS × H			***	**	NS	NS	NS	*	NS	NS	NS
Exp × GS × H			NS	NS	NS	NS	NS	NS	NS	NS	NS

[†] Exp, experiment; GS, growth stage; H, hybrid; Flint, orange-flint; Pop, popcorn; Te, temperate semident; TeTr, temperate and tropical semident; NS, not significant.

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

had the highest kernel weight among genotypes (ca. 240 mg k⁻¹ for the TeTr hybrid and 190 mg k⁻¹ for the other genotypes; averaged across Exp and SD). The only exception was the lowest kernel weight of the Pop hybrid (ca. 134 mg k⁻¹; averaged across Exp and SD) with respect to the rest of genotypes ($P < 0.05$ for Exp × SD × H interaction; Supplemental Table 2). Without considering data set of the Pop hybrid, there was a significant relationship between kernel weight and source/sink ratio around flowering ($r^2 = 0.80$; $P < 0.001$; data not shown), mainly determined by differences among experiments and hybrids.

Among genotypes, the Pop hybrid had the lowest postflowering source/sink ratios (ca. 89 mg k⁻¹; averaged across Exp, SD, and H). Only the Pop and the TeTr hybrids in Exp₁ showed higher values of this trait when sown early (ca. 100 and 223 mg k⁻¹ for the Pop and TeTr hybrids, respectively) than when sown late (ca. 60 and 188 mg k⁻¹ for the Pop and TeTr hybrids, respectively). In Exp₂, the Te hybrid tended to present a higher postflowering source/sink ratio (ca. 156 mg k⁻¹) than the Flint and TeTr ones (ca. 128 mg k⁻¹; averaged between SD), whereas in Exp₁, there were no consistent differences among these

genotypes across sowing dates ($P < 0.01$ for Exp × SD × H interaction; Supplemental Table 2).

Overall, most of these results highlight the lack of sowing date effect on the determinants of neither potential (i.e., source/sink ratio around flowering) nor actual (i.e., postflowering source/sink ratio) kernel weight for the different experiment and hybrid combinations. Therefore, it will be appropriate to normalize the surveyed traits of heat-stressed crops to explore the quantitative impact of extremely high temperatures during different grain-filling stages on hybrids with contrasting kernel type.

Temperature Regimes

In general terms, whenever cloudiness was not extremely high, hourly air temperature at the apical ear level of heated treatment rose above 35°C, while that of control treatment was never above this value (i.e., HSE and CST equal to 0). Within heated shelters, air temperature rose rapidly, at a rate of 3 to 5°C per hour, from 10.00 to 13.00 h and fell at a similar rate from 17.00 to 19.00 h (air temperature remained almost stable from 14.00 to 16.00 h). Changes in air temperature within nonheated shelters were much

less pronounced. Differences in hourly air temperature between temperature regimes were on average $5.7 \pm 0.4^{\circ}\text{C}$ around midday (from 11.00 to 16.00 h) and $0.14 \pm 0.1^{\circ}\text{C}$ for the rest of the day (averaged across Exp, GS, and H). In this sense, heat treatment did not promote considerable changes in mean daily air temperature as compared with the control treatment ($24.8 \pm 0.2^{\circ}\text{C}$ for heated and $23.7 \pm 0.1^{\circ}\text{C}$ for control treatments) but increased T_{MAX} (ca. $36.6 \pm 0.6^{\circ}\text{C}$ for heated and $29.9 \pm 0.5^{\circ}\text{C}$ for control treatments; average across Exp, GS, and H).

The intensity of heat stress depended on the experiment, growth stage, and hybrid (Table 1). Differences in T_{MAX} between experiments were only detected for heated treatments imposed on the Pop, Te, and TeTr hybrids during early stages of the effective grain filling, attaining lower values in Exp₁ (ca. 34°C) than in Exp₂ (ca. 39.5°C ; averaged among H). In Exp₁, T_{MAX} of shelters heated during early grain filling was higher for the Flint and Pop hybrids (ca. 35.7°C) than for the Te and TeTr ones (ca. 33.3°C ; averaged between H). In turn, among shelters heated during late grain filling, those corresponding to the Pop hybrid had the highest T_{MAX} (ca. 39.9°C for the Pop hybrid and approximately 33.8°C for the rest of genotypes). In the same experiment, no significant differences in this descriptor were observed between timings of heating, except for the Pop hybrid that was exposed to a higher T_{MAX} during late (ca. 39.9°C) than during early (ca. 35.1°C) grain-filling stages. In Exp₂, no significant variations in T_{MAX} values among heated treatments were detected (ca. 38°C , averaged across GS and H; $P < 0.01$ for Exp $\times$ GS $\times$ H interaction).

The record of HSE of heated treatments was larger in Exp₂ than in Exp₁ (ca. 10.4 and 6.3 d, respectively; averaged across GS and H). On average, heated treatments during late grain filling of the Flint and Pop hybrids attained a higher number of HSE (ca. 7.3 d) than those of the Te and TeTr ones (ca. 4.1 d; averaged between Exp). The same trend was found for heated treatments during early grain filling only in Exp₁ (ca. 10.2 d for the Flint and Pop hybrids and approximately 7.2 d for the Te and TeTr ones), as all hybrids had a similar number of HSE in Exp₂ (ca. 12.8 d; averaged among H). The record of HSE was always larger for heated treatments applied during early than for those applied during late grain-filling stages (ca. 10.8 and 6 d, respectively; averaged across Exp and H; $P < 0.001$ for Exp $\times$ GS $\times$ H interaction; Table 1). This was related to the fact that kernels of the former treatments commonly arrested their growth after the end of the heating period (i.e., after shelters were dismantled), while kernel growth of the latter treatments ceased, on average, 5.3 d before it (see *Kernel Growth Rate and Duration*, below).

Finally, the Te, TeTr, and Pop hybrids heated particularly during early grain-filling stages in Exp₂ were exposed to more CST (ca. 317°C h ; averaged among H)

than the rest of heated treatments combinations (ca. 84°C h ; averaged across Exp, GS, and H; $P < 0.05$ for Exp $\times$ GS $\times$ H interaction; Table 1).

Oil Yield per Unit Land Area

Extremely high temperatures caused significant losses of oil yield per unit land area (Table 2). The magnitude of this effect was dependent on the experiment, growth stage, and hybrid. Reductions of oil yield per unit land area due to heat stress were less drastic in Exp₁ (ca. -25%) than in Exp₂ (ca. -41% ; averaged across GS and H), with the exception of those of the Flint hybrid for which the opposite trend was observed (ca. -35% in Exp₁ and -24% in Exp₂; $P < 0.001$ for Exp $\times$ H interaction; Table 2). For the Flint, Te, and TeTr hybrids, heat stress during early stages of the effective grain filling promoted a larger reduction of oil yield per unit land area (ca. -44% for the Flint, Te, and TeTr hybrids) than during late stages (ca. -21% for the Flint hybrid and -35% for the Te and TeTr ones; averaged between Exp). The magnitude of oil yield reductions registered for the Pop hybrid heated during early and late periods was similar (ca. -16% ; averaged across Exp and GS) and relatively lower than those of the other hybrids ($P < 0.001$ for GS $\times$ H interaction; Table 2). Hence, the earlier heat stress occurred, the more pronounced differences among hybrids in the response.

Kernel Oil Concentration

Kernel oil concentration was reduced by heat stress during the effective grain-filling period, and the magnitude of this impact differed across experiments, growth stages, and hybrids (Table 2). Except for the Flint hybrid, which showed no differences in kernel oil concentration between experiments, heat stress had a lower effect on this trait in Exp₁ (ca. -7%) than in Exp₂ (ca. -13% ; averaged across GS and H; $P < 0.01$ for Exp $\times$ H interaction; Table 2). For the Te and TeTr hybrids, heat stress during early grain-filling stages caused a greater reduction of kernel oil concentration (ca. -16.5%) than that during late ones (ca. -10% ; averaged across Exp and H). Kernel oil concentrations of the Flint and the Pop hybrids were less affected by heat stress at any stage of the grain-filling period (ca. -5% ; averaged across Exp, GS, and H; $P < 0.01$ for GS $\times$ H interaction; Table 2). Hence, genotypic variation in the sensitiveness to extremely high temperatures was more marked during early than late stages of the grain filling.

Embryo Oil Concentration and Embryo/Kernel Ratio

Both embryo oil concentration and embryo/kernel ratio were negatively affected by extremely high temperatures (Table 2). The impact of heat stress on embryo oil concentration was more severe ($P < 0.05$) in Exp₂ (ca. -8%) than in Exp₁ (ca. -3% ; averaged across GS and H; Table

2). A larger reduction ($P < 0.05$) of this trait due to heat stress was observed for the Te and TeTr hybrids (ca. -9%) than for the Flint and Pop hybrids (ca. -3.5% ; averaged across Exp, GS, and H; Table 2). The embryo/kernel ratio was slightly reduced ($P < 0.05$) only when this constraint occurred early in the grain-filling period (ca. -3.25% ; averaged across Exp and H; Table 2).

Kernel Number per Unit Land Area and Kernel Weight

Kernel number per unit land area was not affected by extremely high temperatures either imposed during early or late stages of the effective grain-filling period. By contrast, most heated treatments reduced kernel weight and differential effects were recorded across experiments, growth stages, and hybrids (Table 2). For the Pop, Te, and TeTr hybrids, the response of kernel weight to heat stress was stronger in Exp₂ than in Exp₁ (ca. -33 and -18% , respectively; averaged across GS and H), unlike the Flint hybrid, which did not exhibit changes in this response through experiments (ca. -24% ; averaged across Exp and GS). Kernel weight of the Pop hybrid was the least affected by high temperature, especially in Exp₁ (ca. -5 and -18.5% in Exp₁ and Exp₂, respectively; averaged between GS; $P < 0.001$ for Exp $\times$ H interaction; Table 2). Only for the Flint and Te hybrids, the impact of heat stress on kernel weight was higher during early (ca. -35%) than late (ca. -23% ; averaged across Exp and H) grain-filling stages ($P < 0.05$ for GS $\times$ H interaction; Table 2).

Kernel Growth Rate and Duration

Heat stress during early and late postflowering stages produced a similar shortening of the grain-filling period (ca. -21% ; averaged across Exp, GS, and H; Table 2). Kernel growth duration of the Flint and Pop hybrids was less ($P < 0.001$) affected (ca. -10.5%) than those of the Te and TeTr hybrids (ca. -33.5% ; averaged across Exp, GS, and H). For all genotypes, heat stress effect on this trait was more pronounced ($P < 0.05$) in Exp₂ (ca. -25%) than in Exp₁ (ca. -16% ; averaged across GS and H; Table 2). Heat stress also altered kernel growth rate but the effect differed between timings of heat stress occurrence ($P < 0.05$). When high temperature was applied during early stages of the grain-filling period, kernel growth rate slightly diminished (ca. -4% ; averaged across Exp and H). On the other hand, when heat stress occurred during late stages of this period, kernel growth rate increased (ca. $+6.5\%$; averaged across Exp and H; Table 2). Kernel growth rate of the Flint hybrid was the most affected ($P < 0.01$) by heat stress (ca. -8% ; averaged across Exp and GS; Table 2).

Postflowering Source/Sink Ratio

Heat stress reduced the postflowering source/sink ratio exclusively through its negative effect on the source (i.e.,

plant growth) during the grain-filling period because the number of sinks (i.e., kernel number) was not altered by this constraint. Reductions of the postflowering source/sink ratio due to heat stress were more marked in Exp₂ (ca. -62%) than in Exp₁ (ca. -42% ; averaged across GS and H). Genotypic differences in the response of this trait were found. In Exp₁, postflowering source/sink ratio of the Pop hybrid was the least susceptible to high temperature (ca. -22%), and those of the Te and TeTr hybrids were the most affected (ca. -49 and 41% , respectively). In Exp₂, the TeTr hybrid exhibited the largest postflowering source/sink ratio reduction (ca. -102%) and the Flint and Pop hybrids the lowest (ca. -35 and -44% , respectively; averaged between GS; $P < 0.001$ for Exp $\times$ H; Table 2). No differential responses of this trait were found between timings of heat stress occurrence.

Functional Relationships: Oil Yield and Numerical Components

When data sets from all treatment combinations (i.e., Exp $\times$ GS $\times$ H combinations) were pooled together, oil yield per unit land area had a greater variation due to heat stress (from -4 to -60%) than those of its numerical components: kernel number per unit land area (from $+7$ to -11% ; Fig. 1a), kernel weight (from -2 to -47% ; Fig. 1b), and kernel oil concentration (from -2 to -22% ; Fig. 1c). The slight variation in kernel number due to high temperature was not reflected on oil yield per unit land area variations (Fig. 1a). Changes in oil yield per unit land area promoted by high temperatures were closely related to variation of kernel weight ($r^2 = 0.94$; $P < 0.001$; Fig. 1b). The slope of the linear regression fitted (i.e., slope equal to 1.2) to data differed significantly ($P < 0.05$) from 1, indicating that reductions in oil yield per unit land area were also determined by changes in another numerical component. Thus, reductions of kernel oil concentration by heat stress also accounted for losses of oil yield per unit land area ($r^2 = 0.80$; $P < 0.001$; Fig. 1c).

Variations of kernel oil concentration were mainly related to changes in embryo oil concentration ($r^2 = 0.64$; $P < 0.001$; Fig. 2a). Particularly, heat stress during early stages promoted changes in the embryo/kernel ratio that partially explained those in kernel oil concentration ($r^2 = 0.46$; $P < 0.05$; Fig. 2b).

Changes of kernel weight due to early and late heating periods were not mediated by those of kernel growth rate (Fig. 3a) but were determined by shortenings of the effective grain-filling period (the slope of this relationship did not significantly differ from 1; $r^2 = 0.48$; $P < 0.01$; Fig. 3b). For this relationship, some data points above the 1:1 line (most of them corresponding to the late heating period) indicate the compensatory effect of the enhanced kernel growth rate promoted by the increased temperature (Fig. 3b).

As a result of the negative effect of heat stress during the grain-filling period of different genotypes in two

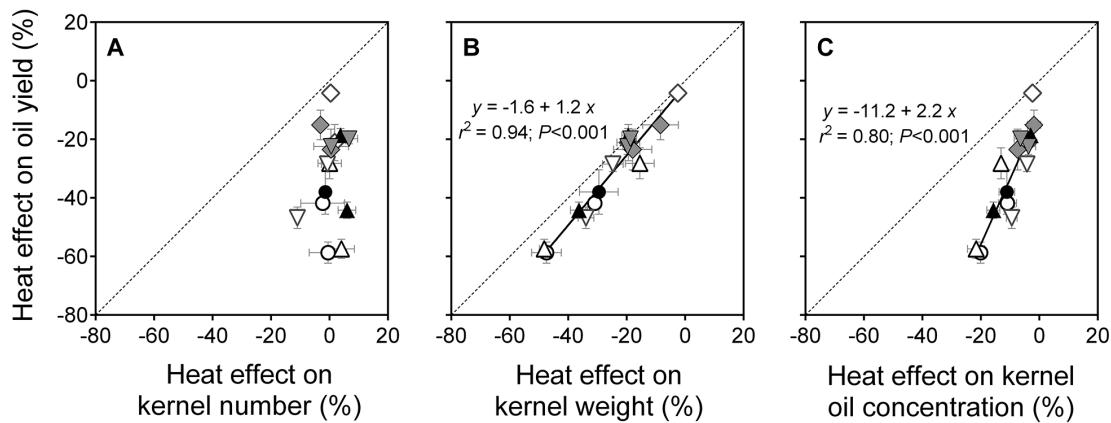


Figure 1. Relationship between the variation (expressed as percentage of control treatment) of oil yield per unit land area and kernel number per unit land area (a), kernel weight (b), and kernel oil concentration (c) of four maize hybrids (orange-flint [Flint]: grey inverted triangles; popcorn [Pop]: grey rhombus; temperate [Te]: black circles; temperate and tropical [TeTr]: black triangles) exposed to heat stress during early (empty symbols) or late (full symbols) stages of the effective grain-filling period in two experiments (2009–2010 and 2010–2011). Each point represents the mean value of three replicates $\pm$ the standard error (SE). Solid lines and inserts in (b) and (c) indicate the linear models fitted to the whole data sets. The dashed line shows the 1:1 relationship between variables.

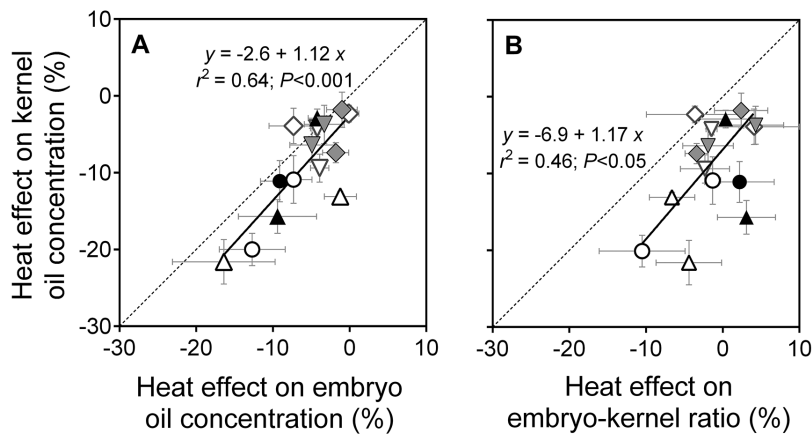


Figure 2. Relationship between the variation (expressed as percentage of control treatment) of kernel oil concentration and embryo oil concentration (a) and embryo/kernel ratio (b) of maize hybrids exposed to heat stress during the effective grain-filling period in two experiments (2009–2010 and 2010–2011). Symbols as in Fig. 1. Solid lines and inserts in (a) and (b) indicate the linear models fitted to the whole data sets. Particularly in (b), data sets corresponding to late stages of the effective grain-filling period (full symbols) were not included in the linear model. The dashed line shows the 1:1 relationship between variables.

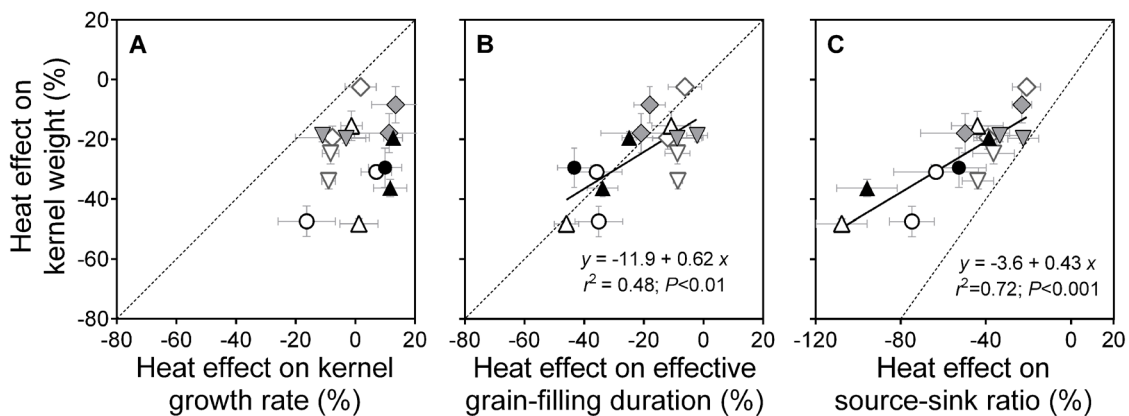


Figure 3. Relationship between the variation (expressed as percentage of control treatment) of kernel weight and kernel growth rate (a), the effective grain-filling duration (b), and the postflowering source/sink ratio (c) of maize hybrids exposed to heat stress during the effective grain-filling period in two experiments (2009–2010 and 2010–2011). Symbols as in Fig. 1. Solid lines and inserts in (a), (b), and (c) indicate the linear models fitted to the whole data sets. The dashed line shows the 1:1 relationship between variables.

experiments, kernels grew under an ample range of post-flowering source/sink ratios (between ca. -108 and -20% ; across Exp, GS, and H combinations; Fig. 3c). Reductions of this trait accounted for most of the kernel weight variation by means of a single linear model fitted to the whole data set ($r^2 = 0.72$; $P < 0.001$, Fig. 3c). As the slope of this model was lower ($P < 0.001$) than 1 (i.e., slope equal to 0.4), reductions of the source/sink ratio during the effective grain-filling period resulted in kernel weight decreases of a lower magnitude (i.e., there was a 40% change of kernel weight per 100% change of the source/sink ratio; Fig. 3c).

Sensitivity to Extremely High Temperatures

Normalized data of kernel weight and kernel oil concentration were negatively related to heat stress descriptors (data not shown). The CST was the most explanatory descriptor of the heat stress effect on the normalized variation of these traits (Fig. 4a, 4b). As data sets corresponding to the Te and TeTr hybrids had a similar ($P > 0.2$) trend, a single linear model was adjusted for both hybrids (Fig. 4a, 4b). Additionally, linear models could not be fitted to the data set from the Flint hybrid, since variations of studied traits were distributed with no clear pattern.

A large proportion of kernel weight variation ($r^2 = 0.44$ – 0.66 ; $P < 0.001$ – 0.05) was accounted for by CST during early and late stages of the effective grain-filling period (Fig. 4a). Among hybrids, the slope value of fitted models to their respective data set did not differ (ca. $-0.07\% \text{ } ^\circ\text{C}^{-1} \text{ h}^{-1}$; $P > 0.5$). However, the model of the Pop hybrid had a higher ($P < 0.001$) γ -intercept (ca. -1%), not different ($P > 0.5$) from 0%, than that of the Te and TeTr hybrids (ca. -23% ; Fig. 4a).

Likewise, linear models were fitted to normalized values of kernel oil concentration and CST during early and late heating periods (Fig. 4b). For the Te and TeTr hybrids, variation in this descriptor gave an accurate ($r^2 = 0.55$; $P < 0.001$) explanation of observed variation in kernel oil concentration. For the Pop hybrid, CST accounted for a minor proportion of kernel oil concentration variation ($r^2 = 0.27$; $P < 0.1$). The slope value did not differ ($P > 0.2$) between fitted models (ca. $-0.03\% \text{ } ^\circ\text{C}^{-1} \text{ h}^{-1}$); but the γ -intercept of the model corresponding to the Pop hybrid was significantly ($P < 0.001$) higher (ca. -0.96%), and not different from 0% ($P > 0.5$), than those of the Te and TeTr hybrids (ca. -9% ; Fig. 4b).

For the Te and TeTr hybrids, comparison among parameters of relationships established between oil yield components and CST revealed that kernel weight had a higher ($P < 0.01$) decrease rate per unit of increase in heat intensity (i.e., slope of the relationship) and a lower ($P < 0.001$) γ -intercept than kernel oil concentration (Fig. 4a, 4b). For the Pop hybrid, similar differences were observed between the slopes of such relationships ($P < 0.1$) but with no changes ($P > 0.5$) between the respective γ -intercepts (Fig. 4a, 4b).

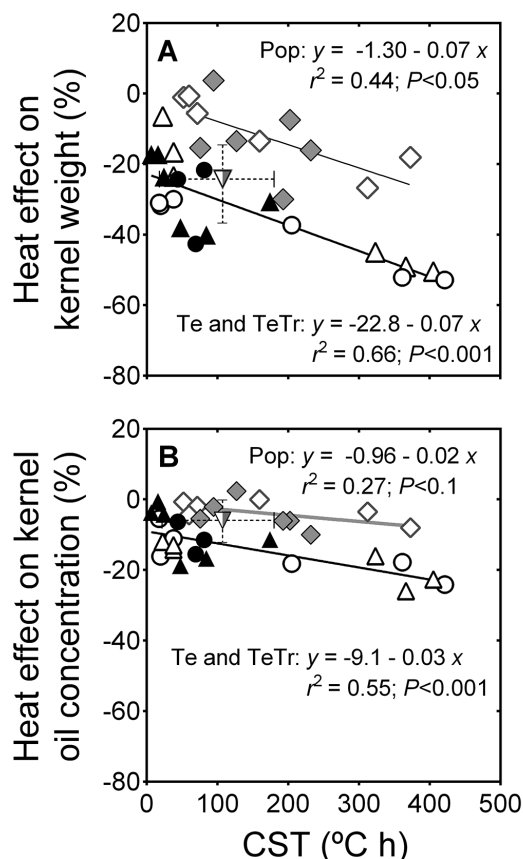


Figure 4. Variations (expressed as percentage of control treatment) of kernel weight (a) and kernel oil concentration (b) as a function of cumulative stressful temperatures (CST; in $^\circ\text{C h}$) experienced by maize hybrids during the effective grain-filling period in two experiments (2009–2010 and 2010–2011). Shape and fill pattern of symbols as in Fig. 1. For the popcorn (Pop), temperate (Te), and temperate and tropical (TeTr) hybrids, each point represents a replicate, and solid lines and inserts indicate the linear models fitted to the respective data sets. For the orange-flint (Flint) hybrid, significant models could not be adjusted to data sets, and the point with vertical and horizontal dashed bars represents the mean value across all replicates and the range of variation of each trait.

DISCUSSION

Extremely high temperatures generate an unfavorable environment for maize growth and reproductive processes that alters grain yield (Cicchino et al., 2010b; Rattalino Edreira et al., 2011a; Rattalino Edreira and Otegui, 2012; Schoper et al., 1986) and kernel composition (Monjardino et al., 2005; Wilhelm et al., 1999). The magnitude of these alterations may depend on (i) the developmental stage of the crop at the occurrence of heat stress, (ii) the intensity of heat stress, and (iii) the sensitivity of the genotype (Hall, 1992; Wahid et al., 2007). In the current research, these conditions were evidenced by the wide range of oil yields promoted by brief episodes of heat stress imposed during different stages of the effective grain-filling period on maize hybrids with distinctive kernel types (flint, popcorn, and semident).

Experimental years exposed the crops to different environmental conditions during periods of heat stress imposition due to the occurrence of the El Niño (Exp₁) and La Niña (Exp₂) phases of the El Niño Southern Oscillation (ENSO) phenomenon (Wyrtki, 1975). Consequently, the heating period was characterized by cloudy skies in Exp₁ and by sunny days in Exp₂ (mean incident photosynthetically active radiation values of 7.2 and 8.9 MJ m⁻² d⁻¹, respectively). However, mean air temperature during this period was slightly higher in Exp₁ (25.9°C) than in Exp₂ (22.4°C). As compared with Exp₁, the higher levels of solar radiation in Exp₂ enhanced the greenhouse effect within heated shelters, increasing the intensity of heat stress and therefore the impact of this constraint on most surveyed traits. Hence, environmental conditions during these experiments were representative of the climatic oscillations that commonly occur in the maize production areas of Argentina.

Episodes of heat stress during the effective grain-filling period, especially those at early stages of this period, caused losses in oil yield per unit in land area through reductions of kernel weight (Fig. 1b) and, to a lesser extent, of kernel oil concentration (Fig. 1c), without affecting kernel number (Fig. 1a). Results of this work extends previous information about heat stress effects (Cicchino et al., 2010a, 2010b; Rattalino Edreira et al., 2011a; Rattalino Edreira and Otegui, 2012) to advanced stages of kernel growth. Moreover, unlike quoted works, this research has provided information about the genotypic sensitivity of oil yield components to the intensity of heat stress occurring during the effective grain-filling period.

Postflowering heat stress reduced kernel weight through a premature cessation of kernel growth (Fig. 3b). Kernel weight reduction was of a high magnitude when the stress was imposed during early stages of this period because crops heated during late stages exhibited an enhanced kernel growth rate (Fig. 3a). Hence, heat stress applied at late stages of the grain-filling period not only arrested kernel growth but also increased the kernel growth rate. This latter effect could be linked to the low number of HSE imposed at late stages and the positive effect of mean air temperature on kernel growth (Jones et al., 1984; Singletary et al., 1994). As for many other abiotic stresses or restrictive growing conditions during the postflowering period (Andrade and Ferreiro, 1996; Echarte et al., 2006; Egharevba et al., 1976; Jones and Simmons, 1983; NeSmith and Ritchie, 1992; Melchiori and Caviglia, 2008; Quattar et al., 1987; Sala et al., 2007; Tanaka and Maddonni, 2009; Westgate, 1994), the most conclusive effect of the extremely high temperatures on kernel growth dynamic was the shortening of the effective grain filling, and this effect was related to impairments of the postflowering source/sink ratio (Fig. 3c). Data points of kernel weight reduction above the 1:1 relationship suggested the importance of assimilate reserves to sustain

kernel growth under heat stress conditions (Badu-Apraku et al., 1983; Rattalino Edreira, 2013).

There are a number of reports in the literature regarding the high homeostasis level of maize kernel oil concentration in the face of variation of cultural practices (Borrás et al., 2002; Cloninger et al., 1975; Earle, 1977; Genter et al., 1956; Jellum and Marion, 1966; Jellum et al., 1973; Singh et al., 2005; Zhang et al., 1993). For its part, treatments of extreme heat used in this research during the effective grain-filling period caused kernel oil concentration reductions of up to approximately 20%. These responses contrast with the lack of changes in this trait under moderate light reductions (Andrade and Ferreiro, 1996; Tanaka and Maddonni, 2008) and heat stress (Wilhelm et al., 1999) but agree with kernel oil concentration decreases under severe water stress (Kettlewell, 1996) and shadings (Early et al., 1967; Tanaka and Maddonni, 2009) during the same period. In the present study, reductions of kernel oil concentration were related to lower embryo oil concentrations (Fig. 2a) and additionally to lower embryo/kernel ratios only if heat stress took place at the early stages of the effective grain-filling period (Fig. 2b). The shortening of both kernel and embryo growth, together with an anticipated cessation of embryo oil deposition, would determine the reduction of both kernel oil concentration components (Tanaka and Maddonni, 2009).

The quantitative approach of changes in kernel weight and kernel oil concentration through a gradient of heat stress intensity revealed similar reduction rates of these traits among genotypes, but different γ -intercepts (Fig. 4a, 4b). Whereas a low-intensity heat stress (CST $\approx$ 50°C h) caused a severe reduction in kernel weight of the Te and TeTr hybrids, the Pop one showed slight changes in this trait. In this way, results suggest an overestimation of the threshold temperature value (i.e., T_T equal to 35°C) assumed in the computation of CST for the Te and TeTr hybrids. Consequently, differences among hybrids in the sensitivity of kernel weight and kernel oil concentration to heat stress were probably based on their distinctive temperature threshold value.

Besides, throughout the wide range of heat stress intensities, fitted models allowed to detect a greater sensitivity of kernel weight than of kernel oil concentration. In fact, when kernel oil concentration values were plotted against those of kernel weight, a single relationship emerged for most treatment combinations (Fig. 5). Moreover, data from previous works addressing the impact of shadings during the effective grain filling on maize kernel oil concentration (Tanaka and Maddonni, 2008, 2009) properly matched the trend (Fig. 5). The adjustment of a single bilinear with plateau model to describe the relationship between variations in both traits revealed that the effect of both abiotic stresses on kernel oil concentration follows a predictable pattern associated with changes in kernel size ($r^2 = 0.73$; $P < 0.001$; Fig. 5). There were

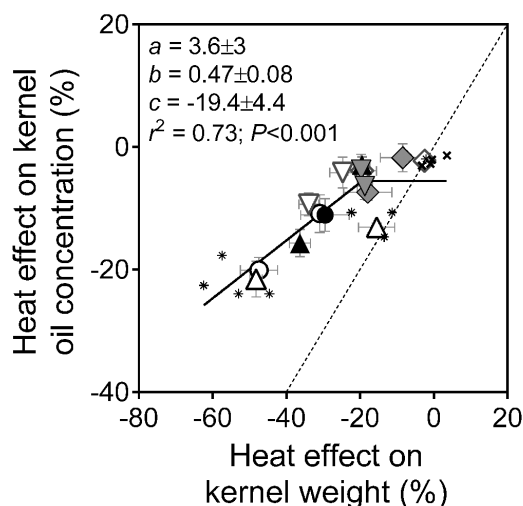


Figure 5. Variations (expressed as percentage of control treatment) of kernel oil concentration plotted against those of kernel weight for maize crops exposed to heat stress in 2009 to 2010 and 2010 to 2011 (Symbols as in Fig. 1), shadings and defoliations in 2005 to 2006 (crosses; data set adapted from Table 1 reported by Tanaka and Maddonni [2008]), and shadings in 2006 to 2007 and 2007 to 2008 (asterisks; data set adapted from Table 1 reported by Tanaka and Maddonni [2009]), during the effective grain-filling period. Solid line and insert (model parameters $\pm$ the standard error [SE]) indicate the bilinear with plateau model fitted to the whole data sets, where a is the y -intercept (in %), b is the slope for kernel weight variations below the break-point, and c is the breakpoint between lines (in %). The dashed line shows the 1:1 relationship between variables.

minimum (i.e., plateau of model equal to approximately -5.5%) decreases in kernel oil concentration when kernel weight reductions were smaller than about 20% (i.e., breakpoint of the relationship equal to -19.4%). On the other hand, for larger kernel weight reductions, as those promoted by the occurrence of abiotic stresses at early stages of kernel growth, kernel oil concentration diminutions became notorious but proportionally lower than those of the former trait (i.e., slope equal to 0.47 ; Fig. 5).

Outcomes from the present study should be validated through experimentation of a larger number of hybrids of each kernel type under similar growing conditions and then coupled with the analysis of climatic constraints for different productive areas (Maddonni, 2012) to define the most suitable strategies to mitigate the impact of heat stress on maize production or kernel quality. Thus, in temperate areas with long frost-free seasons, early sowings which maximize kernel set would expose maize crops to episodes of heat stress during the grain-filling period, affecting kernel weight and kernel composition. The knowledge of genotypic differences in the sensitivity of mentioned traits to this abiotic stress could contribute to mitigate oil yield penalties.

Further research on the threshold temperatures used for the computation of heat stress intensity for different

maize genotypes would be necessary to improve the accuracy of the quantitative approach presented in this work.

CONCLUSIONS

Oil yield of maize crops was affected by episodes of heat stress at both early and late stages of the effective grain-filling period. Quantitatively, those episodes during early grain-filling stages were more drastic than those during late stages, and the most important response to high temperature was the arrest of kernel growth by the limiting assimilates availability (i.e., a reduced postflowering source/sink ratio). Hence, crops yielded lighter kernels with lower kernel oil concentrations. Genotypic differences in the response of oil yield components to heat stress were detected. Oil yield components of the semident hybrids (Te and TeTr hybrids) were the most affected by heat stress and appeared to be sensitive to air temperatures even lower than 35°C . By contrast, the Pop hybrid exhibited the lowest sensitivity of kernel weight and kernel oil concentration to heat stress with a threshold temperature close to 35°C . These results provide information to crop modeling for the estimation of maize oil yield penalties under heat stress-prone environments.

Supplemental Information Available

Supplemental information is included with this article.

Supplemental Table 1. Parameters of the allometric models used to estimate shoot vegetative biomass (in g) at R_1-15 d, R_1 , and R_1+15 d, and ear biomass (in g) only at R_1 and R_1+15 d † in maize hybrids sown at early and late dates in two experiments.

† Shoot vegetative biomass model (biomass = $a + b$ Vol) was based on stem volume (Vol, in cm^3), and ear biomass model (biomass = c Diam) was based on maximum ear diameter (Diam, in mm). Stem volume was calculated from the cylinder formulae, using plant height from ground level to the insertion of the last leaf and stem diameter at the base of the stalk. The coefficients of determination (r^2) of fitted models are detailed.

Exp, experiment; SD, sowing date; H, hybrid; Flint, orange-flint; Pop, popcorn; Te, temperate semident; TeTr, temperate and tropical semident.

Supplemental Table 2. Plant growth rate around silking, kernel number per plant, source/sink ratio around flowering (SSR_{AF}), kernel weight, and postflowering source/sink ratio (SSR_{PF}) of nonheated crops at early and late sowing dates in two experiments.

Exp, experiment; SD, sowing date; H, hybrid; Flint, orange-flint; Pop, popcorn; Te, temperate semident; TeTr, temperate and tropical semident.

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

*** Significant at the 0.001 probability level.

Acknowledgments

The authors wish to thank J.L. Araus for his valuable suggestions and help in performing stable isotopes measurements, D.P. Rondanini for her technical assistance on lipid extraction, and L.M. Burzaco, B. Rattalino Edreira, M. Bielich, A.V. Seco, D. Sammarro, and P.H. Aguirre for their help with field work. This research was financed by the National Council for Research of Argentina (CONICET) and the Regional Fund for Agricultural Technology (FONTAGRO, project 8031). L.I. Mayer and J.I. Rattalino Edreira had a graduate's scholarship from CONICET. G.A. Maddonni is a member of CONICET.

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