

Maize Physiological Responses to Heat Stress and Hormonal Plant Growth Regulators Related to Ethylene Metabolism

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ABSTRACT

Hormonal plant growth regulators (HPGRs) have been evaluated in field grown maize (*Zea mays* L.), but never as a tool for prevention or mitigation of heat stress. We analyzed grain yield determination of maize crops exposed to contrasting temperature regimes (nonheated control plots [T_C]; heated plots [T_H]) and the application of HPGRs associated with ethylene metabolism (ethephon [ETH]; MCP [1-MCP]). Heating extended over daytime hours between V_{11} and tasseling (VT), and products were sprayed immediately before (V_{11}) and/or during (V_{16}) heating. Plants treated with ETH always had reduced height (10–21%) and leaf area (3–10%), but these trends usually had no effect on light interception during treatment period. Biomass production was markedly affected by heating, but a significant interaction effect ($P < 0.01$) indicated that HPGRs caused (i) no effect among T_H plots, and (ii) a decrease (13–19% for ETH and 3.8–9.4% for MCP) among T_C plots. The interaction effect computed for grain yield highlighted that ETH had mild negative effects ($\leq 18\%$) among T_C plots and large positive effects among T_H plots (up to 73%), whereas MCP had no effect among the former and mild positive (V_{16}) or negative (V_{11}) effects among the latter. Variations in grain yield were due to variations in kernel numbers ($r^2 \geq 0.92$), which were explained by ear growth rate around flowering ($r^2 \geq 0.97$). Timely application of HPGRs was critical for improving biomass allocation to the ear (ETH) and having adequate blockage of ethylene receptors (MCP).

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Abbreviations: ANOVA, analysis of variance; ASI, anthesis-silking interval; CGR, crop growth rate during treatment period; EGR_n , ear growth rate during period n; ETH, ethephon; $ETH_{V_{11}}$, ethephon applied at V_{11} ; $ETH_{V_{16}}$, ethephon applied at V_{16} ; Exp.n, Experiment n; fIPAR, fraction of incident photosynthetically active radiation intercepted by the crop; IPAR, amount of incident photosynthetically active radiation intercepted by the crop; HPGRs, hormonal plant growth regulators; KNP, kernel number per plant; KW, individual kernel weight; LAI, maximum leaf area index; MCP, 1-MCP; $MCP_{V_{11}}$, 1-MCP applied at V_{11} ; $MCP_{V_{16}}$, 1-MCP applied at V_{16} ; PAR, photosynthetically active radiation; PGR_n , plant growth rate during period n; PGY, plant grain yield; RUE_n , radiation use efficiency during period n; T_C , nonheated control plots; T_H , heated plots; TT_S , cumulative stressful temperatures; VT, tasseling.

GRAIN YIELD OF CROPS depends on the particular combination of environmental conditions, management practices, and breeding efforts, and is maximized (i.e., potential grain yield) by cropping modern cultivars in the absence of abiotic and biotic stresses (Tollenaar and Lee, 2002; Fischer and Edmeades, 2010). Potential grain yield, however, is seldom reached at the farm level,

Published in Crop Sci. 53:2135–2146 (2013).

doi: 10.2135/cropsci2013.03.0136

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even under irrigated and highly fertilized conditions (Cassman et al., 2003). Reasons for this gap vary, from simple management decisions (e.g., optimum sowing date for a particular season) to reduced resource input rates as compared to experimental stations (e.g., fertilizer and irrigation amounts) due to optimization criteria based on cost/benefit analysis. In the field, therefore, crops are always exposed to some degree of stress (Passioura, 1996). Additionally, reports from international agencies oriented to the analysis of global climate change (IPCC, 2007) forecast an increase in the frequency of high evaporative demand conditions and above optimum temperatures. These scenarios may represent episodes of abiotic stress even for irrigated crops (Loomis and Amthor, 1999; Maddonni, 2012).

Plant responses and adaptation to the environment are mediated, among other things, by hormones (Davies, 2004). One of them is ethylene (C_2H_4). It plays an important role in many different processes, from seed germination and determination of sex in floret primordia to fruit ripening and leaf senescence (Khan, 2006). It is produced by almost all plant organs, but meristematic and nodal regions are usually the most active. Ethylene production by plant tissues can be influenced by exogenous applications of HPGRs related to its metabolism, which may promote (autocatalysis) or inhibit (autoinhibition) its synthesis. Among the former there are products like ethephon (2-chloroethyl phosphonic acid), 1-MCP (1-Methylcyclopropene) and AVG ([S]-trans-2-amino-4-(2-aminoethoxy)-3-butenoic acid hydrochloride) belong to the second group. Mentioned products have been broadly used in pre- and postharvest of vegetable, fruit, and ornamental crops. Their use among extensive crops has been limited almost exclusively to the application of ethephon for preventing lodging in wheat (Wiersma et al., 1986; Nafziger et al., 1986; Knapp and Harms, 1988) and maize (Langan and Oplinger, 1987; Norberg et al., 1988, 1989; Konsler and Grabau, 1989), one of the most consistent limitations to potential grain yield in these species. Research on this topic was developed chiefly during the 1980s and focused predominantly on the response of grain yield to the growth stage when application took place and the rate of product used. Most experiments were based on usual agronomic practices at the time of evaluation and never included a thorough analysis of the variation experienced by all ecophysiological determinants of grain yield (i.e., resource capture, biomass production, and biomass partitioning).

A new era of research on the use of HPGRs related to ethylene metabolism in grain crops started in the 21st century (Rajala and Peltonen-Sainio, 2001; Tripathi et al., 2003, 2004). It has been driven by evidence of the negative effects on crop productivity of ethylene produced under water stress conditions (Morgan et al., 1990; Narayana et al., 1991; Beltrano et al., 1997, 1999; Balota et al., 2004), but particularly by the staggering finding of ethylene male induced sterility in the confined environment of the Mir

space station (Levinskikh et al., 2000). Interest, therefore, shifted from autocatalysis to autoinhibition. Available information on this topic is scarce yet, and results suggest that blocking ethylene receptors may mitigate the negative responses associated to above-optimum temperatures (e.g., enhanced kernel abortion and reduced kernel weight) in genotypes more susceptible to this abiotic stress (Hays et al., 2007). All these studies were performed with wheat, and most analyses corresponded to the individual plant level in research developed in controlled conditions (i.e., greenhouses or chambers). No information is available for maize, a species highly susceptible to heat stress (Herrero and Johnson, 1980; Commuri and Jones, 2001; Cicchino et al., 2010b; Rattalino Edreira and Otegui, 2012, 2013).

In this paper we analyzed the physiological responses of maize crops grown in the field under contrasting temperature regimes (nonheated and heated during daytime hours of the late-vegetative period) to the application of two HPGRs associated with ethylene metabolism (ethephon and 1-MCP).

MATERIALS AND METHODS

Crop Husbandry and Experimental Design

Experiments were developed during two growing seasons (Exp.1 in 2006–2007 and Exp.2 in 2007–2008) in the station of the National Institute of Agricultural Technology (INTA) located at Pergamino (33° 56' S, 60° 34' W; 66 m altitude). The soil is a silty clay loam type (Typic argiudoll, USDA soil survey system), with values of 7.1 for pH (water), 23 g kg⁻¹ for organic matter, and 35 mg kg⁻¹ for mineral P. A temperate hybrid (single-cross AX 842 MG CL, semident, 119 RM, total leaf number approximately 20) was sown late in both experiments (December 12) to avoid the concurrence of contrasting temperature regimes with the summer period of highest irradiance and temperature, which usually takes place during January (Otegui et al., 1996). Experiments were hand-planted at a rate of three seeds per hill, and thinned to a final stand density of 9 plants m⁻² at the three-ligulated leaf stage (V₃; Ritchie and Hanway, 1982). The experimental site was fertilized with 200 kg N ha⁻¹ applied as urea at V₆, and water availability of the uppermost 1 m of soil was kept always near field capacity by means of sprinkler irrigation. Weeds were controlled with 4 L ha⁻¹ atrazine (0.5 a.i.) at sowing, and by hand weeding after the crop was established. Pests and diseases were adequately controlled. The experimental area was always surrounded by two rows of the same hybrid sown 1 wk later for ensuring fresh pollen availability for late-silking plants.

Treatments included the factorial combination of (i) two temperature regimes (T_H and T_C) between V₁₁ and VT of T_C plots, i.e., for approximately 17 d, and (ii) four (Exp.1) or five (Exp.2) applications of HPGRs, described in Table 1. The experimental design was a split plot, with HPGRs in the main plots and temperature regimes in the subplots. There were always three replicates. Main plots had 8 rows of 10 m length and 0.7 m between rows. HPGRs were sprayed in the central six rows of each main plot by means of a controlled pressure

Table 1. Description of experiments and treatments.

Experiment	Temperature regime	Hormonal plant growth regulator
Exp.1: 2006–2007	- T _C : nonheated control - T _H : heated between V ₁₁ and tasseling of T _C plots	- UTC: untreated control - ETH _{V11} : ethephon applied 24 h before the start of heat stress at V ₁₁ - MCP _{V16} : 1-MCP applied during stress (ca. V ₁₆ of T _C plots) - ETH _{V11} +MCP _{V16}
Exp.2: 2007–2008	- T _C - T _H	- UTC - ETH _{V11} - MCP _{V11} : 1-MCP applied 24 h before the start of heat stress at V ₁₁ - ETH _{V16} : ethephon applied during stress (ca. V ₁₆ of T _C plots) - MCP _{V16}

device (Burns, 2008). Applications were performed late in the afternoon (ca. 1800 h) of nonwindy days, with the spraying bar held at less than 10 cm above the top of the canopy. Rates used were 250 g a.i. ha⁻¹ for ethephon (Ethrel 480 SL) and 25 g a.i. ha⁻¹ for 1-MCP (SmartFresh). Applications at V₁₁ (i.e., before stress) were aimed to prevent the negative effects of heating by (i) blocking ethylene receptors (MCP_{V11}), or (ii) reducing plant size (ETH_{V11}). Applications at V₁₆ (i.e., during stress) were aimed to (i) mitigate the effects of heating by blocking ethylene receptors (MCP_{V16}), or (ii) enhance negative heat effects by increasing ethylene production (ETH_{V16}). Applications at V₁₆ took place approximately 1 wk before VT of T_C plots.

Differential temperature regimes were obtained by means of shelters placed along two of the four central rows of each plot for avoiding any possible border effect of HPGRs treatments (i.e., at least two border rows between main plots). Shelters are described in detail in Cicchino et al. (2010a). Briefly, each shelter covered a 1.43 m width per 1.5 m (Exp.1) or 2.86 m length (Exp.2) area, and were all 2.3 m height. They were made of transparent polyethylene film (100- μ m thickness) fixed to a rigid wood structure (sides and roof) anchored firmly to the ground. In those of T_H plots, the film extended down to the soil surface, except for a 10 cm opening at the bottom of one side along a row for granting adequate gas exchange. Shelters of T_C plots were aimed to avoid any confounded effect of the polyethylene film on the amount and quality of light perceived by plants. These shelters had three sides open up to 1.4 m above the soil surface, and the south side completely open for granting no artificial temperature rise (no direct sunlight reached the south side because of northern sun inclination). To avoid water accumulation, the roof of all shelters had a slight slope and was pierced sparsely (4–5 places, 5–8 mm each). Roof piercing also contributed to adequate gas exchange. Heating of T_H plots depended exclusively on temperature rise caused by the greenhouse effect promoted by closed shelters, which did not take place in those of T_C plots (Cicchino et al., 2010a). On clear days, temperature at ear level in T_H plots increased gradually up to values between 35 and 48°C for a few hours around noon (Cicchino et al., 2010a). When crops in T_C plots reached V₁₆, shelters of T_H plots were removed for application of HPGRs and were reinstalled immediately after it. Shelter removal at this stage was not necessary for T_C plots because the open side allowed for correct spraying of plants. All shelters were removed at tasseling (VT) of T_C plots.

Measurements and Statistical Analysis

Daily mean air temperature and incident solar radiation were registered in a CR10X weather station (Campbell Scientific Inc.,

Logan, Utah) located at 300 m of the experimental site. Solar radiation data were converted to photosynthetically active radiation (PAR) by means of a 0.45 conversion factor (Monteith, 1965). Daily PAR values were affected by 0.9 for including the 10% reduction effect of the polyethylene film. Temperature at ear height was monitored hourly in each shelter by means of sensors (TC1047, Microchips Technologies, Chandler, Arizona) connected to data-loggers (Temp-Logger, Cavadevices, Argentina). Sensors were placed in the middle of a white, double-walled plastic cylinder with open bases, which was held in the center of the plot near ear height (i.e., started at the 11th leaf position and was raised periodically up to the 14th–15th leaf position to match ear height). Cumulative stressful temperatures (TT_S, in °C h above an optimum temperature of 33.9°C; Cicchino et al., 2010a, 2010b) were computed for each plot between V₁₁ and VT.

Nine plants were tagged in each shelter at V₁₁. The dates of anthesis (i.e., at least one extruded anther visible) and silking (i.e., at least one extruded silk visible) were recorded on all tagged plants, for estimating the proportion that reached the stage (Uribebarrea et al., 2002). The anthesis–silking interval (ASI; Bolaños and Edmeades, 1993) was computed as the difference in days between median silking and anthesis dates. Median dates corresponded to the date when 50% of the plants reached each stage.

The effect of each treatment combination on canopy size and functionality was evaluated at silking by means of four traits: plant height to the uppermost collar (in cm), maximum leaf area index (LAI; in m² of leaves per m² of soil), leaf conductance, and chlorophyll status. LAI was obtained at anthesis from the product between mean plant leaf area of individual plants and stand density. Plant leaf area of each tagged plant was estimated as the sum of all green leaves, and leaf area of individual leaves was obtained as [leaf length \times maximum leaf width \times 0.75] (Montgomery, 1911). Leaf conductance was measured on green tissue of the ear-leaf blade of three tagged plants of each sub-plot, by means of (i) a LiCor 6400 (LiCor, Lincoln, Nebraska) in Exp.1, and (ii) a Decagon SC-1 (Decagon Devices, Pullman, Washington) in Exp.2. For the former, irradiance was set at 1800 μ mol cm⁻² s⁻¹ and CO₂ at 400 ppm. For the latter, measurements took place around noon of a clear day. Chlorophyll status was estimated indirectly by SPAD (Konica-Minolta, Japan) and obtained as the average of one measurement performed on green tissue near the middle of the ear-leaf blade of each tagged plant.

Plant biomass of each tagged plant was estimated for different stages around the critical period for kernel number determination (Andrade et al., 1999; Otegui and Bonhomme, 1998). These stages were: (i) immediately before the start of heating (V₁₁), (ii) immediately after the end of heating (VT of T_C plots), and (iii) 15

d after silking (R_2) of T_C plots. Estimations were based on morphometric models well described elsewhere (Vega et al., 2000; Borrás and Otegui, 2001; Maddonni and Otegui, 2004; Echarte and Tollenaar, 2006; D'Andrea et al., 2008), including studies on heat stress (Cicchino et al., 2010b; Rattalino Edreira and Otegui, 2013). Briefly, these models were based on relationships established between (i) whole plant biomass (excluding the ear) and stem volume (linear relationship), and (ii) ear biomass and maximum ear diameter (exponential relationship). Stem volume was estimated as the cylinder volume, obtained from plant height to the uppermost visible collar and stem diameter at ground level (average of maximum and minimum diameters). Models were fitted to data obtained from an independent set of plants, harvested at each growth stage of interest from heated and nonheated conditions (i.e., independent models for T_C and T_H plots). Fits were always highly significant ($r^2 > 0.8$; $P \leq 0.01$). Biomass of tagged plants was estimated from established relationships and morphometric measurements performed in situ on these plants at the same growth stages (i.e., nondestructive approach). These data were averaged for obtaining crop biomass (in g m^{-2}) as the product between plant biomass and stand density, and crop growth rate (CGR, in $\text{g m}^{-2} \text{d}^{-1}$) was computed for the treatment period (V_{11} –VT). Plant and ear growth rates (PGR and EGR, respectively, in $\text{g pl}^{-1} \text{d}^{-1}$) were computed for the period between V_{11} and R_2 , representative of the whole critical period for kernel set (PGR_{CP} and EGR_{CP}).

The fraction of incident PAR intercepted by the canopy (fIPAR) was estimated for each treatment combination at mentioned stages (V_{11} , VT, and R_2 of T_C plots), following the approach described elsewhere (Gallo and Daughtry, 1986; Maddonni and Otegui, 1996). Briefly, two measurements were performed in each subplot by means of a 1 m line quantum sensor (Cavabar, Cavadevices, Argentina). The sensor was placed diagonally across the interrow space and immediately below the green bottommost leaves of the plants. The average of these measurements (PAR_{in}) was related to those obtained at the top of the canopy (PAR_{out}) for computing fIPAR as $[1 - (\text{PAR}_{\text{in}}/\text{PAR}_{\text{out}})]$. Daily fIPAR for the period between V_{11} and R_2 was obtained from linear interpolation, and applied to daily values of PAR for computing cumulative intercepted PAR (IPAR) for each period of interest (IPAR_{PRE}: for V_{11} to VT; IPAR_{POST}: for VT to R_2 ; IPAR_{CP}: for the whole critical period, between V_{11} and R_2). Radiation use efficiency (RUE, in g MJ^{-1}) was estimated as (i) the quotient between crop biomass and IPAR for the period between V_{11} and VT (RUE_{PRE}), and (ii) the linear relationship between crop biomass and IPAR for the whole critical period (RUE_{CP}).

All tagged plants were harvested at physiological maturity. Total plant biomass (in g pl^{-1}), plant grain yield (PGY, in g pl^{-1}), and grain yield components (kernel number per plant and individual kernel weight) were obtained for each plant at this stage. We computed (i) harvest index (HI) as the quotient between PGY and total plant biomass, and (ii) prolificacy as the number of grained ears per plant. Kernel number per plant (KNP) was obtained by manual counting of all harvestable grains produced by each plant, and individual kernel weight (KW, in mg) estimated from the quotient between PGY and KNP. For each treatment combination, mean values of total plant biomass, PGY and KNP were multiplied by stand density for computing final crop biomass production (in g m^{-2}), crop grain yield (in g m^{-2}), and kernel number per m^2 .

The effect of treatments and their interactions was evaluated by analysis of variance (ANOVA, Infostat, 2008). The model for the split plot design is described in Eq. [1], with fix effects for both treatments (HPGRs in the main plot and temperature regimes in the subplot)

$$y_{ijk} = \mu + \tau_i + \beta_j + (\tau\beta)_{ij} + \gamma_k + (\tau\gamma)_{ik} + (\beta\gamma)_{jk} + (\tau\beta\gamma)_{ijk} + \varepsilon_{ijk} \quad [1]$$

where each measured trait (y_{ijk}) can be described as the overall mean (μ) plus a block effect (τ_i), a main plot effect (β_j), a subplot effect (γ_k), interactions among them and an error term (ε_{ijk}). Significant differences between means were established by means of Duncan's multiple range test ($P < 0.05$). Regression analysis was applied to the relationship between variables.

RESULTS

Overall environmental conditions have been addressed in previous work (Cicchino et al., 2010a, 2010b). Briefly, mean air temperature and incident PAR during crop growth (mid-December to mid-April) were higher for Exp.2 (21.2°C and 9.45 $\text{MJ m}^{-2} \text{d}^{-1}$, respectively) than for Exp.1 (20.35°C and 8.44 $\text{MJ m}^{-2} \text{d}^{-1}$, respectively). This trend was also verified for air temperature during treatment period (24.2°C for Exp.2 and 22.9°C for Exp.1), but not for incident PAR during this phase (9.5 $\text{MJ m}^{-2} \text{d}^{-1}$ for Exp.2 and 10.2 $\text{MJ m}^{-2} \text{d}^{-1}$ for Exp.1).

For heated plots (T_H), the number of days with maximum temperature (Tmax) above 33.9°C (Cicchino et al., 2010a) at ear level was larger during treatment period of Exp.2 (16 d) than of Exp.1 (10 d). Mean air temperatures at ear level during this period were (i) 25.8°C for T_H and 23.1°C for T_C in Exp.1, and (ii) 26.6°C for T_H and 24.0°C for T_C in Exp.2. Cumulative stressful temperatures (hours with Tmax > 33.9°C, in °C h) during treatment period differed between plots and experiments (Cicchino et al., 2010b). This index was larger during Exp.2 (823°C h for T_H and 251°C h for T_C) than during Exp.1 (295°C h for T_H and 15°C h for T_C). Application of different HPGRs had no effect on described temperature trends.

Crop Development

Heat stress always caused a significant ($P < 0.01$) delay in flowering events (Table 2). This trend averaged (i) 3.75 d in Exp.1 and 1.96 d in Exp.2 for anthesis date, and (ii) 4.48 d in Exp.1 and 4.2 d in Exp.2 for silking date. There was, however, a significant ($P < 0.05$) interaction effect (HPGRs × TR) on silking date of Exp.2. In this experiment, silk exposure was earlier among (i) nonheated plants treated with 1-MCP at V_{11} , and (ii) heated plants treated with ethephon before the start of heat stress (ETH_{V11}). Treatments had no effect on the proportion of plants that reached anthesis, and application of HPGRs had no effect on the proportion of nonheated plants that reached silking. But an interaction effect was detected for the proportion of plants that reached silking in Exp.2 (Table 2), which indicated that (i) application of ethephon always allowed all plants to

Table 2. Response of phenological events (days to anthesis and silking), the anthesis-silking interval (ASI) and the proportion of plants that reached anthesis and silking to the application of HPGRs and contrasting temperature regimes (TR). Results of analysis of variance (ANOVA, *F* test) are indicated for each source of variation.

Experiment	Temperature regime	HPGRs [†]	Days from sowing to			Proportion of plants that reached		
			Anthesis	Silking	ASI	Anthesis	Silking	
Exp.1	Nonheated	UTC [‡]	61.7	63.0	1.33	1.00	1.00	
		ETH _{V11}	63.0	62.7	-0.33	1.00	1.00	
		MCP _{V16}	63.0	63.7	0.67	1.00	1.00	
		ETH _{V11} + MCP _{V16}	64.3	64.3	0.00	1.00	1.00	
	Heated	UTC	67.3	69.3	2.00	0.83	0.97	
		ETH _{V11}	67.3	67.0	-0.33	0.97	1.00	
		MCP _{V16}	66.7	68.3	1.67	1.00	1.00	
		ETH _{V11} + MCP _{V16}	65.7	67.0	1.33	1.00	1.00	
Source of variation		HPGRs	NS	NS	*	NS	NS	
		TR	**	**	*	NS	NS	
		HPGRs×TR	NS	NS	NS	NS	NS	
Exp.2	Nonheated	UTC	57.0	57.3 cd [§]	0.33	1.00	1.00 a	
		ETH _{V11}	57.0	57.3 cd	0.33	1.00	1.00 a	
		MCP _{V11}	55.3	55.7 d	0.33	1.00	1.00 a	
		ETH _{V16}	56.0	58.0 cd	2.00	1.00	1.00 a	
		MCP _{V16}	57.0	57.7 cd	0.67	1.00	1.00 a	
		UTC	58.0	62.0 ab	3.33	0.97	0.90 ab	
	Heated	ETH _{V11}	59.0	59.7 bc	0.67	1.00	1.00 a	
		MCP _{V11}	58.0	61.7 ab	3.67	1.00	0.80 b	
		ETH _{V16}	57.7	62.3 a	3.67	1.00	0.97 ab	
		MCP _{V16}	58.7	61.3 ab	2.67	1.00	0.90 ab	
			HPGRs	NS	NS	**	NS	NS
			TR	**	**	**	NS	**
	HPGRs×TR	NS	*	NS	NS	*		

*Significant at the 0.05 probability level.

**Significant at the 0.01 probability level. NS: not significant ($P > 0.05$).

[†]Description in Table 1.

[‡]Untreated control.

[§]Data followed by the same letter within a column, experiment and factor do not differ at $P = 0.05$ (Duncan's multiple range test).

reach silking (i.e., no difference with respect to nonheated plots), and (ii) application of 1-MCP before the start of heat stress (MCP_{V11}) caused an increase in the proportion of heated plants that did not reach silking (20%) as compared to nonsprayed control plants (10%).

Treatments had a clear effect on the ASI (Table 2). Heat stress caused a marked increase of this interval, which was larger during Exp.2 (2.07 d; $P < 0.01$) than during Exp.1 (0.76 d; $P < 0.05$). Among HPGRs, ethephon application at V₁₁ was always accompanied by a reduction of the ASI (Table 2).

Plant Size and Light Interception

No difference was detected among plots in plant height and light interception efficiency (fIPAR) immediately before the start of treatment application at V₁₁ (Table 3). From this stage onward, above-optimum temperatures had a negative effect on stem elongation and leaf expansion, evident as a significant ($P \leq 0.02$) reduction in plant height and leaf area (Table 3). However, the decrease in maximum values of these traits due to temperature stress was (i) never larger than 21%,

and (ii) larger for plant height (10–21%) than for leaf area (3–10%). Application of ethephon always caused a reduction in maximum plant height (Table 3), which tended to be larger for the early (V₁₁) than for the late (V₁₆) treatment. Application of 1-MCP had no effect on plant size.

The reduction in leaf area caused by treatments had no significant effect on light interception efficiency at VT (Table 3), except for a moderate decrease (9.5%; $P < 0.05$) in this trait observed among plots treated with ethephon at V₁₁ during Exp.2. This response translated into a slight reduction (4.8%; $P < 0.05$) in the amount of cumulative IPAR between V₁₁ and VT (IPAR_{PRE}; Table 3).

Biomass Production and Radiation Use Efficiency

Heat stress caused a marked reduction in crop growth rate during the treatment period (Table 4), which averaged 72.7% across levels of HPGRs and experiments. SPAD and leaf conductance measurements performed at the end of the heating period followed the same trend. Above-optimum

Table 3. Response of maize plant height, maximum leaf area index (LAI), fraction of light interception (fIPAR), and cumulative photosynthetically active radiation intercepted by the crop during treatment period (IPAR_{PRE}) to the application of HPGRs and contrasting temperature regimes (TR). Results of analysis of variance (ANOVA, *F* test) are indicated for each source of variation.

Experiment	Factor	HPGRs [†]	Plant height			LAI	fIPAR		IPAR _{PRE}
			V ₁₁	VT [‡]	Maximum		V11	VT	
			cm						MJ m ⁻²
Exp.1	HPGRs	UTC [§]	37.7	147	207	5.37 a [¶]	0.58	0.92	96
		ETH _{V11}	34.9	117	181	4.73 b	0.51	0.92	94
		MCP _{V16}	36.0	141	206	5.31 a	0.51	0.91	93
		ETH _{V11} + MCP _{V16}	32.8	114	184	4.74 b	0.46	0.89	90
	TR	Non heated	36.7	137 a	204 a	5.31 a	0.53	0.92	95
		Heated	34.0	122 b	185 b	4.77 b	0.50	0.91	92
Source of variation	HPGRs		NS	NS	NS	*	NS	NS	NS
	TR		NS	*	**	**	NS	NS	NS
	HPGRs×TR		NS	NS	NS	NS	NS	NS	NS
Exp.2	HPGRs	UTC	47.1	121 a	188 a	5.91	0.71	0.91 ab	149 ab
		ETH _{V11}	44.5	94 b	154 b	5.53	0.71	0.86 b	144 b
		MCP _{V11}	53.7	133 a	199 a	6.06	0.71	0.95 a	152 a
		ETH _{V16}	50.3	127 a	157 b	6.00	0.71	0.95 a	152 a
		MCP _{V16}	46.5	119 a	186 a	5.90	0.71	0.91 ab	149 ab
		Non heated	48.4	124 a	197 a	5.98 a	0.71	0.92	150
	TR	Heated	48.5	114 b	156 b	5.78 b	0.71	0.91	149
		Source of variation		NS	**	**	NS	NA [#]	*
TR		NS	**	**	**	NA	NS	NS	
HPGRs×TR		NS	NS	NS	NS	NA	NS	NS	

*Significant at the 0.05 probability level.

**Significant at the 0.01 probability level. NS: not significant (*P* > 0.05).

[†]Description in Table 1.

[‡]Vt, Tasseling.

[§]UTC, untreated control.

[¶]Data followed by the same letter within a column, experiment and factor do not differ at *P* = 0.05 (Duncan's multiple range test).

[#]NA: not available because a general measurement was performed before the start of heat stress with no distinction among plots.

temperatures produced a 35.6% decline in SPAD and a 42.5% decline in leaf conductance (*P* < 0.01; data not shown). These responses were accompanied by a negative effect on shoot biomass production of heated plots (Table 4), which was more pronounced at VT (average of -52.4%) than at physiological maturity (average of -28.9%).

A significant (*P* < 0.01) interaction effect was computed for biomass production and some related traits (crop and plant growth rates) during Exp.2 but not during Exp.1 (Table 4). In Exp.2, the application of HPGRs had no effect on these traits among heated plots, but all ethephon treatments (ETH_{V11} and ETH_{V16}) and the late application of 1-MCP (MCP_{V16}) caused a decrease among the nonheated ones (Table 4). The magnitude of this negative effect was larger for ethephon treated plots (16 to 17% for biomass at VT, 15.5 to 17% for biomass at R₂, and 13 to 19% for biomass at physiological maturity) than for those treated with 1-MCP at V₁₆ (3.8% for biomass at VT, 4% for biomass at R₂, and 9.4% for biomass at physiological maturity). A different trend was detected by the interaction effect computed for ear growth rate during Exp.2 (Table 4), which captured the mentioned negative effect of ETH_{V16} among nonheated plots but (i) no negative effect of ETH_{V11} and MCP_{V16} in

this thermal condition, and (ii) positive effects of ETH_{V11} among heated plots and of MCP_{V11} among nonheated plots. No interaction effect was detected for SPAD and leaf conductance measurements (data not shown).

Reduced effects of above-optimum temperature on leaf area and light interception were accompanied by a large decline in biomass production. This was evidence of negative effects of stress on radiation use efficiency (RUE), which dropped 68% between nonheated (RUE_{CP} = 4.08 g MJ⁻¹; *P* < 0.001) and heated plots (RUE_{CP} = 1.3 g MJ⁻¹; *P* < 0.001) for the critical period around silking (i.e., between V₁₁ and R₂). Cumulative stressful temperatures (TT_S) during the heating period (i.e., between V₁₁ and VT) explained variations in radiation use efficiency calculated for the same stage (RUE_{PRE} = 7.56 - 0.89 ln TT_S; *r*² = 0.66, *P* < 0.001). Experiments and HPGRs had no effect on these trends.

Grain Yield Determination

Maximum grain yields were always obtained in nonheated plots (Table 5). Above-optimum temperatures caused a drastic decrease in grain yield (-29% in Exp.1 and -54% in Exp.2). This response was due almost exclusively to reduced kernel numbers (-30% in Exp.1 and -50% in Exp.2; Fig. 1a), because

Table 4. Response of maize crop biomass at different growth stages, crop growth rate during heating period (CGR), and plant (PGR_{CP}) and ear (EGR_{CP}) growth rates during the critical period to the application of HPGRs and contrasting temperature regimes (TR). Results of analysis of variance (ANOVA, *F* test) are indicated for each source of variation.

Experiment	Temperature regime	HPGRs [†]	Crop biomass				CGR	PGR _{CP}	EGR _{CP}
			V ₁₁	VT [‡]	R ₂	Maturity			
			g m ⁻²				g m ⁻² d ⁻¹	g plant ⁻¹ d ⁻¹	
Exp.1	Nonheated	UTC	309	978	1563	2063	37.1	3.98	0.86
		ETH _{V11}	294	784	1444	1891	27.2	3.65	0.92
		MCP _{V16}	301	885	1441	1827	32.5	3.62	0.72
		ETH _{V11} + MCP _{V16}	273	719	1341	1709	24.9	3.39	0.74
	Heated	UTC	295	463	623	1247	9.4	1.04	0.27
		ETH _{V11}	279	423	651	1343	8.0	1.18	0.44
		MCP _{V16}	216	440	575	1474	9.9	1.14	0.34
		ETH _{V11} + MCP _{V16}	282	427	692	1428	8.1	1.30	0.50
Source of variation	HPGRs	NS	NS	NS	NS	NS	NS	NS	
	TR	NS	**	**	**	**	**	**	
	HPGRs×TR	NS	NS	NS	NS	NS	NS	NS	
Exp.2	Nonheated	UTC	375	1224 ab [§]	1488 ab	2347 a	40.5 a	3.83 ab	0.98 ab
		ETH _{V11}	356	1031 bc	1257 b	1896 b	32.1 bc	3.13 bc	0.87 bc
		MCP _{V11}	422	1352 a	1611 a	2305 ab	44.3 a	4.13 a	1.09 a
		ETH _{V16}	391	1017 c	1235 b	2042 ab	29.8 c	2.93 c	0.74 c
		MCP _{V16}	361	1177 abc	1427 ab	2125 ab	38.8 ab	3.70 ab	0.90 b
		UTC	376	586 d	684 c	1363 c	10.9 d	1.07 d	0.37 e
	Heated	ETH _{V11}	361	569 d	698 c	1541 c	9.9 d	1.17 d	0.57 d
		MCP _{V11}	425	608 d	713 c	1443 c	8.7 d	1.00 d	0.35 e
		ETH _{V16}	385	553 d	682 c	1554 c	8.0 d	1.03 d	0.42 e
		MCP _{V16}	378	587 d	695 c	1486 c	9.9 d	1.10 d	0.40 e
		HPGRs	NS	**	**	NS	**	**	*
		TR	NS	**	**	**	**	**	**
HPGRs×TR	NS	**	**	**	**	**	**		

*Significant at the 0.05 probability level.

**Significant at the 0.01 probability level. NS: not significant ($P > 0.05$).

[†]Description in Table 1.

[‡]Vt, Tasseling.

[§]Data followed by the same letter within a column, experiment and factor do not differ at $P = 0.05$ (Duncan's multiple range test).

heating before anthesis had null (Exp.1) or only moderate (–10% in Exp.2) effects on individual kernel weight (Table 5). Application of HPGRs had no clear effect per se on grain yield, but a significant interaction ($P < 0.01$) was detected between treatments during Exp.2. On one hand, ethephon application caused a decrease in grain yield (between 15 and 18%) and kernel numbers (between 12 and 16%) among nonheated plots. On the other hand, there was a clear positive effect on grain yield of the early application (V₁₁) of ethephon among heated plots (increase of 73% respect to the heated untreated control [UTC]). This response followed the trend observed for kernel number m⁻² (increase of 78% respect to the heated UTC) for which similar interaction effects were significant in both experiments ($P \leq 0.05$; Table 5).

Observed trends could not be totally explained by differences in plant growth rate (Table 4) or in biomass partitioning to reproductive structures (Table 5), either during the critical period for kernel set (represented by EGR_{CP} PGR_{CP}⁻¹) or at physiological maturity (represented by harvest index). For the former, there was a clear positive effect of

heat stress and the early application of ethephon on biomass allocation to the ear (Table 5). For the latter, there was (i) no variation in harvest index among nonheated plots, (ii) a marked negative effect of heat stress on harvest index of untreated control plots (–20% in Exp.1 and –40% in Exp.2), and (iii) positive (e.g., ETH_{V11} in both experiments, and MCP_{V16} and ETH_{V11}+MCP_{V16} in Exp.1) or negative (e.g., MCP_{V11}) effects of some products on harvest index of heated plots. Nevertheless, previously described variations in ear growth rate during the critical period explained observed variations in kernel numbers per plant (Fig. 1b). Independent models gave an improved fit to data from each experiment ($r^2 \geq 0.966$; $P < 0.01$) because an uneven distribution of residuals was detected (52 ± 25.7 for Exp.1 and -42 ± 40.8 for Exp.2) when a single fit was tested for the whole data set ($r^2 = 0.71$; $P < 0.01$).

DISCUSSION

The effects of heat stress during late vegetative growth on maize grain yield components and its physiological determinants have been thoroughly addressed in a

Table 5. Response of maize grain yield, grain yield components (kernel number m⁻² and individual kernel weight), and biomass partitioning to reproductive organs to the application of HPGRs and contrasting temperature regimes (TR). Biomass partitioning to reproductive organs was computed as the quotient between (i) ear and plant growth rates for the critical period between V₁₁ and R₂ (EGR_{CP} PGR_{CP}⁻¹), and (ii) grain yield and total shoot biomass at physiological maturity (harvest index). Results of analysis of variance (ANOVA, *F* test) are indicated for each source of variation.

Experiment	Temperature regime	HPGRs [†]	Grain yield	Kernel number m ⁻²	Kernel weight	Biomass partitioning		
						EGR _{CP}	PGR _{CP} ⁻¹	Harvest index
			g m ⁻²		mg			
Exp.1	Nonheated	UTC	1017	4410 a [‡]	230	0.22	0.49 a	
		ETH _{V11}	954	4500 a	212	0.25	0.51 a	
		MCP _{V16}	873	4284 a	203	0.20	0.48 a	
		ETH _{V11} + MCP _{V16}	864	3996 a	216	0.22	0.51 a	
	Heated	UTC	486	2124 c	229	0.26	0.39 b	
		ETH _{V11}	684	3348 b	205	0.37	0.51 a	
		MCP _{V16}	711	2916 b	242	0.30	0.48 a	
		ETH _{V11} + MCP _{V16}	738	3627 b	203	0.38	0.51 a	
Source of variation		HPGRs	NS	NS	NS	NS	*	
		TR	**	**	NS	**	NS	
		HPGRs×TR	NS	*	NS	NS	*	
Exp.2	Nonheated	UTC	1251 a	4311 a	291	0.26	0.53 a	
		ETH _{V11}	1026 b	3609 ab	284	0.28	0.54 a	
		MCP _{V11}	1206 a	4284 a	281	0.26	0.52 a	
		ETH _{V16}	1062 b	3807 ab	279	0.25	0.52 a	
		MCP _{V16}	1143 ab	4221 a	270	0.24	0.54 a	
		Heated	UTC	441 d	1692 d	259	0.34	0.32 bc
	ETH _{V11}		765 c	3015 bc	255	0.49	0.50 a	
	MCP _{V11}		315 d	1314 d	240	0.35	0.22 c	
	ETH _{V16}		477 d	1944 d	240	0.41	0.31 bc	
	MCP _{V16}		594 d	2196 cd	270	0.36	0.40 ab	
	Source of variation			HPGRs	NS	*	NS	NS
			TR	**	**	**	**	**
		HPGRs×TR	**	**	NS	NS	*	

*Significant at the 0.05 probability level.

**Significant at the 0.01 probability level. NS: not significant (*P* > 0.05).

[†]Description in Table 1.

[‡]Data followed by the same letter within a column, experiment and factor do not differ at *P* = 0.05 (Duncan's multiple range test).

previous paper (Cicchino et al., 2010b). Main conclusions from that study focused on the importance of reduced ear growth rate and radiation use efficiency on final kernel set and grain yield of crops exposed to above-optimum temperatures. Contrary, this constraint had no negative effect on these traits mediated by changes in biomass partitioning to the ear around flowering.

In current research we combined the described temperature regimes (heated and nonheated during approximately 15 d immediately before anthesis) with the application of two products related to ethylene metabolism, one a promoter (ethephon) and the other an inhibitor (1-MCP) of its synthesis. An expected effect of the former is the decrease in tissue expansion that affects growing organs at time of application (Earley and Slife, 1969; Cox and Andrade, 1988; d'Andria et al., 1997; Shekoofa and Emam, 2008), which was confirmed by the reduction in plant height and leaf area index registered in most plots treated with this product (Table 3). These responses

produced a decline in traits related to light interception and biomass production particularly among early-sprayed (V₁₁) nonheated plots. These trends are in agreement with previous reports of negative effects of ethephon on mentioned traits, but in experiments developed under potential rather than stress conditions (Cox and Andrade, 1988; Kasele et al., 1994; d'Andria et al., 1997). The decline in light interception and biomass production took place in spite of the large stand density used in our research (9 pl m⁻²) as compared to old studies (3.7–7.6 pl m⁻²), which should have allowed crops to hold LAI values above the threshold that is considered critical for maximum light interception in this species (Maddonna et al., 2001). The observed trends in canopy size were accompanied by a mild decrease in kernel numbers (always ≤ 16%) and grain yield (always ≤ 18%) of nonheated ethephon treated plots, and a large improvement (always ≥ 73%) in these traits among the heated ones sprayed at V₁₁, in agreement with previous reports. Early studies referred losses up to 33% (Earley and

Slife, 1969) among nonstressed crops and increases up to 200% among the stressed ones (d'Andria et al., 1997), which were attributed primarily to variations in kernel numbers (Cox and Andrade, 1988; Kasele et al., 1994; d'Andria et al., 1997). Based on this evidence and no further thorough physiological analyses, ethephon use did not expand among commercial maize crops. However, important conclusions can be drawn on this topic using an adequate physiological framework; i.e., capacity for resource capture, resource use for biomass production, and biomass partitioning to reproductive structures. Ethephon applications had effects on all three processes, and the balance between them conditioned the final result. Negative effects on canopy size vary with the timing of product application along the cycle (Earley and Slife, 1969) and should be expected to be highest when it matches the expansion of the largest leaves. These leaves are located around the ear and its exact number depends primarily on cycle duration up to anthesis (Dwyer et al., 1992); e.g., tropical hybrids > temperate hybrids. It has been demonstrated that this response and its negative consequences on biomass production can be partially or totally compensated by increasing stand density (Cox and Andrade, 1988; Langan and Oplinger, 1987) and/or reducing row spacing (Langan and Oplinger, 1987). The negative effects on kernel numbers deserve two considerations. On one hand, they could be attributed to mentioned reductions in crop growth and biomass production, in this case promoted by decreased light interception. However, the relationship between kernel number per plant and plant growth rate during the critical period (Andrade et al., 1999) was not as robust as expected due to the narrow range of variation of the independent variable among heated plots (relationship not shown). On the other hand, we confirmed the tight association between kernel number per plant and ear growth rate during the critical period (Pagano and Maddonni, 2007), and detected the benefit of early applications of ethephon on biomass allocation to the ear at all growing conditions (i.e., heated and nonheated). The physiological processes behind this response may include (i) reduced competition for assimilates between the ear and other organs (stem and tassel) that are undergoing active growth (Fischer and Palmer, 1984; Otegui, 1997), as observed for other crops and HPGRs (Gomez et al., 2011), and (ii) direct effects of ethephon on pollen development, which have not been reported for maize but were observed in other species (Berhe and Miller, 1978). The anticipated silking date and reduced ASI observed among early-treated ethephon plots (V_{11}) support these contentions and highlight the importance of the timing of the application on final results (there were larger benefits for $ETH_{V_{11}}$ than for $ETH_{V_{16}}$ for the hybrid tested in current research). The described responses translated into enhanced kernel numbers and harvest index that partially compensated (nonheated) or overcompensated (heated)

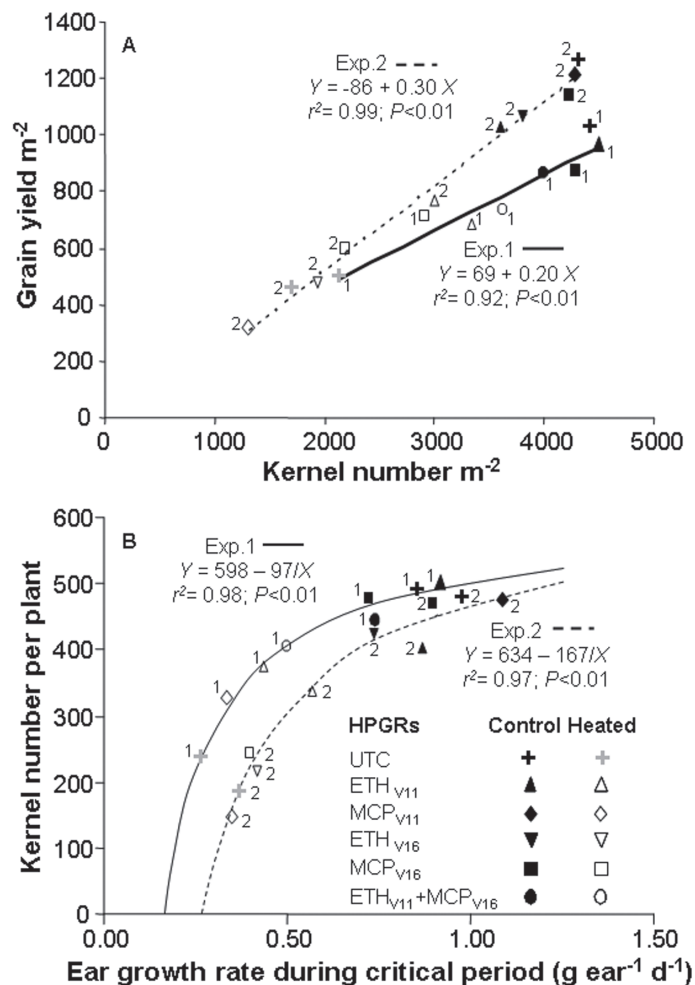


Figure 1. Response of grain yield to kernel number m^{-2} (a) and of kernel number per plant to ear growth rate during the critical period (b). Data correspond to the combination of different thermal regimes (control and heated) and hormonal plant growth regulators (HPGRs) during two experimental years (Exp.n). Detail of treatments is given in Table 1. Numbers (1 and 2) next to symbols indicate the experiment.

reductions in biomass production, with the concomitant stability (nonheated) or increase (heated) in grain yield.

Contrary to ethephon, 1-MCP had (i) a mild positive effect on crop growth rate before anthesis when applied to nonstressed plots at V_{11} in Exp.2, which had no effect on final biomass production and grain yield, and (ii) an apparent benefit (not significant at $P = 0.10$) on biomass production and grain yield when sprayed on heated plots during stress (V_{16} , in both experiments) but not immediately before stress (V_{11} , in Exp.2); i.e., better for mitigation than for prevention of stress. The first result may be indicative of the occurrence of transient stress conditions among nonheated plots (Loomis and Amthor, 1999) or simply of an artifact in computations (e.g., slight differences among plots assigned to each treatment that were not detected by the ANOVA of initial crop biomass; Table 4). The second result deserves different interpretations. One relies on the timing and rate of product application, which may have been insufficient for compensating the

synthesis of new ethylene receptors (Feng et al., 2000; Able et al., 2002; Pesis et al., 2002) with the concomitant increase in the negative effects of the hormone at some point during the heating period (earlier for V_{11} than for V_{16} treated plots). Another one is based on possible negative effects of having ethylene receptors blocked at the start of stress (preventive effect) as compared to the advantage of allowing activation of protective, early stress-response mechanisms (Passioura, 1996) followed by the subsequent blockage of ethylene receptors with 1-MCP for avoiding other deleterious stress responses (mitigation effect). Further research should address these gaps of knowledge.

CONCLUSIONS

The current study addressed the combined effects of preanthesis heat stress and different applications of HPGRs related to ethylene metabolism (ethephon and 1-MCP) on maize grain yield determination. We could reveal several aspects of ethephon use that remained unclear up to now, particularly those related to contrasting responses in kernel set and final grain yield. Apparent controversies disappeared when results were evaluated within a physiological framework that considered product effects on crop capacity for light capture and biomass production, and subsequent biomass partitioning to reproductive structures. Based on our findings and previous results, use of ethephon can be recommended in commercial maize fields when heat or water stress conditions are expected during the critical period for kernel set. But we highlighted the importance of adequate decisions on (i) management practices related to crop structure (stand density and row spacing) for minimizing negative results on LAI that may decrease the amount of light intercepted by the crop and consequently biomass production, particularly in nonstressed conditions, and (ii) timely application for improving biomass allocation to the ear (i.e., before the start of active ear growth). The results for the inhibitor of ethylene receptors (1-MCP) were not as clear as those described for ethephon, and the apparent benefit of 1-MCP use for stress mitigation (i.e., during stress) rather than for stress prevention (i.e., before stress) must be taken only as the standing point of future research.

Acknowledgments

The authors gratefully acknowledge Luis B. Blanco, Mariela Chintio, Joaquín Martínez Bercovich, María Rossini, Alan Severini, Walter Tanaka, and Gustavo A. Maddonni for their assistance with field work. This research was financed by the National Council for Research (CONICET PIP 00125), the University of Buenos Aires (UBACyT G08 and 00454), AgroFresh, and the Regional Funding for Agricultural Technology (FONTAGRO 8031). M.A. Cicchino held a graduate's scholarship from INTA and J.I. Rattalino Edreira held a graduate's scholarship from CONICET. M.E. Otegui is a member of CONICET.

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