

## **EFFECTS OF HIGH ALTERNATING DAY/NIGHT TEMPERATURES ON GRAIN GROWTH AND QUALITY**

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### **Abstract**

We studied the effects of high alternating day/night temperatures (ADNT) on grain growth and oil quality of an oil-type hybrid. Heat treatments were applied to the capitulum in only one of three successive six-day periods during grain filling named HT1 (12-18 daa [days after anthesis]), HT2 (18-24 daa) and HT3 (24-30daa). Final grain weight was strongly affected by peak (i.e., mean grain temperature from 11 to 16 h) temperatures >35C applied early in grain filling (HT1), whereas later stresses (HT2 and HT3) caused smaller reductions. These effects were attributable, in the main, to changes in grain filling duration. Peak temperatures >35C also affected other grain properties, including grain oil content, oil fatty acid composition, and hull to kernel ratio; and the timing (i.e., HT1, HT2 or HT3) of the treatment producing the greatest effect could change with the attribute considered. We compared the results obtained here with previously published work using constant temperatures throughout the day applied to an inbred line, which exhibited a shorter period of oil accumulation. Both sets of experiments were consistent insofar as a temperature of about 35C represents the response threshold for many effects. The comparison also served to show that the differences between genotype responses could be understood in terms of the degree of overlap between the stress treatment and the active grain-growth process during the stress period. This emphasizes the need to relate stress responses to grain growth patterns, which can differ between genotypes.

### **Introduction**

The temperature effectively sensed by sunflower grains and other organs exposed to high radiation loads and with restricted capacity for transpiration can considerably exceed air temperatures during the sunlit period of the day. Ploschuk and Hall (1995) found receptacle

and grain temperatures exceeded air temperatures by 10C and 5C, respectively, under sunny conditions on a day in which diurnal air temperatures did not exceed 25C. Temperature is not only a major environmental factor regulating the rate and duration of grain filling (Chimenti et al., 2001), but also can modify grain yield and quality (Rondanini et al., 2003).

Rondanini et al. (2003) examined the effects of high temperature on grain growth by exposing heads to a range of constant temperatures applied throughout the day for periods of seven days. The main objective of the present study was to investigate the effects of high alternating day/night temperatures, applied at three different subphases of the grain-filling period, on grain growth and oil quality. Rondanini et al. (2003) used the inbred line HA 89; here we used the hybrid Paraíso 30, in which grain oil deposition continues longer during grain-filling than in the inbred line. In the present report we present the results obtained with Paraíso 30 and emphasize the contrasts between the earlier work and the new.

## Materials and Methods

Two experiments using alternating day/night temperatures (ADNT) were carried out at Facultad de Agronomía, Universidad de Buenos Aires (34° 35'S) in summers of 2001 and 2002. In the first of these we used the hybrid Paraíso 20 [Nidera Seeds], in the second the hybrid Paraíso 30 [Nidera Seeds] was used. Because results for basic responses were similar in both years and more detailed measurements of grain size distribution (see below) were made in the second year, this paper concentrates on the latter experiment. Plants were grown in the field in large pots arrayed as a crop until 10 days after anthesis (daa), at which stage the plants were transferred to a greenhouse with a 26/20C (day/night) temperature regime. Heat treatments were applied by heating the capitulum in one of three successive six-day periods during grain filling named HT1, HT2 and HT3 (for details, see Table 1 and illustration of grain temperature dynamics in Figure 1). During each treatment period, thermostatically controlled heat chambers (Rondanini et al., 2003) were fitted to selected capitula. Control plant heads and the rest of each treated plant remained at greenhouse temperature.

Table 1. Achieved mean daily (constant temperature treatments, Rondanini et al., 2003) grain temperatures (°C) and achieved peak (from 11 to 16 h) and base (from 19 to 7 h) average grain temperatures (ADNT treatments), and timing of temperature treatments (HT1, HT2, HT3) during grain filling in constant temperature (CT, Rondanini et al., 2003) and ADNT treatments.

	Constant Temperature	Alternated Temperature
	Mean daily temperature (°C)	Peak/base temperature (°C)
<b>HT1</b>	<b>12-19 daa</b>	<b>12-18 daa</b>
	25	27/21
	33	33/31
	36	37/33
	40	43/40
<b>HT2</b>	<b>19-26 daa</b>	<b>18-24 daa</b>
	24	26/20
	32	34/29
	35	40/35
	38	45/37
<b>HT3</b>	<b>26-33 daa</b>	<b>24-30 daa</b>
	24	26/21
	35	35/27
	39	42/32
	41	45/38

The daily grain temperature dynamics (Figure 1) in plants subjected to ADNT treatments showed a quasi-plateau (here termed peak) from 11 to 16 h, a lower temperature during the night (from 1900 to 700 h, here termed base) and gradual changes from base to peak and vice-versa. This pattern is rather similar to that found by Ploschuk and Hall (1995) in plants growing in the field on sunny days (Figure 1). In this paper, peak and base temperatures are the mean values for the respective periods. Individual grain weight dynamics were followed during the grain-filling period according to Ploschuk and Hall (1995). Oil content was determined using a combined solvent/supercritical CO<sub>2</sub> two-step extraction method for small samples described in Rondanini et al., 2003. Fatty acid methyl esters were quantified by GLC. Treatments were arranged in a DCA with four replicates. A bilinear model was fitted to weight/time relationships (see Rondanini et al., 2003). ANOVA and Tukey's test (SAS Institute, 1988) were applied to experimental results.

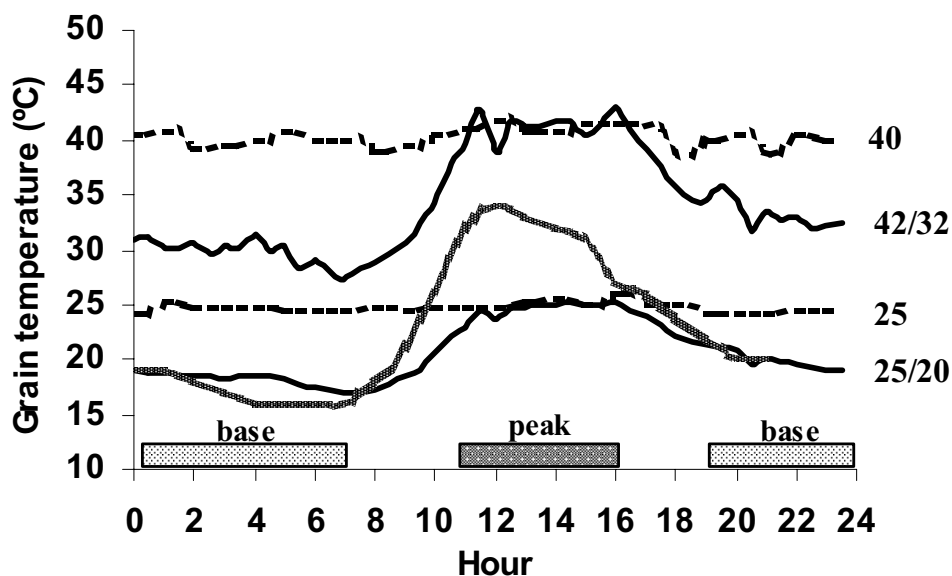


Figure 1. Examples of daily grain temperature dynamics measured 25 daa for constant (dashed line) and alternating day/night (full line) temperature treatments. Values on the right indicate achieved thermal regime (C), either as 24 h means (CT) or peak/base means (ADNT treatments). Bars indicate the periods used for calculating peak and base temperature values. Grain temperature dynamics measured in the field (Ploschuk and Hall, 1995) on a sunny day are also shown (grey line). Data for constant temperature treatments are from Rondanini et al. (2003).

## Results and Discussion

Mean (24 h) grain temperatures achieved in the ADNT treatments (data not shown, but close to the average of peak and base temperatures shown in Table 1) and in constant temperature treatments (Rondanini et al., 2003 and Table 1) were fairly similar in each of the three treatment periods, but resulted from rather different daily grain temperature patterns (Figure 1). A further important difference between the two sets of experiments was that in the

grain of HA 89, oil deposition ceased before the whole grain had reached its maximum weight (Figure 2A), while both end points were rather close together in Paraíso 30 (Figure 2B).

Final grain weight was affected by peak temperatures  $> 35^{\circ}\text{C}$  in ADNT treatments (Figure 3A). The decrease in grain weight was greater in HT1 (55%, on average) than HT2 and HT3 (40% and 30%, respectively) (Figure 3A). Although the nature of temperature treatments was different in ADNT with respect to CT (Rondanini et al., 2003), the relative grain weight reductions evoked by the earliest stress (HT1) were quite similar in both experiments (Figure 3A). Similarly, in both experiments temperatures (peak or mean) of about  $35^{\circ}\text{C}$  seem to mark the response threshold. There was some indication that the HT2 stress had less effect with ADNT than with CT treatments. Grain-filling duration had a stronger association to final grain weight than grain-filling rate in both ADNT and CT treatments (Figure 3B).

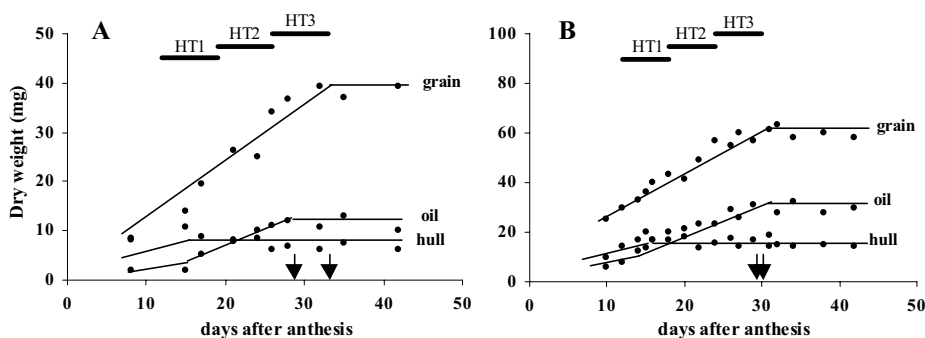


Figure 2. Dynamics of the growth of the grain and its components (hull and oil) from capitula of control plants of HA 89 (A) and Paraíso 30 (B). Bars indicate the periods in which high temperature treatments (HT) were applied. Arrows indicate timing of end of the oil synthesis and grain growth period. Note difference in “y” axis scales. Data for HA 89 from Rondanini et al., 2003.

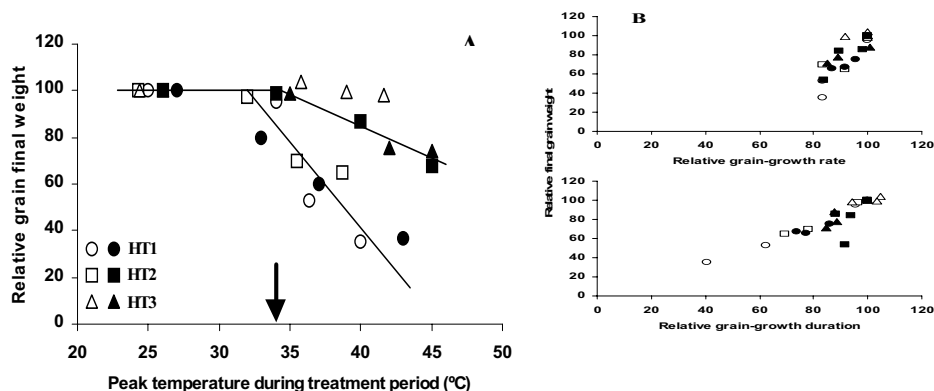


Figure 3. A) Relative grain final weight (control final grain weight=100%) of peripheral grains of capitula ( $n=4$ ) exposed to constant (empty symbols) and alternating day/night (full symbols) temperature treatments during brief periods of the grain filling phase. For timing of treatments see Table 1 and Figure 1. Vertical arrow indicates the suggested threshold separating high and very high temperature conditions for sunflower. Data for constant temperature treatments from Rondanini et al., 2003. B) Relationships between relative final grain weight and relative grain-growth rate (above) and relative grain-filling duration (below) for peripheral grains exposed to brief periods of heat stress. Symbols and data sources as in A.

Final grain weight frequency distribution was altered by ADNT treatments exposed to peak temperatures >35C, with the distribution being skewed towards the left and average grain weight being reduced (Figure 4). The proportion of very small (10-30 mg) grains (named half-full grains) was increased and these showed alterations to hull colour and integrity (data not shown). These effects of treatment on grain weight distribution were more noticeable in HT1 than in later stresses (data not shown).

Data in Table 2 illustrate some effects of constant and alternating day/night temperatures on grain variables. Effects tended to be greatest in HT1 and least in HT3 and to increase with applied temperature. Usually, temperatures (mean or peak) greater than 35C were those that produced the effects of greatest magnitude. Constant temperatures tended to produce greater alterations in grain variables than alternating ones, but the oil content per grain in HT3 was an exception here, possibly because HT3 affected a greater portion of the oil deposition window in Paraíso 30 than in HA 89 (Figure 2).

Table 2. Final oil content, hull to kernel ratio and percentage of oil in mature peripheral grains from capitula of sunflower exposed to constant and alternated high temperature treatments applied during one of three separate periods (HT, for timing see Table 1) during grain filling. Values followed by different letters indicate significant differences ( $P<0.05$ ) between temperatures within each treatment period. Data for constant temperature treatments are from Rondanini et al. (2003).

Constant Temperature				Alternating Temperatures			
Treatment period and mean daily temperature (C)	Oil content (mg/grain)	Hull: kernel ratio	Oil grain %	Treatment period and peak/day temperature (°C)	Oil content (mg/grain)	Hull: kernel ratio	Oil grain %
<b>HT1=12-19 daa</b>				<b>HT1= 12-18 daa</b>			
25	13.9 a	0.20 a	35.0 a	27/21	27.2 a	0.26 a	45.1 a
33	13.8 a	0.17 a	36.4 a	33/31	16.6 b	0.25 a	36.9 a
36	4.9 b	0.43 b	23.3 b	37/33	12.1 c	0.30 b	29.9 b
40	3.0 b	0.58 c	21.5 b	43/40	11.6 c	0.32 c	29.0 b
<b>HT2= 19-26 daa</b>				<b>HT2= 18-24 daa</b>			
24	13.6 a	0.19 a	35.3 a	26/20	27.4 a	0.27 a	46.5 a
32	12.9 a	0.18 a	34.5 a	34/29	23.8 ab	0.26 a	46.6 a
35	8.9 b	0.26 b	33.0 ab	40/35	22.4 ab	0.25 a	45.3 a
38	8.0 b	0.29 b	31.8 b	45/37	19.7 b	0.25 a	43.5 a
<b>HT3= 26-33 daa</b>				<b>HT3= 24-30 daa</b>			
24	14.6 a	0.20 a	39.1 a	26/21	26.9 a	0.26 a	44.8 a
35	14.5 a	0.19 a	37.3 a	35/27	20.8 b	0.30 b	39.4 a
39	13.6 a	0.19 a	36.7 a	42/32	18.9 b	0.34 c	40.3 a
41	13.7 a	0.18 a	37.3 a	45/38	16.8 b	0.38 c	39.0 a

Plots of oleic to linoleic acid ratios in mature grains against base temperature (ADNT) or night (CT) temperature (Figure 5) were used to examine effects of treatment on oil quality. We used night (or base) temperatures as the independent variable given that Izquierdo et al. (2002) identified this period of the day as the crucial one for oil quality determination. Stress applied in HT1 had no effect on this ratio in either experiment, but response patterns to later stresses differed between CT and ADNT (cf. responses to HT2 and HT3 in both experiments). A possible explanation for this contrasting behaviour lies in whether enough time elapses between the end of the stress period and the end of the oil synthesis period (earlier in HA 89 than in Paraíso 30, see Figure 2) for recovery of enzyme activity to affect oil composition. Response patterns of an indicator of oleate desaturase dynamics during grain filling (data not shown) are consistent with this interpretation. Additionally, modifications (when present) in

oleic content from ADNT treatments were lower than ones in CT treatments (Figure 5). This may be related to genotype responses and/or related to lower night temperatures applied in ADNT treatments (Table 1).

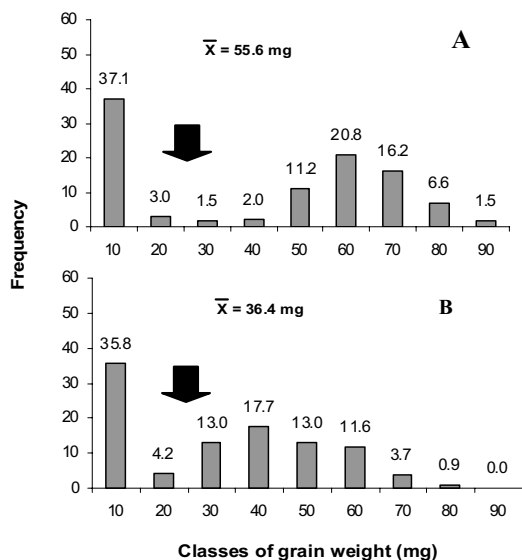


Figure 4. Grain weight frequency distribution for heads of control (A) plants and of plants exposed to alternating peak/base temperatures of 45/37 from 12 to 18 daa (HT1) (B). Average grain weight ( $\bar{x}$ ) was estimated after excluding the empty grains (0-10 mg). Arrows indicate the very small grain category (20-30 mg) termed half-full.

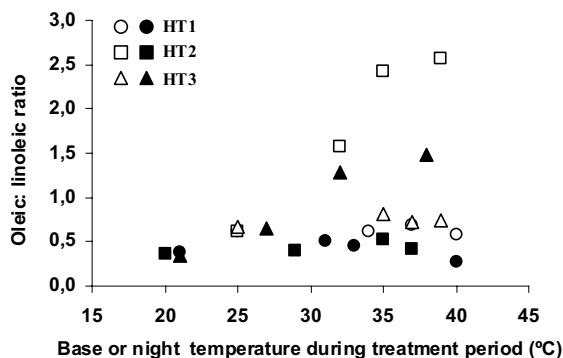


Figure 5. Changes in the oleic: linoleic ratio at grain maturity in oil from peripheral grains exposed to constant (empty symbols) and alternating day/night temperatures (full symbols). For timing of treatments see Table 1 and Fig. 1. Data for constant temperature treatments from Rondanini et al., 2003.

## Conclusions

Our results confirm that there is a direct detrimental effect of brief periods of grain temperature  $> 35^{\circ}\text{C}$ , even if only applied during a few hours per day, on grain weight, oil content and fatty acid composition in sunflower. Fatty acid composition was also affected by

temperatures lower than 35C. The differences observed in the responses shown by genotypes used in this and previous work are a complex but orderly functional response that can be related to the grain component growth process (i.e., hull, kernel, oil) affected by each stress period. This emphasizes the need to relate periods of greater sensitivity to stress to grain growth patterns, which can be different between genotypes. Future work will be focused on examining the minimum duration (number of successive days of stress) of stress conditions required to alter grain yield and/or quality.

### **Acknowledgments**

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### **References**

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