TEMPERATURE-DEPENDENT HETEROSIS IN MAIZE

By J. R. McWilliam* and B. Griffing*†

[*Manuscript received January* 8, 1965]

Summary

The existence of temperature-dependent heterosis in maize and its contribution to hybrid vigour was investigated in controlled environments.

Additional heterosis was found when plants were grown at temperatures above the growth optimum (33/27 °C), or following high-temperature shocks. This temperature-dependent heterosis was manifest because the hybrids grew relatively better than the inbreds at high temperatures, and recovered more rapidly after heat shock treatments. Temperature-dependent heterosis was also expressed at temperatures below 21/16 °C, which was due largely to the greater sensitivity of the inbreds to low temperatures.

The results indicate that the level of heterosis demonstrated by hybrid maize is strongly temperature-dependent, and that the higher yields of the hybrids were due to the greater phenotypic stability over the entire temperature range. The genetic basis for high-temperature-dependent heterosis is thought to stem from the random fixation of genes sensitive to high temperatures in the inbreds, giving rise to thermolabile enzymes which lead to mutant expression only at elevated temperatures.

This differential heterosis phenomenon may well provide a physiological interpretation for at least part of the heterosis observed in field-grown crops of maize which are subjected to high-temperature stresses.

I. Introduction

Temperature-dependent heterosis has recently become of interest both from a theoretical point of view, in that a precise biochemical explanation can be offered for the phenomenon, and also from an applied viewpoint, since it may be an important contributor to the heterotic response of field-grown crops subject to heat-wave conditions.

The experimental evidence for temperature-dependent heterosis has been reviewed by Langridge (1962) and Griffing and Langridge (1963). In their work with *Arabidopsis thaliana* grown in controlled environments, Griffing and Langridge compared the growth of five homozygous races, some F_1's, and all possible F_2's at a series of temperatures ranging from 16 to 31°C. In general the heterozygous generations displayed greater phenotypic stability than the parents over the temperature range. In the lower and optimal temperature ranges the degree of heterosis was relatively slight, but at the higher temperatures the degree of heterosis was greatly increased. The superior phenotypic stability of the hybrids was directly related to the phenomenon of temperature-dependent heterosis.

Langridge (1962) has suggested that the biochemical basis of this phenomenon stems from the accumulation of random sets of genes sensitive to high temperatures in

*Division of Plant Industry, CSIRO, Canberra.

†Present address: Department of Zoology and Entomology, Ohio State University, Columbus, Ohio.

the homozygous races. These genes produce unstable enzymes which lead to mutant expression in the phenotype only at elevated temperatures. In the hybrids the effect of dominance leads to thermostable representatives of each enzyme, and thus high-temperature stability of phenotype.

Lewis (1955), studying heterosis in tomatoes, explored the consequences of shifting parents and hybrids from one environmental regime to another. He discovered that in some cases the hybrids maintained their growth rate after the shift, whereas the parents exhibited a lag phase.

On the basis of these results, Griffing and Langridge (1963) hypothesized that the overall phenomenon of temperature-dependent heterosis may contribute to the hybrid vigour of field crops which are subject to high temperatures during periods of critical growth. Not only would hybrids grow better at high temperatures, but they would also tend to recover from the heat shock more quickly than the inbreds.

The objective of the present study was to test this hypothesis under more realistic conditions. Maize was chosen for study since it is widely used commercially in the form of hybrids, and is normally grown in climates which occasionally experience periods of extreme heat. In the present experiments, heat waves were simulated by heat shocks given under controlled conditions in the Canberra phytotron.

II. Materials and Methods

The experiments were carried out in the Controlled Environment Research Laboratory at Canberra, the design and facilities of which have been described by Morse and Evans (1962).

The experimental material consisted of different sets of maize, *Zea mays* (L.), each containing two different inbreds and their reciprocal F_1's. All plants were grown in controlled-environment glasshouses with natural day length extended to 16 hr by incandescent lamps, which provided an intensity of 25 f.c. at plant height.

Plants were initially irrigated twice daily, once with Hogland's nutrient solution and once with water. A third watering with nutrient solution was given after the tenth leaf stage to reduce possible nutrient and moisture stress.

In all experiments the glasshouse temperatures during the day (8.30 a.m.–4.30 p.m.) were 5 degC higher than those at night. High-temperature shock treatments, either of long or short duration, were given by placing plants in either naturally or artificially lit growth cabinets.

All growth data were transformed to logarithms for analysis since plants when harvested were in the exponential growth phase.

(*a*) *Heterosis at Different Temperatures*

Two commercial inbred lines of maize from the United States of America were used; 38–11 (G_{12}) of northern flint origin (United States Department of Agriculture), and K4 (G_{10}) of southern dent origin from Missouri.

Both inbreds and their reciprocal hybrids were grown from seed at nine different day/night temperatures from 15/10°C rising by intervals of 3 degC to 39/34°C.

Since no glasshouse was available at 39/34°C, plants were grown in two naturally lit growth cabinets at this temperature. The plants were re-randomized regularly between cabinets and in other respects were maintained under the same conditions as those in the glasshouse. Seeds were sown in 10-in. diameter plastic buckets in perlite and thinned to three seedlings soon after emergence. Twenty plants were harvested from each inbred and hybrid at each temperature.

TABLE 1

DETAILS OF HEAT-SHOCK TREATMENTS OF LONG DURATION AND THEIR RESPECTIVE CONTROLS

S, heat shock; H, harvest; A, first heat shock period; B, second heat shock period; C, third heat shock period

	Days after Sowing			
	21	33	45	57
Experiment 1				
Control 1 (C_1)		H		
Control 2 (C_2)			H	
Control 3 (C_3)				H
Treatment 1 (T_1)	S	H		
Treatment 2 (T_2)	S	S	H	
Treatment 3 (T_3)	S	S	S	H
Experiment 2				
Control				H
Treatment 1 (T_1)—A	S			H
B		S		H
C			S	H
Treatment 2 (T_2)–AB	S	S		H
AC	S		S	H
BC		S	S	H
Treatment 3 (T_3)–ABC	S	S	S	H

Harvests were made when the majority of plants at a given temperature had reached the sixteenth leaf stage.

Relative growth rates of inbreds and hybrids were calculated from log (dry weight of tops), corrected for initial weight of the embryo, and heterosis was expressed as the difference in growth rate between the mean of the F_1's and the mid-parent value ($\bar{F}_1 - MP$).

(*b*) *Heat Shocks of Long Duration*

The same inbreds and hybrids were grown under similar cultural treatments as those described in Section II(*a*). Plants were germinated and grown for 21 days at 27/22°C, and then those to be treated were exposed to one, two, or three 12-day heat shock treatments. Heat shocks were given in naturally lit growth cabinets at 41/32°C and the photoperiod was extended to 16 hr to match the photoperiod of the controls. During heat shock treatments, attempts were made to reduce moisture stress by frequent watering and by maintaining a high humidity in the cabinet. Two experiments were conducted: (1) exposure to three successive heat shocks, with a harvest after each heat shock; and (2) all possible combinations of one, two, or three heat shocks with a single harvest at the end of the treatment period.

Details of the heat shock treatments and of the controls for these two experiments are set out in Table 1.

The means for the log (dry weight of tops) of 10 plants from each inbred and reciprocal hybrid were used to calculate the heterotic response ($\bar{F}_1 - MP$), following each of the temperature shock treatments. The magnitudes of this response were then compared with that displayed by the appropriate controls to measure the extent to which the heterosis was temperature-dependent.

(*c*) *Heat Shocks of Short Duration*

(i) *Material.*—Four sets of maize were used, each set consisting of two different inbreds and their reciprocal F_1 hybrids. In two sets the inbreds were derived from varieties originating in Queensland. The other two sets involved inbreds from the corn belt of the United States of America. The material and their pedigrees are as follows:

Set No.	Genotypic Designation	Origin
S_1	G_1 = L12 G_2 = L12 × L30 G_3 = L30	Lawes, Qld.
S_2	G_4 = L26 G_5 = L26 × L105 G_6 = L105	Lawes, Qld.
S_3	G_7 = K1 G_8 = K1 × C121 G_9 = C121	Missouri, U.S.A. Tennessee, U.S.A.
S_4	G_{10} = K4 G_{11} = K4 × 38–11 G_{12} = 38–11	Missouri, U.S.A. United States Department of Agriculture

(ii) *Treatment.*—All plants were raised as described previously with the exception that pot size was reduced to 7 in., and only one plant was grown per pot.

Heat shocks were given by placing plants in an artificially lit, controlled-environment cabinet (type LB) (Morse and Evans 1962) for 2 hr, at a temperature of 50°C. All pots were saturated before applying the heat shock, and high humidities were maintained during the treatment period. Recovery time for the cabinet to return to 50°C after loading with pots was approximately 30 min. This time was not regarded as part of the treatment.

The details of the controls and heat shock treatments are set out in Table 2. All four sets of maize were used in the first three treatments (T_1, T_2, T_3) and their respective controls (C_1, C_2, C_3). Treatments T_4 and T_5, however, involved only No. S_4.

TABLE 2

DETAILS OF HEAT SHOCK TREATMENTS OF SHORT DURATION AND THEIR RESPECTIVE CONTROLS

S, heat shock; H, harvest

Treatment	Days after Sowing			
	15	26	38	49
Controls				
C_1		H		
C_2			H	
C_3				H
Treatments				
T_1	S	H		
T_2	S	S	H	
T_3	S	S	S	H
T_4	S		H	
T_5	S			H

(iii) *Analysis.*—Heat shock treatments were compared with their appropriate controls using log (dry weight of the entire plant) as the variable. Each comparison was replicated three times and there were five plants of each genotype represented in each treatment. The comparison of each treatment with its control was analysed separately.

To investigate the temperature-dependent heterosis phenomenon, various definitions were made. For the ith set, the following sums were used to define a temperature-dependent heterosis contrast (an arbitrary notation is followed here which does not follow the C_iT_j notation indicated earlier in Table 2):

	T_0 (control)	T_i (heat shock)
G_{i0} (inbred)	$X_{i00\cdot\cdot}$	$X_{i01\cdot\cdot}$
G_{i1} (F_1)	$X_{i10\cdot\cdot}$	$X_{i11\cdot\cdot}$
G_{i2} (inbred)	$X_{i20\cdot\cdot}$	$X_{i21\cdot\cdot}$

where $X_{ijk\cdot\cdot}$ is the sum over replications and plants within replications. This sum involves 15 observations.

The temperature-dependent heterosis contrast was defined as the interaction of quadratic effect among the genotypes and the treatment effect, i.e.

$$H_i = (2X_{i11\cdot\cdot} - X_{i21\cdot\cdot} - X_{i01\cdot\cdot}) - (2X_{i10\cdot\cdot} - X_{i20\cdot\cdot} - X_{i00\cdot\cdot}).$$

For temperature-dependent heterosis to be mainfest, this contrast must have a positive value.

To test the hypothesis that this contrast was significantly different from zero, the following mean square (M.S.) was required:

$$\text{M.S. } (H_i) = (H_i)^2/180.$$

To test whether or not there was temperature-dependent heterosis when all four sets were considered, the following contrast and mean square were used:
Contrast:

$$H. = H_1 + H_2 + H_3 + H_4.$$

Mean square:

$$\text{M.S. } (H.) = (H.)^2/720.$$

The contrast and mean square to test whether or not the temperature-dependent heterosis was the same for the two Australian sets were:
Contrast:

$$H_A = (H_2 - H_1).$$

Mean square:

$$\text{M.S. } (H_A) = (H_A)^2/360.$$

The contrast and mean square to test whether or not the temperature-dependent heterosis was the same for the two United States sets were:
Contrast:

$$H_U = (H_4 - H_3).$$

Mean square:

$$\text{M.S. } (H_U) = (H_U)^2/360.$$

Finally, the contrast and mean square to test whether or not the temperature-dependent heterosis in the Australian sets was the same as that in the United States sets were:

Contrast:

$$H_{AU} = (H_4 + H_3 - H_2 - H_1).$$

Mean square:

$$\text{M.S. } (H_{AU}) = (H_{AU})^2/720.$$

Treatments T_1, T_2, and T_3 utilized all sets of genotypes and, therefore, all of the above contrasts and tests were applied to the treatment combinations C_1T_1, C_2T_2, and C_3T_3. However, only set No. S_4 was used in treatments T_4 and T_5. Therefore, only the temperature-dependent contrast (H_4) and its mean square was utilized in the analysis of treatment combinations C_2T_4, C_3T_5, T_4T_2, and T_5T_3.

III. Results

(a) *Expression of Heterosis at Different Temperatures*

The growth rates of inbreds and their reciprocal hybrids at each of nine temperature regimes are given in Table 3. For both groups the optimal growth temperature was in the range 33/28–36/31°C. The growth rates of the hybrids were superior to the parents at all temperatures and in particular at temperatures below 21/16°C and above 33/28°C (Fig. 1).

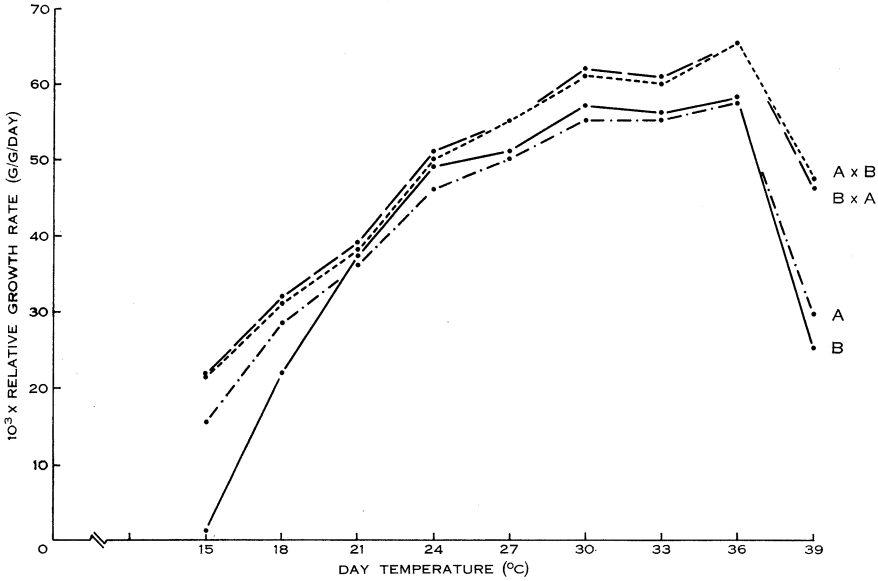

Fig. 1.—Relative growth rate of inbreds and their reciprocal hybrids over a range of temperatures. A = inbred G_{10}; B = inbred G_{12}. Night temperatures were 5 degC lower than day temperatures.

At these extreme temperatures the growth rates of all plants were reduced but the effect on the inbreds was far more severe. Throughout the temperature range there was little difference in the performance of the reciprocal hybrids. The inbreds, however, differed in their performance at both high and low temperatures, and particularly at temperatures below 21/16°C. The cause of this was the poor performance of inbred G_{12} which was chlorotic at 18/13°C and failed to survive beyond the three-leaf stage at 15/10°C. The performance of G_{12} was atypical in this respect, as 10 unrelated inbreds, subsequently tested over this temperature range, showed only mild chlorotic symptoms below 18/13°C and all survived at 15/10°C, although the growth rate was much reduced. All plants failed when the temperature was reduced to 12/7°C, although the hybrids made some growth and survived for a longer period than the inbreds. An examination of inbreds growing at temperatures between 18/13–15/10°C showed a general chlorophyll deficiency; however, it was more severe in the case of G_{12}, and was associated with a high proportion of abnormal chloroplasts.

TABLE 3

AVERAGE VALUES FOR RELATIVE GROWTH RATE* OF INBREDS AND HYBRIDS, AND OF HETEROSIS ($\bar{F}_1 - MP$) AT EACH TEMPERATURE

Genotypes	$10^3 \times$ Relative Growth Rate (g/g/day)								
	15/10 °C	18/13 °C	21/16 °C	24/19 °C	27/22 °C	30/25 °C	33/28 °C	36/31 °C	39/34 °C
$A(G_{10})$	15·37±0·51	28·57±0·25	36·35±0·28	46·24±0·40	50·38±0·36	55·68±0·36	54·67±0·67	57·74±0·62	29·56±0·74
$B(G_{12})$	1·57±0·32	22·35±0·72	37·07±0·26	49·43±0·38	51·13±0·42	57·15±0·40	55·67±0·23	58·31±0·41	25·26±0·62
F_1 ($A \times B$)	21·75±0·38	31·44±0·21	38·42±0·32	50·35±0·31	54·90±0·23	61·09±0·23	59·88±0·16	65·47±0·22	48·20±0·48
F_1 ($B \times A$)	21·99±0·30	32·14±0·23	39·03±0·24	50·96±0·33	54·98±0·24	62·24±0·27	60·77±0·20	65·51±0·26	47·10±0·45
($\bar{F}_1 - MP$)	13·39±0·39	6·33±0·42	2·02±0·27	2·83±0·36	4·19±0·33	5·25±0·32	5·16±0·38	7·47±0·41	20·24±0·58

* Relative growth rate $R = 1/t \log_e (w_t/w_0)$.

The heterotic response ($\bar{F}_1 - MP$) has been plotted over the entire temperature range in Figure 2. At low temperatures, below 21/16° C, there was a significant increase in the degree of heterosis by comparison with that displayed at optimum temperatures. This additional heterosis was accentuated by the poor performance of G_{12} at these low temperatures. At 21/16° C all plants were normal in appearance and displayed the usual level of heterosis exhibited by hybrid maize. This level of heterosis increased slightly at temperatures above 24/19° C but thereafter remained relatively unchanged up to 33/28° C. Above 33/28° C there was a highly significant increase in the level of heterosis.

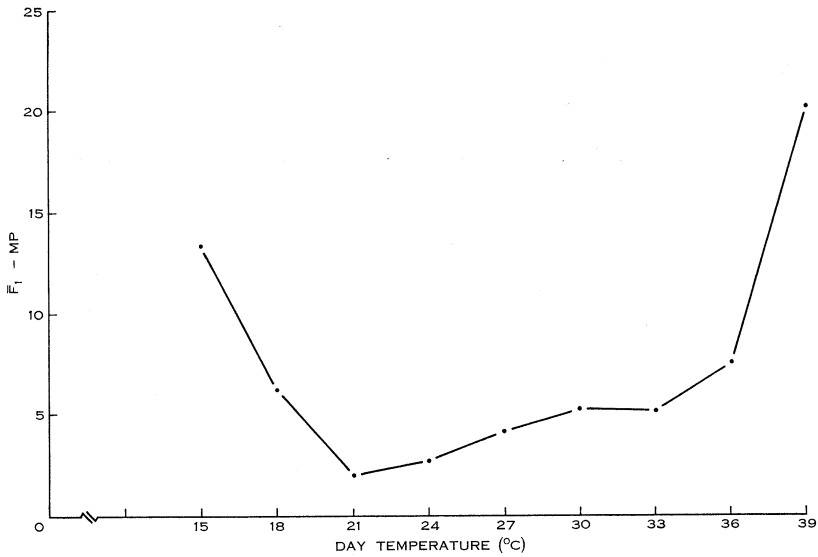

Fig. 2.—Heterosis expressed as growth rate ($\bar{F}_1 - MP$) plotted over the temperature range. All values $\times 10^3$. Night temperatures were 5 degC lower than day temperatures.

(*b*) *Expression of Heterosis following Heat Shocks of Long Duration*

(i) *Experiment 1.*—Plants were harvested after each heat shock to measure the cumulative effect of a succession of heat shocks of long duration. The results of this experiment are illustrated in Figure 3.

Following the first heat shock there was an increase in heterosis but this increase was not significantly different from the controls. Following a second and third heat shock, however, there was a marked increase in the temperature-dependent component of heterosis. These cumulative responses of two and three shocks produced a highly significant effect over no shocks.

(ii) *Experiment 2.*—All plants were harvested immediately after the final heat shock period. These exposures to single or multiple heat shocks, given for different intervals during the treatment period, provided further evidence for the cumulative effect of such treatments. Also they provided a comparison of the effect of heat

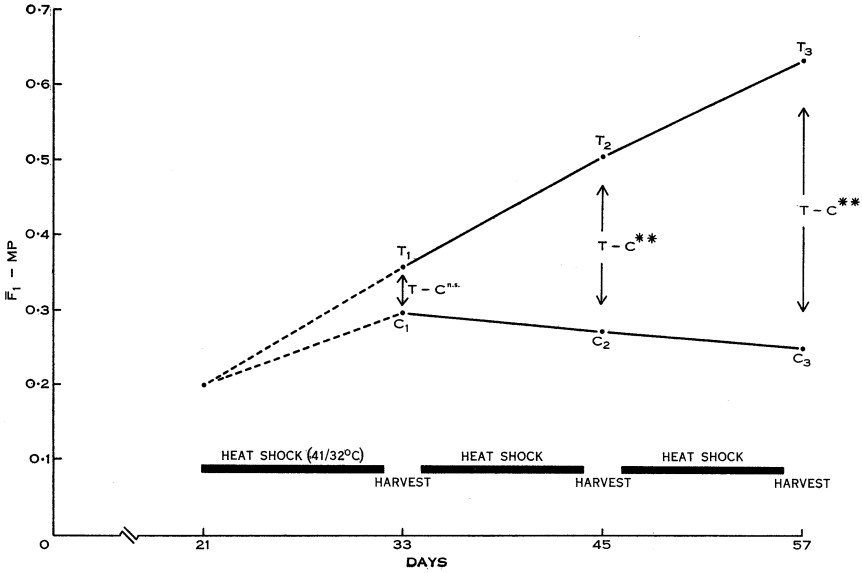

Fig. 3.—Cumulative effect of successive heat shocks of long duration (41/32 °C) on the expression of heterosis ($\bar{F}_1 - MP$). Values based on log(dry weight of tops).

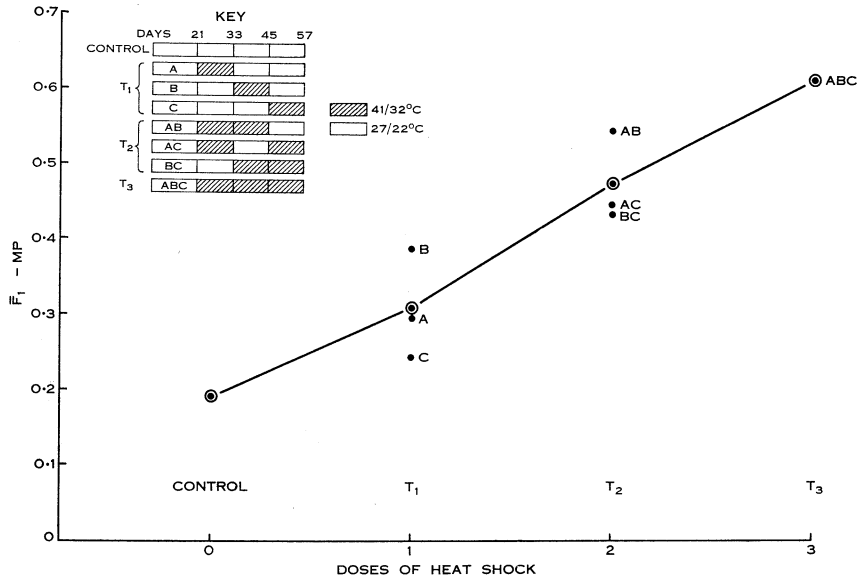

Fig. 4.—Effect of single or multiple heat shocks of long duration (41/32 °C) given at different stages of development. Values based on log(dry weight of tops); solid line joins means of each heat-shock treatment.

shocks applied at different stages during the development of the plants. The results of these treatments are given in Table 4 and the analyses of the data in Table 5.

TABLE 4

AVERAGE VALUES (FOR LOG DRY WEIGHT) OF MID-PARENTS AND F_1'S FOLLOWING HEAT-SHOCK TREATMENTS OF LONG DURATION

	Log Dry Weight (g)							
	Control	Heat-shock Treatment T_1			Heat-shock Treatment T_2			Heat-shock Treatment T_3
		A*	B*	C*	AB	AC	BC	ABC
MP	1·502	1·188	1·070	1·253	0·672	0·868	0·848	0·473
$\bar{F}_1$	1·691	1·486	1·458	1·496	1·217	1·316	1·283	1·089
$\bar{F}_1 - MP$	0·189	0·298	0·388	0·243	0·545	0·448	0·435	0·616
F_1 ($G_{10} \times G_{12}$) − F_1 ($G_{12} \times G_{10}$)	−0·079	−0·122	−0·054	+0·078	−0·085	−0·006	−0·123	−0·143

* A, B, C, heat shock during periods 1, 2, and 3, respectively (see Table 1).

TABLE 5

ANALYSIS OF VARIANCE (FOR LOG DRY WEIGHT) OF MAIZE HYBRIDS AND INBREDS FOLLOWING EXPOSURE TO A RANGE OF HEAT-SHOCK TREATMENTS

Source	Degrees of Freedom	Mean Square
A (first treatment period)	1	0·6566***
B (second treatment period)	1	0·9045***
C (third treatment period)	1	0·3436***
G_Q†	1	1·2494***
G_R‡	1	0·0169**
A × G_Q§	1	0·0531***
A × G_R‖	1	0·0014
B × G_Q	1	0·0812***
B × G_R	1	0·0002
C × G_Q	1	0·0130**
C × G_R	1	0·0020
Error	160	0·0015

** $0·001 < P < 0·005$. *** $P < 0·001$.

† G_Q, quadratic contrast among genotypes ($\bar{F}_1 - MP$).

‡ G_R, contrast among reciprocal F_1's.

§ A × G_Q, measures temperature-dependent heterosis when heat shock is applied in A.

‖ A × G_R, measures temperature-dependent reciprocal effects when heat shock is applied in A.

As found in the previous experiments, increasing the number of heat shocks caused an almost linear increase in the average level of temperature-dependent heterosis when plotted on a logarithmic scale (Fig. 4). By varying the timing of the single or double heat shocks with respect to the harvest date, it was possible to influence the magnitude of the additional heterosis. The response was greater in those treatments (for example T_1A, T_1B, T_2AB) which received a 12-day recovery period before harvest by comparison with those receiving a similar duration of heat shock (e.g. T_1C, T_2BC, T_2AC) without a recovery period. The same effect can be seen in experiment 1: one shock with a harvest immediately after the treatment gave a non-significant increase in heterosis, whereas in experiment 2 the same shock treatment harvested after a recovery period gave a highly significant increase.

TABLE 6

NUMERICAL VALUES FOR TEMPERATURE-DEPENDENT HETEROSIS AND OTHER CONTRASTS FOR VARIOUS SHORT DURATION HEAT-SHOCK COMBINATIONS

Values based on log (dry weight of entire plant)

Contrast Designation	Treatment Contrasts†						
	C_1 T_1	C_2 T_2	C_3 T_3	C_2 T_4	C_3 T_5	T_4 T_2	T_5 T_3
H_1	−0·049	1·800	7·438**				
H_2	3·560*	1·888	6·510**				
H_3	−1·754	0·981	7·373**				
H_4	0·857	3·776**	8·451**	1·751	0·162	2·025	8·289*
$H_.$	2·614	8·445**	29·772**				
H_A	3·609	0·088	−0·928				
H_U	2·611	2·795	1·978				
H_{AU}	−4·408	1·069	1·876				

* Significant at the 5% level. ** Significant at the 1% level.

† Degrees of freedom for error mean square are:

C_1T_1, C_2T_2, C_3T_3 = 288. C_2T_4, C_3T_5, T_4T_2, T_5T_3 = 70 (approx.).

This suggests that the hybrids not only grow faster than the inbreds during the high-temperature treatment, but also recover more rapidly following the treatment period.

All heat shocks given at any of the three stages during the 36-day growth period gave a significant temperature-dependent heterotic response (Table 5). Of the three treatments the third shock was the least effective presumably because of the absence of a recovery period. There was in addition a significant reciprocal effect between hybrids, but this difference was not temperature-dependent.

(c) *Expression of Heterosis following Heat Shocks of Short Duration*

The results are summarized in Table 6.

Following a single heat shock (C_1T_1 contrast) only two out of the four sets showed a temperature-dependent heterotic effect. Of these, only that due to set No. S_2 was significant. The average effect over all sets was not significant.

With two heat shocks (C_2T_2 contrast) all sets gave a temperature-dependent heterotic response although only set No. S_4, taken by itself, yielded a significantly large value. The value averaged over all sets was significant.

All sets of genotypes yielded highly significant temperature-dependent heterotic responses when exposed to three successive heat shocks (C_3T_3 contrast).

There was no apparent difference between sets either within or between places of origin.

The results for all three heat shocks, with respect to one of the American sets (S_4), are illustrated in Figure 5.

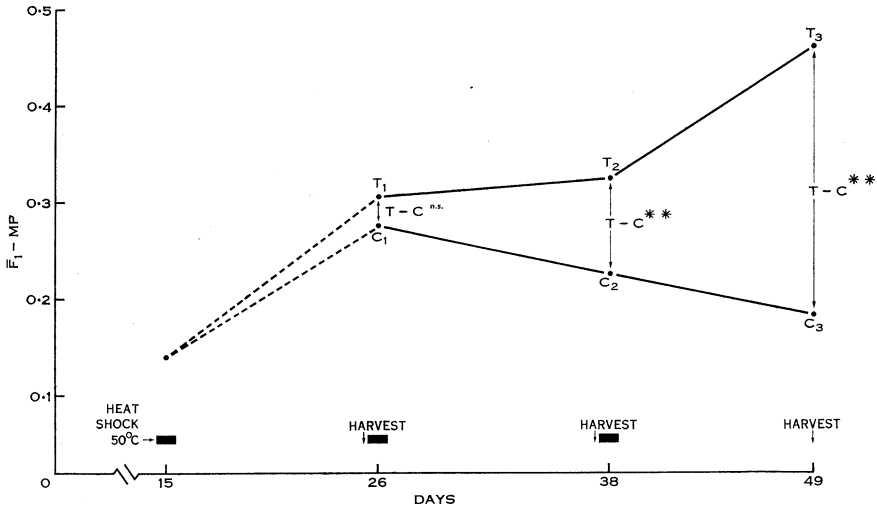

Fig. 5.—Cumulative effect of a succession of heat shocks of short duration (50 °C) on the expression of heterosis ($\bar{F}_1 - MP$). Values for S_4 only, based on log(dry weight of entire plant).

One shock followed by an extended post-shock growing period (comparisons C_2T_4 and C_3T_5) produced a temperature-dependent heterotic effect, though it was not sufficiently great to be significant. This result agrees with the previous single-shock comparison and also shows that the heat shock does not produce a delayed detrimental effect.

If the first treatment is followed by one or two more heat shocks (comparisons T_4T_2,T_5T_3), the second shock produces a further increase, though not significantly great, and the third shock produces an additional highly significant response, over one shock. This cumulative response of all three shocks also produces a highly significant effect over no shocks compared with the untreated control, as shown in the C_3T_3 contrast.

IV. Discussion

The experimental evidence reported in this paper consistently showed that the level of heterosis demonstrated by hybrid maize is strongly temperature-dependent. To explain this pattern of heterosis over the wide range of temperatures used it is necessary to postulate the action of a number of gene systems.

Firstly within the temperature range for normal growth of both inbreds and hybrids (21/16–33/28° C), the level of heterosis remained relatively constant, and is determined largely by the effect of the deleterious recessive growth genes accumulated in the homozygotes.

At temperatures below 21/16° C there was a marked increase in the level of heterosis. This increase was significant even without the inclusion of G_{12}, which was somewhat atypical in its reaction to low temperature. The basis for this low-temperature heterosis appears to be attributable largely to the presence of mutant genes in the homozygotes restricting the full formation of chlorophyll. In extreme cases, as with G_{12}, low temperatures appear to be preventing the full formation of chloroplasts rather than blocking synthesis of chlorophyll molecules. This situation has also been found in tomatoes grown at low root temperatures (Shakhov and Golubkova 1960).

When grown in fluctuating temperatures, the existence of additional heterosis at high temperature supports the finding of Griffing and Langridge (1963), that at temperatures above the apparent growth optimum for the species the amount of heterosis becomes progressively larger. The genetic basis for this observed temperature-dependent heterosis may be the random fixation of high-temperature-sensitive alleles in the inbreds (Langridge 1962).

When inbreds and hybrids of maize were grown at high temperatures, the growth of the inbreds was retarded at temperatures much lower than those which caused retardation of growth in hybrids. The hybrids germinated and grew quite actively at the highest growth temperature used (39/34° C), but the inbreds at this temperature showed evidence of high-temperature lesions, and in a test at an even higher temperature (42/37° C) they did not survive. Thus the striking feature of the hybrids when compared with the inbreds was the greater phenotypic stability exhibited by hybrids over the entire temperature range.

High-temperature shocks of long or short duration also resulted in additional heterosis, and there was little difference in the response to either form of shock treatment. Also repeated heat shocks were cumulative in their effect, and were effective when applied over a wide range of growth stages.

In his studies with *Arabidopsis*, Langridge (1963*b*) found that other environmental stresses (including high-moisture tension) were not so effective in producing heterosis. In the present experiments precautions were taken to minimize factors limiting growth, including moisture stress.

Much of the injury sustained during a brief or prolonged exposure of plants to supra-optimal temperatures is thought to occur because of the inactivation of thermolabile enzymes (Langridge 1963*a*). In this study with maize, the high-temperature-dependent heterosis appears to be a consequence of the fixation of alleles in the homozygote which induce the formation of temperature-unstable enzymes. In the hybrid the effect of dominance leads to thermostable enzymes at these same loci.

In conclusion the experimental results of this study support and enlarge the hypothesis under consideration, in that the higher yields of hybrids were due to greater

phenotypic stability over the *entire* temperature range. In genetic terms this phenotypic stability was due to both high- and low-temperature-dependent heterosis. The high-temperature-dependent heterosis was manifest because the hybrids grew relatively better than the inbreds at high temperatures and also because the hybrids appeared to recover from the heat treatments more rapidly than did the inbreds.

V. Acknowledgments

We wish to acknowledge the competent assistance of Mrs. Elizabeth Zsiros and Mr. H. Schroeder. We are also grateful to Dr. J. Anderson for conducting chlorophyll analyses, and to Mr. P. Grogan, Queensland Agricultural College, Lawes, for supplying seed of the various inbred and hybrid lines of maize. This investigation was supported in part by the United States Public Health Service Grant G.M.10032–01.

VI. References

Griffing, B., and Langridge, J. (1963).—Phenotypic stability of growth in the self-fertilized species *Arabidopsis thaliana*. Statist. Genet. Plant Breed. NAS–NRC Publ. No. 982. pp. 368–94.

Langridge, J. (1962).—Genetic and molecular basis for heterosis in *Arabidopsis* and *Drosophila*. *Am. Nat.* **96**: 5–27.

Langridge, J. (1963*a*).—Biochemical aspects of temperature response. *A. Rev. Pl. Physiol.* **14**: 441–62.

Langridge, J. (1963*b*).—The genetic basis of climatic response. In "Environmental Control of Plant Growth". Ch. 20. pp. 367–79. (Academic Press Inc.: New York.)

Lewis, D. (1955).—Gene interaction, environment and hybrid vigour. *Proc. R. Soc.* B **144**: 178–85.

Morse, R. N., and Evans, L. T. (1962).—Design and development of Ceres—an Australian phytotron. *J. Agric. Engng. Res.* **7**: 128–40.

Shakhov, A. A., and Golubkova, B. M. (1960).—An electron microscope study of chloroplasts of plants with root systems kept at low temperatures. *Dokl.* (*Proc.*) *Acad. Sci. USSR Bot. Sci. Sect.* (Eng. Transl.) **135**: 259–62.