ENHANCED HETEROSIS AND STABILITY IN THE GROWTH OF AN INTERSPECIFIC *PHALARIS* HYBRID AT HIGH TEMPERATURE

By J. R. McWilliam,* B. D. H. Latter,* and M. J. Mathison†

[*Manuscript received November* 1, 1968]

Summary

Interspecific hybrids between a range of ecotypes of *Phalaris tuberosa* and *Phalaris arundinacea* were grown with the parental species in controlled environments over a range of temperatures from 10 to 35°C.

Heterosis was strongly dependent for its expression on the temperature at which the plants were grown, maximum heterosis being found at temperatures well above the growth optimum. This superiority of the hybrids at high temperature is largely a function of their greater phenotypic stability under stress.

A growth analysis revealed no significant heterosis in net photosynthesis, but indicated that the greater total photosynthetic area of the hybrids was a factor contributing to their superiority over the parents in relative growth rate and total dry weight at high temperature.

A genetic hypothesis to explain differential heterosis over a range of temperatures based on the accumulation of alleles specifying heat-stable enzymes is discussed.

I. Introduction

There is now considerable evidence in both self- and cross-fertilized species that heterozygotes exhibit greater phenotypic stability than homozygotes when exposed to a spectrum of environmental conditions. This additional stability, or "individual buffering" of heterozygotes (Allard and Bradshaw 1964), has been demonstrated in a number of plants including tomatoes (Lewis 1955), maize (Shank and Adams 1960), wheat (Palmer 1952), lima beans (Allard and Workman 1963), *Mimulus* (Hiesey 1963), and tobacco (Bucio Alanis and Hill 1966). Evidence for population buffering in heterozygous material comes mainly from the data of Sprague and Federer (1951) and Jones (1958) who showed that double crosses in maize are more stable than single crosses over a range of diverse environments. The work of Allard (1961) with lima beans and Finlay (1963) with barley also indicates that complex hybrid mixtures in self-pollinating species are higher yielding and more stable than their parents over a range of environments.

There is evidence that buffering can be a property of specific genotypes, either homozygous or heterozygous (Finlay and Wilkinson 1963; Griffing and Langridge 1963; Johnson, Shafer, and Schmidt 1968; Pederson 1968), and that some of this variation is additive genetic and responds to selection (Griffing and Langridge 1963; Scott 1967).

* Division of Plant Industry, CSIRO, P.O. Box 109, Canberra City, A.C.T. 2601.

† Department of Agriculture, Box 901E, G.P.O., Adelaide, S.A. 5001.

Much of the advantage claimed for both heterogeneous and heterozygous populations appears to result from their superiority in stress environments (Finlay 1963; Frey and Maldonado 1967). In particular, higher temperatures have been shown to enhance heterozygote superiority in *Arabidopsis* (Griffing and Langridge 1963; Pederson 1968) and in maize (McWilliam and Griffing 1965), largely because of the ability of the hybrids to tolerate supra-optimal temperatures.

In this study we have examined the productivity and stability of an interspecific hybrid from the cross *Phalaris tuberosa* L. ($2n = 28$) $\times$ *Phalaris arundinacea* L. ($2n = 42$) when grown over a wide range of controlled temperatures. The successful hybridization of these two cross-pollinated perennial grasses to yield a vigorous sterile F_1 hybrid ($2n = 35$) has been described previously (McWilliam 1962). The hybrid combines the seasonal growth rhythm of both parents, and under field conditions displays wide adaptation and heterosis for autumn and summer growth.

The availability of a range of ecotypes of both species, and the ease of hybridization, provided an opportunity to make a study of genotype–environment interactions involving both heterosis and buffering capacity (stability), at a more complex level of heterozygosity than has been attempted previously with plants.

II. Materials and Methods

(a) *Plant Material*

Crosses between ecotypes of the two perennial grasses *P. tuberosa* and *P. arundinacea* were made in a systematic fashion as indicated in Figure 1. The five ecotypes of *P. tuberosa*, each represented by 10 plants, were selected to represent the range of variation present in this Mediterranean species (Cooper and McWilliam 1966). Similarly five ecotypes of *P. arundinacea* were sampled from a collection of hexaploid introductions from southern Europe and the United States of America (McWilliam and Neal-Smith 1962).

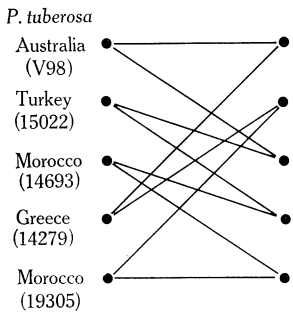


Fig. 1.—Origin and CPI numbers (in parentheses) of ecotypes used for hybridization. *P. arundinacea* was used as the pollen parent, and each ecotype was crossed to two other *P. tuberosa* ecotypes. The direction of the crosses are indicated by the solid lines.

F_1 hybrid seed was obtained from controlled crosses between individual plants of each species. Seed representative of each ecotype was also obtained from crosses among the 10 genotypes making up each ecotype. To achieve crosses between early and late flowering ecotypes, flowering times were synchronized by manipulating temperature and photoperiod. In all interspecific crosses *P. arundinacea* was used as the pollen parent, and all plants were either heterozygous or homozygous for a single dominant gene marker (red root tip; McWilliam and Shepherd 1964). As all the *P. tuberosa* parents lacked the gene, rapid and positive identification of the hybrids was possible.

Equal weights of seed from individual crosses within and between ecotypes of the two species were bulked to give a total of 20 populations made up of 10 F_1 hybrids, and 5 of each parental species. Seed of all populations was germinated and grown under controlled conditions in the Canberra phytotron.

(*b*) *Growth Analysis*

Seedlings were grown singly in 4-in. pots in a mixture of perlite and vermiculite, and irrigated daily with a modified Hoagland nutrient solution. One or two additional irrigations with water were given each day at the higher temperatures to prevent any development of water stress. Initially all plants were grown in short days (8 hr) at a constant temperature of 21°C in growth cabinets, at 3000 f. c. under artificial light supplied by fluorescent and incandescent lamps (total incident energy of 15 cal/cm^2/hr). Twenty-one days after sowing (third leaf stage) lots of 20 seedlings from each of the 20 hybrid and parent populations were transferred to each of six constant temperatures (10, 15, 20, 25, 30, and 35°C). These were provided in humidity-controlled growth cabinets using the same non-inductive day length (8 hr), with light conditions as described previously, and with an average moisture deficit of 4–8 g m^3 maintained over all temperatures. All pots in the growth cabinets were completely randomized every 48 hr to eliminate any possibility of a position effect.

After a period of preconditioning which varied from 2 days at the highest temperature to 10 days at the lowest, the plants in each population were carefully paired and half were harvested at a stage corresponding to the mean time of appearance of the fourth leaf. The remaining plants were grown for a further period varying from approximately 3 to 6 weeks, depending on the growth temperature, and were harvested at the same average state of development, viz. the appearance of the eighth leaf on the main shoot. Roots and shoots were harvested separately and oven-dried at 80°C. Leaf areas were determined using an air-flow planimeter (Jenkins 1959) calibrated for the range of leaf areas encountered. From these data and from measurements made during the experiment the following growth characters were calculated: relative growth rate (mg/mg/day); $\log_e$[total final weight (mg)]; rate of leaf area appearance (leaves/day); area of leaves 5, 6, and 7 on the main shoot (cm^2), and tiller number at the final harvest.

(*c*) *Photosynthesis and Respiration*

Plants involved in the growth analysis were also used to measure rates of net photosynthesis and dark respiration. Measurements at each temperature were made on fully adapted plants which had been grown throughout the experiment at that temperature.

Over most of the temperature range (15–30°C) measurements were made by enclosing the fully expanded sixth leaf on the main shoot in a Perspex assimilation chamber. A group of four plants was measured at the same time, and two such groups were sampled from each parental and hybrid population. At the extreme temperatures (10 and 35°C), because of the difficulty associated with the smaller leaves, measurements were made on entire plant tops which were sealed in an assimilation chamber. Light intensity at all temperatures was 3000 f.c. Net photosynthesis was measured as the differences in CO_2 concentration between air entering and that leaving the assimilation chamber using an infrared gas analyser sensitive to 1 p.p.m. CO_2. The air renewal rate was 4 litres/min for single leaves and 8 litres/min for entire plants, and the CO_2 depletion in the assimilation chambers was usually less than 40 p.p.m. Rates of net photosynthesis and dark respiration were calculated in milligrams of CO_2 per square decimetre of leaf surface per hour from the measured air renewal rate, CO_2 uptake, and enclosed leaf area.

III. Results

(*a*) *Growth Analysis*

(i) *Leaf Growth*

The total area of leaves 5, 6, and 7 on the main shoot (leaf area) and their rate of appearance at each of six temperatures is illustrated in Figures 2(*a*) and 2(*b*).

For both characters there was a temperature optimum around 25°C and a marked reduction in performance at both temperature extremes. At temperatures above 15°C the hybrids produced a significantly higher mean leaf area than the parents, which were similar over most of the temperature range. The rate of leaf appearance

in the hybrids was also greater than in *P. tuberosa* at all temperatures, but was similar to *P. arundinacea* over the range from 20 to 30°C, and only exceeded it at the temperature extremes.

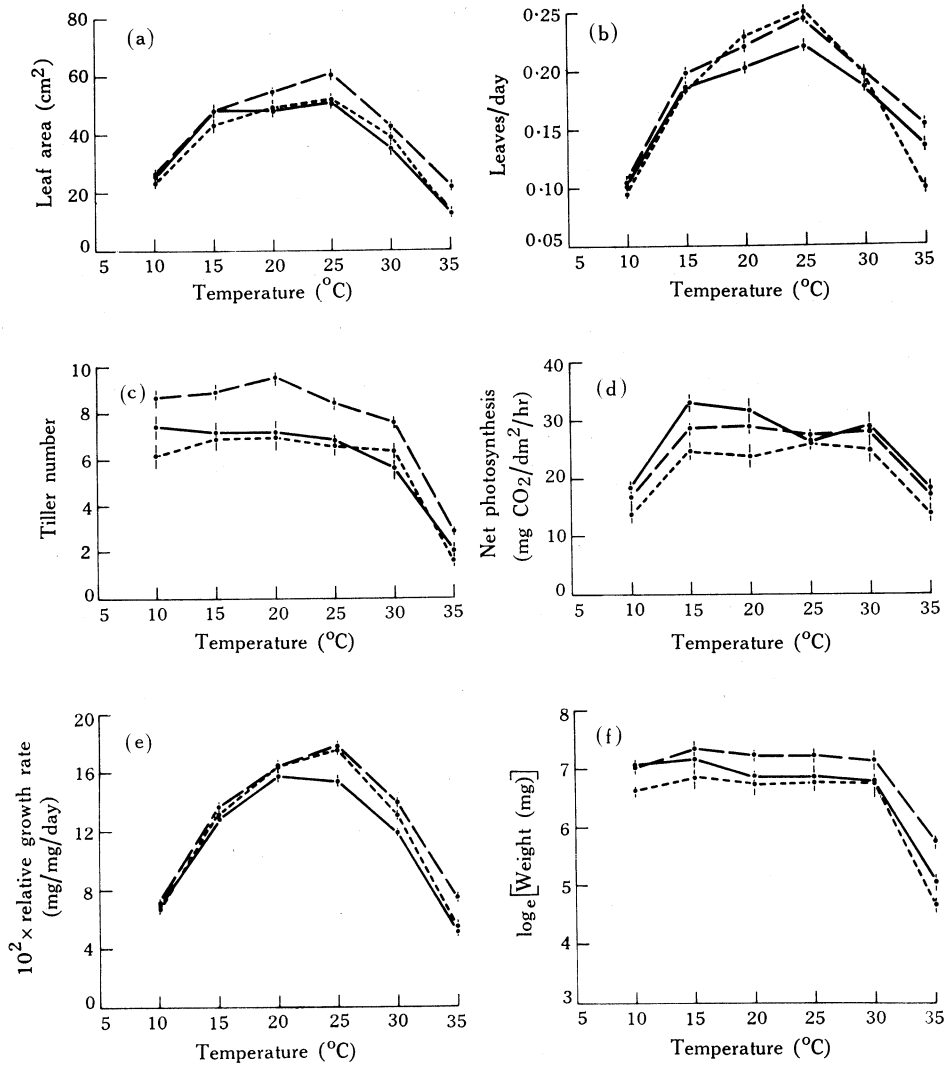


Fig. 2.—Mean performance of parental and hybrid populations for six growth characters measured over a range of constant temperatures from 10 to 35°C. (*a*) Leaf area. (*b*) Rate of leaf appearance. (*c*) Tillering. (*d*) Net photosynthesis. (*e*) Relative growth rate. (*f*) Final weight. Confidence intervals of $\pm 2 \times$S.E. are shown for each mean value. ——— *P. tuberosa*. - - - *P. arundinacea*. — — — Hybrid.

(ii) *Tiller Production*

The hybrids produced a higher number of tillers than the parents at all temperatures as indicated in Figure 2(*c*). All populations also showed a similar decrease

in tillering capacity at higher temperatures, which is consistent with the tillering pattern of temperate grasses (Mitchell 1956; Cooper and McWilliam 1966).

(iii) *Photosynthesis*

Rates of net photosynthesis for parental and hybrid populations are shown in Figure 2(*d*). Assimilation in all populations was reduced at both 10 and 35°C by comparison with the rates at intermediate temperatures. This reduction is possibly attributable to the effects of temperature stress at these extremes, but it is also a reflection of the lower rates of photosynthesis measured for whole plants, by comparison with rates based on single leaves as used at all other temperatures.

The highest rates of net photosynthesis were recorded for *P. tuberosa* and the lowest for *P. arundinacea*. These differences were significant over most of the temperature range. The rates for the hybrids were generally intermediate although at the higher temperatures they were not significantly different from those obtained for *P. tuberosa*. The absence of any marked superiority of the hybrids at 35°C distinguishes photosynthesis from the other growth characters studied.

Dark respiration rates increased with increasing temperature as expected, but differences between the hybrids and parents were small and mostly non-significant.

(iv) *Growth Rate and Final Yield*

Curves for relative growth rate and final dry weight based on whole plants (roots+shoots) are shown in Figures 2(*e*) and 2(*f*). Growth over the range of temperatures followed the usual pattern for temperate grasses, with an optimum in the vicinity of 25°C and a marked reduction at both high and low temperatures.

Over the temperature range from 10 to 20°C there was little difference in the relative growth rates of all three populations. The hybrids maintained a higher rate but the differences were smaller and barely significant. Above 20°C the hybrids maintained a significantly higher growth rate, particularly at 30 and 35°C. The growth rate of *P. arundinacea* was also superior to *P. tuberosa* at 25 and 30°C.

The faster growth rate of the hybrids is also reflected in the greater total production of dry matter (eighth leaf stage) at all temperatures above 10°C. Comparisons between populations at different temperatures are not particularly meaningful, because the duration of the growth period at each temperature varied. However, comparisons of total yield in milligrams at any given temperature show that the hybrids produced from 30 up to 50% more dry matter than the mean of the parents over the temperature range 15–30°C, and 130% more at 35°C.

(*b*) *Heterosis*

A quantitative measure of hybrid performance for all growth characters at each temperature has been calculated as the difference between the mean performance of the hybrid and the mid-parent ($F_1 - MP$), expressed as a percentage of the mid-parent value at each temperature (Fig. 3). This gives a measure which is independent of the magnitude of the growth response and for the purpose of this paper is referred to as heterosis. A similar pattern of heterosis was obtained when the hybrid difference

was based on the high-parent rather than the mid-parent, indicating that the method used provides a realistic measure of true heterosis.

Only two of the four growth characters (tiller number and leaf area) which were contributing to relative growth rate and final yield showed appreciable heterosis (Figs. 2 and 3). For leaf area the heterosis was significant at temperatures above

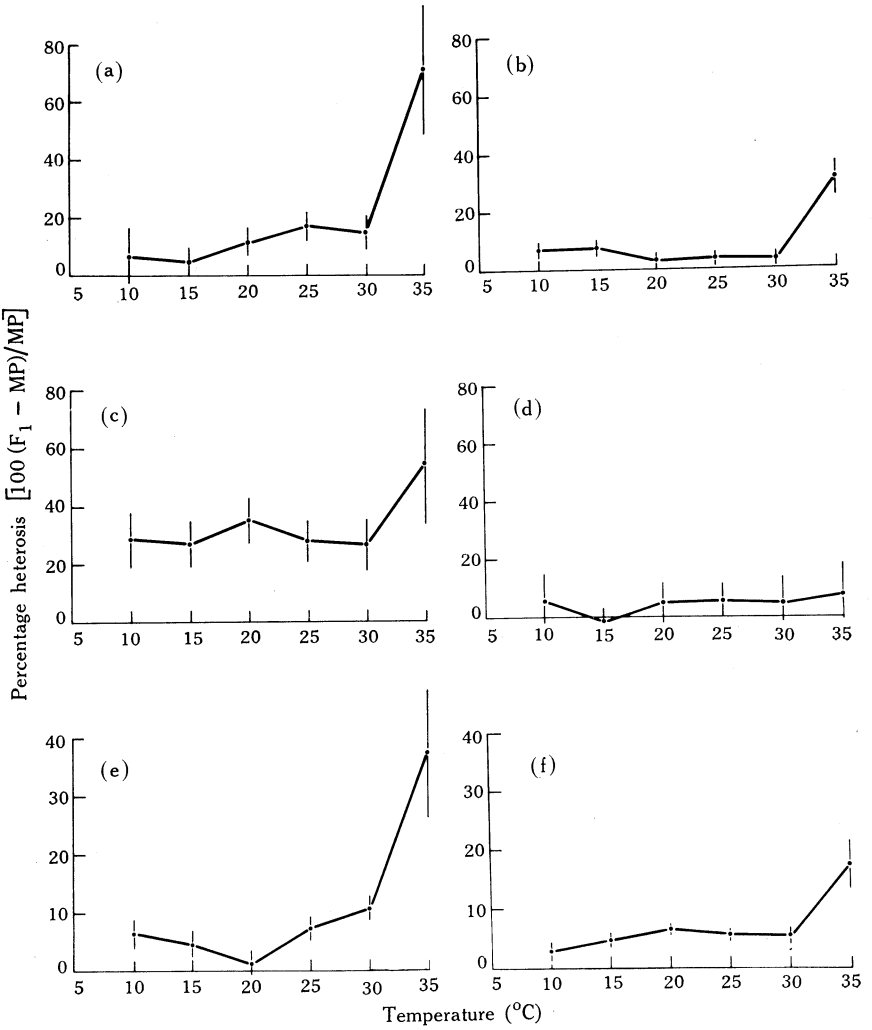


Fig. 3.—Relationship between hybrid performance and temperature as expressed for each of six growth characters. Relative hybrid performance is calculated as $\overline{F}_1 - MP$ and expressed as a percentage of the mid-parent value at each temperature. (*a*) Leaf area. (*b*) Rate of leaf appearance. (*c*) Tillering. (*d*) Net photosynthesis. (*e*) Relative growth rate. (*f*) Final weight. Confidence intervals of $\pm 2 \times$ S.E. are shown for each estimate.

15°C and increased with increasing temperature. The same pattern was true for tiller number, except that the level of heterosis was significant at all temperatures and the increase with temperature was confined to the response at 35°C. The rate of leaf

appearance showed little or no heterosis [Figs. 2(*b*) and 3(*b*)] except at the highest temperature. The results for net photosynthesis [Figs. 2(*d*) and 3(*d*)] were similar except that there was no significant heterosis even at the highest temperatures. Averaged over all temperatures, however, there was a small positive superiority of F_1 over mid-parent value averaging $4{\cdot}4 \pm 1{\cdot}6\%$.

The effect of heterosis in growth components such as leaf area and tiller number is reflected in heterosis for relative growth rate [Figs. 2(*e*) and 3(*e*)] expressed at temperatures above 20°C; relative hybrid performance showed a marked degree of temperature dependence, with a maximum response at 35°C. This pattern is also shown by final weight [Figs. 2(*f*) and 3(*f*)], though the use of the log scale greatly reduces the magnitude of the heterotic response.

(*c*) *Phenotypic Stability*

The stability of phenotypic expression of the various growth characters in parents and hybrids has been examined by comparing the variances of these populations at each of the six experimental temperatures. At most temperatures the variances associated with characters in the hybrid fell within the range of the parental variances (with the exception of tiller number which was more variable in the hybrid at all temperatures) and in only a few cases were the hybrids, on average, more stable than either parent.

At the highest temperature (35°C), however, when the plants were under considerable temperature stress, there was a more consistent pattern in the stability of the individual hybrid populations by comparison with the parents.

For characters such as leaf area and rate of leaf appearance, which show markedly increased heterosis at high temperature, the hybrids were as variable as *P. tuberosa*, but clearly more stable as a group than *P. arundinacea*. Tiller number in the hybrids, which also showed marked heterosis at 35°C, was as variable in the hybrids as in either parent. This applied also to net photosynthesis for which there was no heterosis expressed. Relative growth rate and total productivity in the hybrids, however, were more stable than in either parent under temperature stress, with the biggest difference being between the hybrids and *P. arundinacea*. These points are illustrated in Figures 4 and 5, in which the frequency distribution of relative growth rates and total weights of individual plants of the combined parental and hybrid populations are given at each of three temperatures.

The progressive increase in the hybrid mean relative to the parents with increasing temperature, and the greater stability of the hybrids at 35°C is apparent. At this temperature values for relative growth rate and for total weight of all hybrid and parental populations showed a negative correlation with the population standard deviation ($r = 0{\cdot}62$ and $0{\cdot}74$ respectively, both values being significant at the 1% level), indicating that those populations which are most depressed by high temperature are in general those which are the most variable under temperature stress.

(*d*) *Relationship between Parental and Hybrid Performance*

The extent of the correlation between the relative growth rate of individual parental populations and their hybrid progenies at different temperatures is shown

in Figure 6. For this purpose the mean of individual hybrids has been plotted against those of the respective mid-parents at each of four temperatures.

With respect to relative growth rate, there appears to be little relationship between the performance of parents and their hybrids. At all temperatures, including those not illustrated (15 and 30°C), the correlations were small and not significant.

Fig. 4

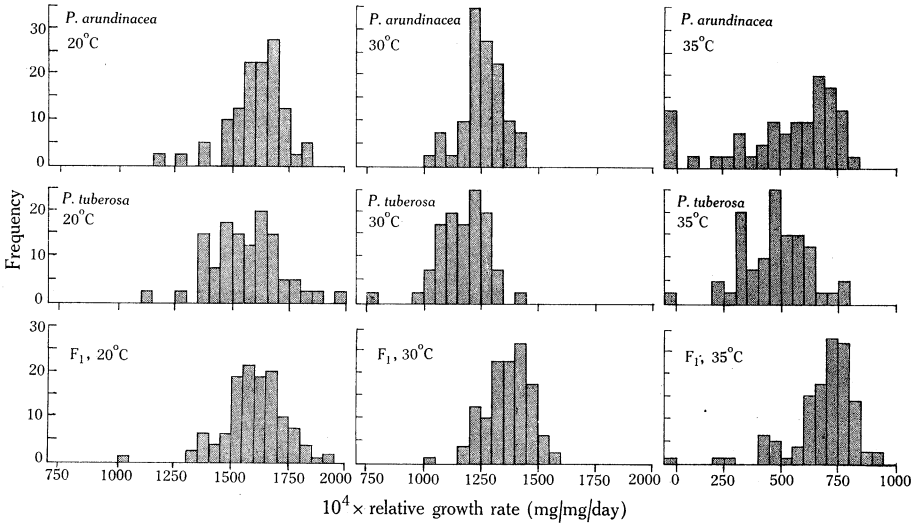
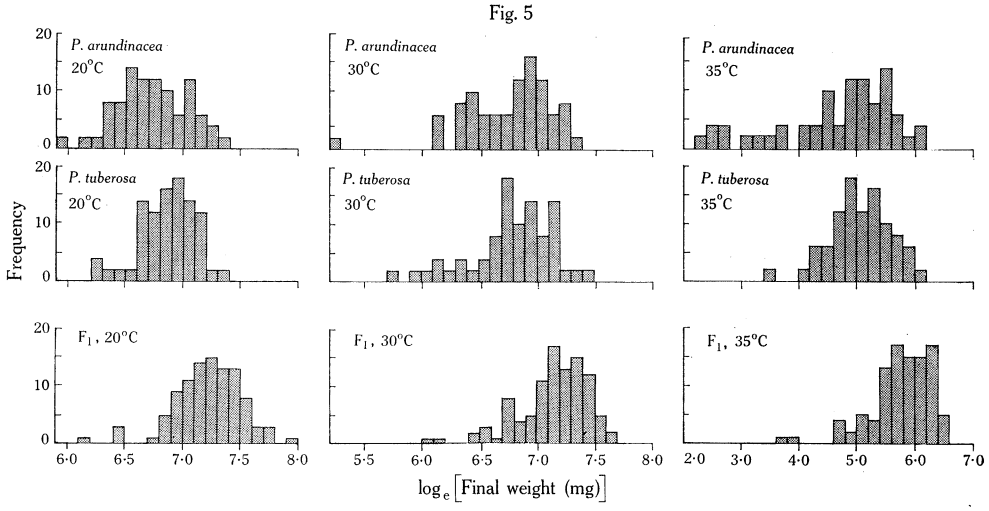


Fig. 5



Figs. 4 and 5.—Frequency distribution of $10^4 \times$ relative growth rates (Fig. 4) and total (final) weights (Fig. 5) for individual plants in parental and hybrid populations at optimum (20°C) and high temperatures (30 and 35°C).

This lack of correlation indicates quite clearly that genetic factors contributing to high parental performance are not necessarily those concerned with superior hybrid performance.

IV. Discussion

A striking feature of the present study is the superiority of the hybrids over the parents at 35°C by comparison with the relative performance of these groups at lower temperatures. This reflects the greater capacity of hybrids to tolerate the high temperature stress, and provides an explanation for the increased heterosis expressed by hybrids at these temperatures. Figures 4 and 5 indicate that the superiority of the hybrid populations under high temperature stress (35°C) is due largely to the individual buffering properties of the genotypes concerned.

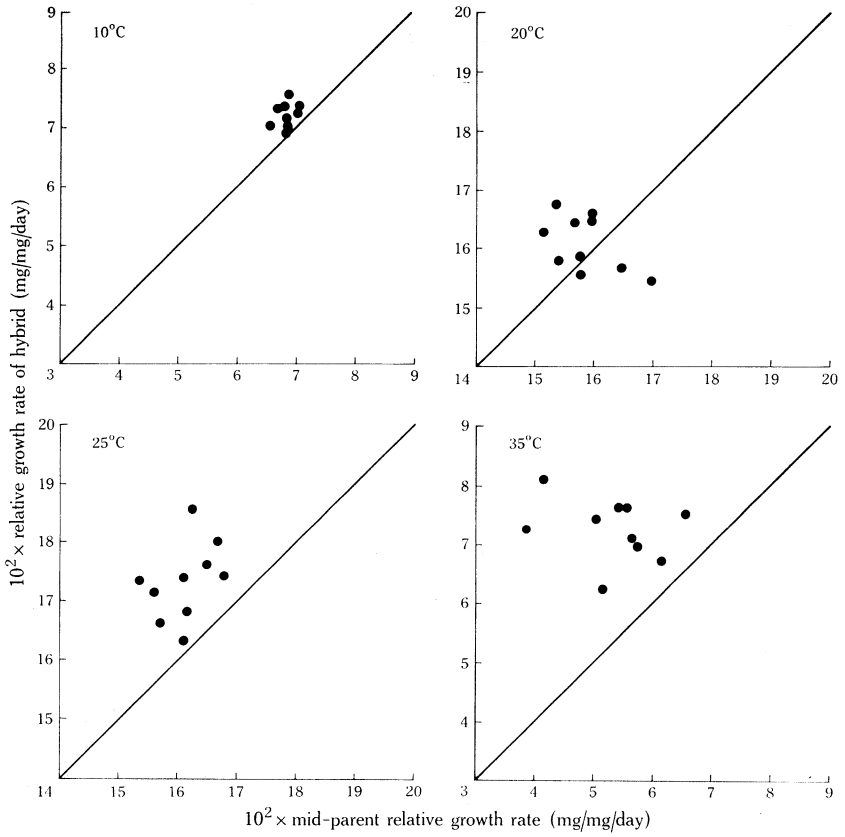


Fig. 6.—Relationship between mean relative growth rates of individual hybrid populations and those of the respective mid-parent values at each of four temperatures.

The hybrids show marked superiority in relative growth rate at high temperatures, and also in a number of other growth characters including tiller number, leaf area, and rate of leaf appearance on the main shoot. Mean leaf area (total area of the fifth, sixth, and seventh leaf on the main shoot) is itself closely correlated with relative growth rate (Fig. 7). Although it is not possible from this study to determine causal relationships, leaf growth is known to be one of the most important determinants of yield (Watson 1956), and it would appear that at least part of the

superiority of the hybrids at high temperature is due to their ability to develop a greater photosynthetic area under conditions of temperature stress. A similar conclusion has been reached by MacColl (1965) to explain the superior winter growth of Italian and H1 hybrid ryegrass.

Although this implies greater total assimilation per plant, there is no evidence for heterosis in the rate of photosynthesis per unit of leaf area. In fact, the data for net photosynthesis and its standard deviation within parental and hybrid sets indicates that this is probably not an important factor in hybrid superiority or stability under conditions of temperature stress. A similar conclusion has been reached by Miflin and Hageman (1966) who found no heterosis in the activity of chloroplasts isolated from various maize inbreds and hybrids.

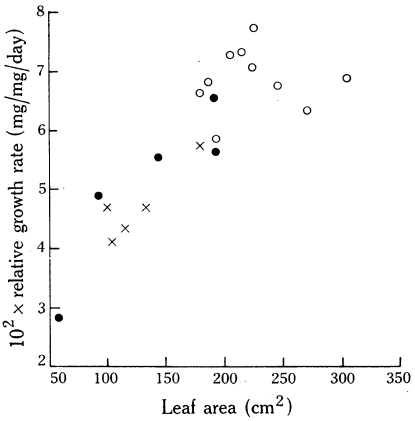


Fig. 7.—Relationship between relative growth rate and leaf area (leaves 5, 6, and 7 on the main shoot) in parental and hybrid populations at 35°C. ● *P. arundinacea*. × *P. tuberosa*. ○ F_1. Regression coefficient is 0·84 (significant at 1% level).

The observed pattern of response of parental and hybrid material under high-temperature stress is consistent with a genetic model involving partial dominance of alleles producing relatively heat-stable enzymes. The lack of conspicuous heterosis in relative growth rate around the optimal temperatures indicates selective neutrality of such alleles under favourable environmental conditions, and the independence of parental and hybrid performance under stress temperatures suggests an essentially random distribution of the more stable alleles among the parental ecotypes.

The pattern of temperature response in the interspecific hybrids and parental species in this study has a close parallel in the *Arabidopsis* experiments of Langridge (1962) and Griffing and Langridge (1963). There appears to be little evidence, therefore, to suggest that temperature-dependent heterosis is related to the level of heterozygosity of the parental material, which reinforces the suggestion that the phenomenon is to be explained in terms of the specific allelic constitution of the parents.

The present study differs from that of Griffing and Langridge (1963) in one important respect, viz. the magnitude of the reduction in performance of the hybrids at high stress temperatures by comparison with the better parental species. The temperature response in the hybrid for most characters, over the range from 30 to 35°C, is of the same order as that of the parent showing the least reduction in the mean (Fig. 1). The hypothesis proposed by Griffing and Langridge (1963) involved complete dominance of the more stable over the less stable allele in the heterozygote,

and such a model would only be acceptable for the present data if one parent always provided the more stable allele.

It is clear from Figure 4 that for relative growth rate at 35°C, *P. tuberosa* does not provide all the stable alleles to the hybrid populations, and that *P. arundinacea* shows a considerably greater genetic diversity in temperature stability than *P. tuberosa*. A model of partial dominance of the products of heat-stable alleles appears to be compatible with the observations recorded. This may well be close to expectation from molecular considerations since the high-temperature stress involved in this study is one which evidently causes many enzymes to be limiting, even in the hybrids.

V. Acknowledgments

We wish to acknowledge the competent assistance of Mr. H. E. Schroeder, and we are also indebted to the staff of the Phytotron for care of the plants, and for the provision and maintenance of the equipment.

VI. References

Allard, R. W. (1961).—Relationship between genetic diversity and consistency of performance in different environments. *Crop Sci.* **1**, 127–33.

Allard, R. W., and Bradshaw, A. D. (1964).—Implications of genotype–environment interactions in applied plant breeding. *Crop Sci.* **4**, 503–8.

Allard, R. W., and Workman, P. L. (1963).—Population studies in predominantly self-pollinated species. IV. Season fluctuations in estimated values of genetic parameters in lima bean populations. *Evolution* **17**, 470–80.

Bucio Alanis, L., and Hill, J. (1966).—Environmental and genotype–environmental components of variability. II. Heterozygotes. *Heredity, Lond.* **21**, 399–405.

Cooper, J. P., and McWilliam, J. R. (1966).—Climatic variation in forage grasses. II. Germination, flowering and leaf development in Mediterranean populations of *Phalaris tuberosa*. *J. appl. Ecol.* **3**, 191–212.

Finlay, K. W. (1963).—Adaptation—its measurement and significance in barley breeding. Proc. 1st Int. Barley Genet. Symp., Wageningen. pp. 351–9.

Finlay, K. W., and Wilkinson, G. N. (1963).—The analysis of adaptation in a plant-breeding programme. *Aust. J. agric. Res.* **14**, 742–54.

Frey, K. J., and Maldonado, V. (1967).—Relative productivity of homogeneous and heterogeneous oat cultivars in optimum and suboptimum environments. *Crop Sci.* **7**, 532–5.

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

Hiesey, W. M. (1963).—The genetic–physiologic structure of species complexes in relation to environment. Proc. 11th Int. Conf. Genet., The Hague. pp. 437–45.

Jenkins, H. V. (1959).—An airflow planimeter for measuring the area of detached leaves. *Pl. Physiol., Lancaster* **34**, 532–6.

Johnson, V. A., Shafer, S. L., and Schmidt, J. W. (1968).—Regression analysis of general adaptation in hard red winter wheat (*Triticum aestivum* L.). *Crop Sci.* **8**, 187–91.

Jones, D. F. (1958).—Heterosis and homeostasis in evolution and applied genetics. *Am. Nat.* **92**, 321–8.

Langridge, J. (1962).—A genetic and molecular model for heterosis in *Arabidopsis* and *Drosophila*. *Am. Nat.* **96**, 5–27.

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

MacColl, D. (1965).—Growth analysis of ryegrass families of contrasting growth habit. Rep. Welsh Pl. Breed. Stn, 1964. pp. 23–24.

McWILLIAM, J. R. (1962).—Interspecific hybridization in *Phalaris:* hybrids between *Phalaris tuberosa* and the hexaploid race of *Phalaris arundinacea Aust. J. agric. Res.* **13**, 585–98.

McWILLIAM, J. R., and GRIFFING, B. (1965).—Temperature-dependent heterosis in maize. *Aust. J. biol. Sci.* **18**, 569–83.

McWILLIAM, J. R., and NEAL-SMITH, C. A. (1962).—Tetraploid and hexaploid chromosome races of *Phalaris arundinacea* L. *Aust. J. agric. Res.* **13**, 1–9.

McWILLIAM, J. R., and SHEPHERD, C. J. (1964).—The nature and genetic control of a red anthocyanin pigment in the root meristems of *Phalaris. Aust. J. biol. Sci.* **17**, 601–8.

MIFLIN, B. J., and HAGEMAN, R. H. (1966).—Activity of chloroplasts isolated from maize inbreds and their F_1 hybrids. *Crop Sci.* **6**, 185–7.

MITCHELL, K. J. (1956).—Growth of pasture species under controlled environment. I. Growth at various levels of constant temperature. *N.Z. Jl Sci. Technol.* A **38**, 203–15.

PALMER, T. P. (1952).—Population and selection studies in a *Triticum* cross. *Heredity, Lond.* **6**, 171–85.

PEDERSON, D. G. (1968).—Environmental stress, heterozygote advantage and genotype–environment interaction in *Arabidopsis. Heredity, Lond.* **23**, 127–38.

SCOTT, G. E. (1967).—Selection for stability of yield in maize. *Crop Sci.* **7**, 549–51.

SHANK, D. B., and ADAMS, M. W. (1960).—Environmental variability within inbred lines and single crosses of maize. *J. Genet.* **57**, 119–26.

SPRAGUE, G. F., and FEDERER, W. T. (1951).—A comparison of variance components in corn yield trials. II. Error, year × variety, location × variety, and variety components. *Agron. J.* **43**, 535–41.

WATSON, D. J. (1956).—Leaf growth in relation to crop yield. In "The Growth of Leaves". (Ed. F. L. Milthorpe.) pp. 178–91. (Butterworths Scientific Publications: London.)