THE EARLY STAGES OF GRAIN DEVELOPMENT IN WHEAT: RESPONSE TO LIGHT AND TEMPERATURE IN A SINGLE VARIETY

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Summary

For wheat plants (cv. Gabo) grown under natural daylight at a temperature of 21/16°C, increase in dry weight of the stem exceeded that of the ear for the first 10 days following anthesis. Higher temperatures (27/22°C) resulted in a greater rate of grain development, with a corresponding increase in the rate of cell division in the endosperm tissue, and a shortening of the stem growth period. Despite initial differences in the rates of cell division with variation in temperature, the final number of cells formed in an endosperm did not vary significantly between temperature treatments. Dry weight accumulation in the stem was, in contrast to the grain, highest at lower temperatures (15/10°C).

Low light intensity (17·5% of daylight) reduced dry weight accumulation by both the stem and ear, and resulted in a reduction in the final number of endosperm cells formed in the grain. Both dry weight analyses and studies of the distribution of ^{14}C-labelled photosynthate from the flag leaf indicated that the top internode of this variety, which elongated a further 12–16 cm after anthesis, competed with the grain for assimilates under light-limiting conditions.

Grain set was maximal when temperatures were low, and light intensity high from the time of anthesis, and ranged from 28 grains per ear at 27/22°C under 17·5% sunlight to 49 grains per ear at 15/10°C under full sunlight.

A comparison was made of temperature and light variations during the first 10 days after anthesis with similar treatments during the stage of starch deposition 15–25 days after anthesis. High temperature during either period reduced grain yield per ear at maturity with the greater effect during starch deposition. However, low yield which resulted from high temperature during the early period was caused by a reduction in seed set, which was partially compensated by increased grain size. In contrast high temperatures during the later stages reduced the weight of individual grains. Low light at both stages of development significantly reduced grain weight per ear at maturity.

I. INTRODUCTION

Complex anatomical and biochemical changes occur in the wheat grain following anthesis and fertilization of the ovary. For the first 3–4 days there is a rapid multiplication of the endosperm nuclei with the later formation of cell walls, and not until about 6 days after anthesis does starch deposition commence in the endosperm (Harlan 1920; Woodman and Engledow 1924; Hoshikawa 1961). The later-formed starch-containing cells are apparently produced by inward division of the peripheral cells of the endosperm, which on completion of the stage of cell division, 18–20 days after anthesis, form the outer aleurone layer cells (Hoshikawa 1961). At anthesis, the stem would appear to be the organ most actively accumulating dry material, with

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extension growth in the uppermost internode and accumulation of reserve carbohydrate in the lower parts (Hsia, Waon, and Wang 1963; Wardlaw and Porter 1967). There was in fact some evidence from earlier work (Carr and Wardlaw 1965) that the top internode might actively compete with the developing grain for a limited supply of carbohydrate under stress conditions.

Despite the possible importance of the early stages of grain development in controlling the ultimate size of the starch storage tissue of the grain, variations during the cell-division stage of grain development have received little attention, in comparison with the later stages of rapid starch deposition. This lack has occurred largely because of difficulties associated with the interactions between treatments and effects of seed set at the time of anthesis. The following experiments were designed to examine more closely how the cell-division stage of grain development, in a single variety, would respond to changes in light and temperature, and to observe any interaction between stem and grain development.

II. Methods and Materials

(*a*) *Cultural Conditions*

Wheat plants (*Triticum aestivum* cv. Gabo) were grown singly in perlite in 5-in. pots. Air temperatures were controlled at 21°C for 8 hr of the daylight period and at 16°C for the remainder of the 24-hr cycle, and natural daylength was extended to 16 hr by low-intensity incandescent lamps. All plants were supplied with standard nutrient solution in the morning and with water each afternoon. Removal of tillers 5 weeks after sowing, and again at anthesis, limited each plant to a single main culm.

Tiller removal was used to facilitate the handling of plants during ^{14}C-labelling experiments and to ensure that at no stage were they subject to water stress, particularly at higher temperatures. Earlier work (Wardlaw, Carr, and Anderson 1965) indicated that this treatment would not alter the distribution of photosynthate to the ear.

Treatments were generally applied in the first 10 days following anthesis. In the first experiment a comparison was also made with identical treatments for the period 15–25 days after anthesis, to assess the relative effects of the treatments in the period of starch deposition. In this experiment light and temperature treatments were carried out in controlled-temperature glasshouses under natural daylight, with light intensity controlled by Zarlon shade cloth. The plants reached authesis in March and there was very little cloud until after the second period. Subsequent experiments were carried out from anthesis in artificially lit cabinets under a bank of VHO daylight fluorescent tubes, supplemented with incandescent lamps (Morse and Evans 1962).

(*b*) *Growth Measurements*

Length of the stem and of the top internode was measured at intervals from anthesis to maturity. Dry weights of ear structure, grains, stem internodes, tillers, and roots were measured directly on samples taken at different stages of development after anthesis, following drying at 80°C for 48 hr.

(*c*) *Carbon-14 Determinations*

To assess the effects of environmental factors on the distribution of photosynthetic assimilates, the flag leaf blades of different plants were exposed to $^{14}CO_2$ at various stages after anthesis. $^{14}CO_2$ was generated from 50 mg of $BaCO_3$ containing 100 μCi of ^{14}C. The initial concentration of CO_2 was 0·2% (v/v), and uptake by groups of six leaves was allowed to continue for 30 min with an air flow rate of 2 litres/min. Plants were harvested 48 hr after the commencement of the uptake of ^{14}C and immediately cut into parts for drying. The distribution of radioactivity was then determined by counting powdered 30-mg aliquots as described in previous papers (O'Brien and Wardlaw 1961; Wardlaw 1965).

(d) Endosperm Cell Counts

The number of cells in the endosperm tissue of a grain was determined at different stages of development, using the method described by Rijven and Wardlaw (1966). The endosperm tissue was dissected from the grain, stained with Feulgen's reagent, and macerated by fungal cellulase. The stained nuclei were then precipitated from an aliquot of the resulting suspension and counted under a microscope. Some difficulty was encountered with fully developed endosperm tissue, because of deformation of nuclei by pressure from starch granules, but it is felt that this did not invalidate the comparison between treatments.

III. Results

(a) Effect of Light and Temperature Changes on Stem and Ear Dry Weight in Relation to the Stage of Grain Development

At anthesis the stem was the most actively growing organ in Gabo wheat; however, its rate of growth declined as that for the grain increased, so that the curves intersected after day 15 at a temperature of 21/16°C (Fig. 1). The most rapid accumulation of dry matter by the ear occurred between 15 and 20 days after anthesis.

Fig. 1

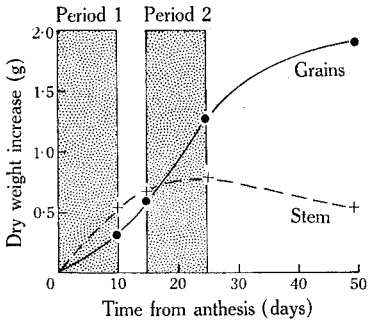

Fig. 2

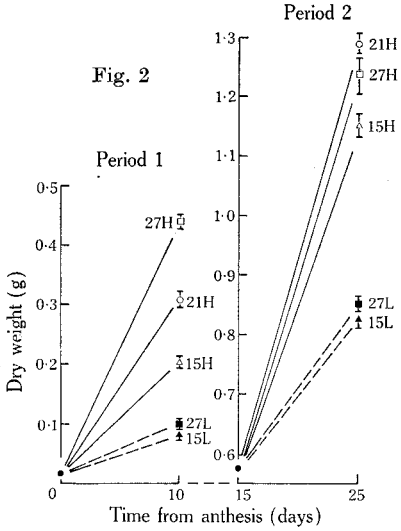

Fig. 1.—Dry weight increase following anthesis of stem and grain of wheat grown at 21/16°C. Period 1 covers the initial phase of endosperm cell division; period 2 covers the stage of most rapid starch deposition within the endosperm.

Fig. 2.—Change in dry weight per ear as affected by temperature and light. The plants were subject to the following differential light and temperature treatments during either period 1 (0–10 days from anthesis) or period 2 (15–25 days from anthesis): 27/22°C, full sunlight (27H); 27/22°C, 17·5% sunlight (27L); 21/16°C, full sunlight (21H); 15/10°C, full sunlight (15H); 15/10°C, 17·5% sunlight (15L). Each result is the mean of 10 replicates and each vertical line represents 2 × S.E.

In this experiment different levels of light and temperature were arranged either during the first 10 days after anthesis (period 1), or during the 10 days from 15 to 25 days after anthesis (period 2). The experimental details are outlined in the legend to Figure 2. Harvests were made at the commencement of treatment, at the end of the treatment period, and at grain maturity.

Decreasing the temperature under high light conditions from 21/16°C to 15/10°C resulted in a reduced rate of grain dry weight increase in both periods (Fig. 2). Increasing the temperature to 27/22°C increased the rate of development of the grain during period 1, but reduced it slightly during period 2. The effect of temperature was relatively small under light-limiting conditions.

There was a marked interaction between light and temperature on grain weight at maturity, in that the reduction in grain weight per ear (Table 1), or the reduction in individual grain weight (Table 2), due to low light, was greatest at high temperatures.

TABLE 1

EFFECT OF DIFFERENTIAL LIGHT AND TEMPERATURE TREATMENTS, DURING EITHER PERIOD 1 OR PERIOD 2, ON TOTAL GRAIN WEIGHT PER EAR AT MATURITY

Each result is the mean weight ±S.E. of 20 replicates

Treatment	Dry Weight (mg)	
	Period 1	Period 2
27/22°C, full sunlight	1725±48	1529±25
27/22°C, 17·5% sunlight	261±39	896±35
21/16°C, full sunlight	1902±49	1902±49
15/10°C, full sunlight	1855±43	2061±46
15/10°C, 17·5% sunlight	1537±46	1682±46

Since there was little indication of temperature effects on dry weight during the actual low light treatments, the ultimate difference can best be explained by the different effects of temperature on the amount of development occurring under low light conditions.

TABLE 2

EFFECT OF DIFFERENTIAL LIGHT AND TEMPERATURE TREATMENTS, DURING EITHER PERIOD 1 OR PERIOD 2, ON WEIGHT PER GRAIN AT MATURITY

Each result is the mean weight ±S.E. of 20 replicates and each replicate is the mean of four grains taken from the first floret of four central spikelets in a head

Treatment	Dry Weight (mg)	
	Period 1	Period 2
27/22°C, full sunlight	45±0·6	38±0·6
27/22°C, 17·5% sunlight	11±1·3	25±0·6
21/16°C, full sunlight	45±0·6	45±0·6
15/10°C, full sunlight	40±0·6	49±1·0
15/10°C, 17·5% sunlight	39±2·5	36±0·7

There was a difference between treatment periods in the response of individual grains to temperature (Table 2). Individual grain weight at maturity was greatest with

high temperatures during period 1 and low temperatures during period 2. On a grain weight per ear basis (Table 1), however, this effect was only partially observed and reflects the effect of treatments in period 1 on the number of grains set per ear.

No alteration in grain set per ear was observed by altering temperature and light during period 2, but grain set was reduced by both low light conditions and high temperature during period 1 as shown in the following tabulation (each result being the mean of 20 replicates ±S.E.):

Temperature	Grains per Ear	
	Full Sunlight	17·5% Sunlight
27/22°C	41±1·2	28±1·2
21/16°C	45±1·2	—
15/10°C	49±1·1	40±1·3

Stem weight showed the opposite temperature response to that of the grain (Fig. 3) in that decreasing the temperature increased stem weight in both period 1 and

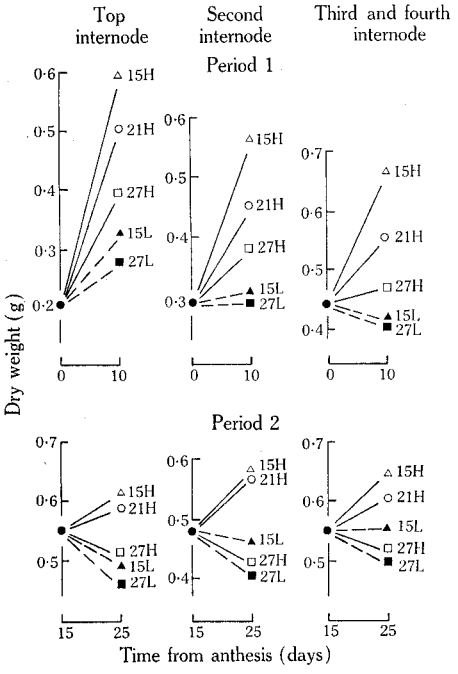

Fig. 3.—Change in stem dry weight in response to temperature and light. Each result is the mean of 10 replicates.

period 2. The effect of lowering light intensity, as with the grains, was to reduce stem weight, but the magnitude of the effect varied with both the stage of development and the particular internode under observation. In period 1 there was a continued increase in dry weight of the top internode, even under low light conditions which limited grain development, while the second internode showed little change and the lower internodes decreased in weight.

The final length of the top internode, which was still actively expanding at the time of anthesis, varied between treatments. Extension growth following anthesis ranged from an average of 17·9 cm at 15/10°C under low light conditions, down to 14·7 cm at 27/22°C, also under low light. Plants held from anthesis at 15/10°C had the greatest final stem length, due to greater expansion of the top internode. Although the rate of extension was slower at low temperatures the period of growth was extended (Fig. 4).

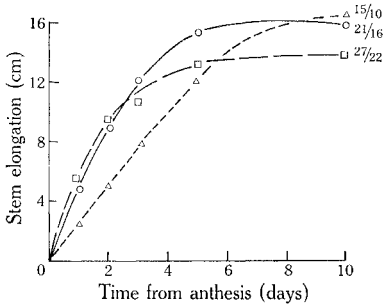

Fig. 4.—Effect of temperature on stem elongation following anthesis.

(*b*) *Stem and Ear Interactions*

Plants were held from anthesis until harvest at either 27/22°C or 15/10°C, under high (3500 f.c.) or low (500 f.c.) light conditions extending for a 16-hr daylength, in an artificially lit cabinet. Flag leaf blades of individual plants were allowed to assimilate

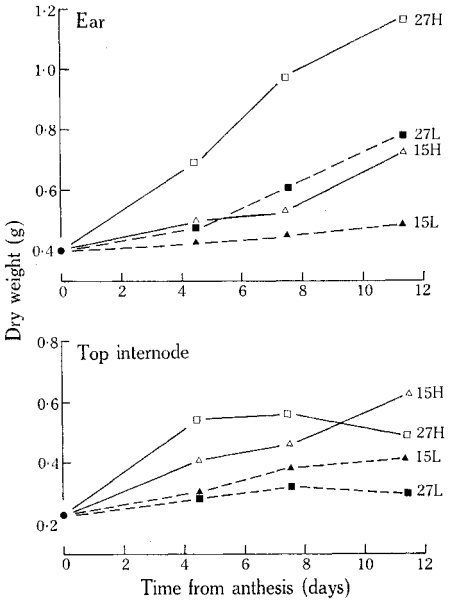

Fig. 5.—Dry weight changes in the ear and top internode of Gabo wheat in response to variations in light and temperature following anthesis. Data from ^{14}C-labelling experiment. Each result is the mean of four replicates. High light (H), 3500 f.c.; low light (L), 500 f.c.

$^{14}CO_2$ at one of three stages of grain development: 2–3 days, 5–6 days, and 9–10 days after anthesis. To obtain adequate replication it was necessary to extend the $^{14}CO_2$ labelling over a period of 2 days. The plants were subsequently harvested 48 hr after the uptake of $^{14}CO_2$.

Dry weight changes in response to temperature were very similar to those in the first experiment. However, the earlier harvests at 4 and 5 days and 7 and 9 days indicated that the higher temperatures initially stimulated growth of the top internode (Fig. 5). By the final harvest 11 and 12 days after anthesis the weight of the top internode was higher at the lower temperature. As was noted previously, there was continued growth in dry weight of the top internode, under light conditions that markedly reduced grain development.

The total ^{14}C activity per plant remaining 48 hr after labelling (Table 3) reflects the difference in photosynthetic activity under high and low light conditions. However, this comparison will be inaccurate due to differences in respiratory losses and also due to small differences in the area of assimilation.

TABLE 3

PERCENTAGE DISTRIBUTION OF ^{14}C FLAG LEAF ASSIMILATES IN RESPONSE TO LIGHT AND TEMPERATURE CONDITIONS FOLLOWING ANTHESIS

Each result is the mean ±S.E. of six replicates

Time from Anthesis (days)	Part	27/22°C		15/10°C	
		3500 f.c.	500 f.c.	3500 f.c.	500 f.c.
2–3	Flag leaf	12·1±0·6	12·0±1·3	12·8±0·8	14·6±0·7
	Ear	9·6±1·9	36·2±1·9	6·1±0·3	19·3±1·3
	Top internode	22·8±3·8	31·1±3·5	26·5±1·2	49·3±1·6
	Lower stem	32·9±3·5	8·1±2·2	45·3±1·2	6·7±0·9
	Roots and crown	22·7±4·1	12·6±3·5	9·4±0·8	10·1±1·2
Relative total activity (counts/min):		9333±923	4031±256	10,599±236	3324±104
9–10	Flag leaf	11·0±0·7	14·4±0·7	14·2±0·6	13·9±2·5
	Ear	48·6±4·0	70·7±1·8	12·5±1·0	31·0±4·8
	Top internode	10·6±0·5	4·6±0·7	19·8±1·6	29·9±4·5
	Lower stem	19·6±2·3	3·5±0·3	37·3±1·5	15·5±2·2
	Roots and crown	10·1±1·5	6·8±1·4	16·4±2·5	9·7±1·4
Relative total activity (counts/min):		8789±458	3703±92	9324±569	3461±357

The most dramatic response to light was a reduction in the movement of ^{14}C assimilates to the lower parts of the stem and roots under low light conditions and increased movement to the ear. Based on absolute levels of ^{14}C, there was at high temperature a greater total contribution of assimilates to the ear from the flag leaf under low light than at high light, despite the drop in photosynthetic rate (ear counts were 921 counts/min under high light, and 1451 counts/min under low light at the first harvest interval). Although the total accumulation of ^{14}C by the top internode was reduced under low light, the proportion of the available flag leaf assimilates moving to the top internode was increased (Table 3).

High temperature hastened the development of the ear and increased the movement of ^{14}C assimilates to this from the flag leaf. At the same time development of the top internode was completed more rapidly at high temperatures (cf. Fig. 4), and this can be seen by the rapid reduction in the incorporation of ^{14}C by the top internode with time from anthesis at high temperatures in comparison with the more continuous demand at low temperatures (Table 3), particularly under low light.

Variations in light and temperature had very little effect on the turnover of ^{14}C assimilates in the flag leaf, measured as percentage retention of ^{14}C after 48 hr (Table 3).

(c) *Endosperm Cell Development*

The rate of cell division in the endosperm was reduced by low temperatures (15/10°C) and increased by high temperatures (27/22°C) following anthesis (Fig. 6). In this experiment temperature differentials were only maintained for the first 5 days following anthesis and the number of cells developed by the endosperm 15 days after anthesis were similar for all treatments. However, Hoshikawa (1962) also noted that cell numbers were similar when temperature differential treatments were continued throughout the period of endosperm cell development.

Fig. 6

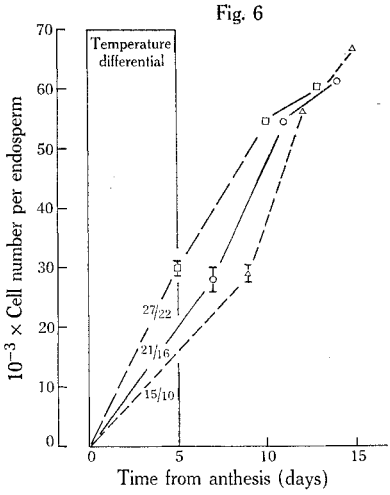

Fig. 7

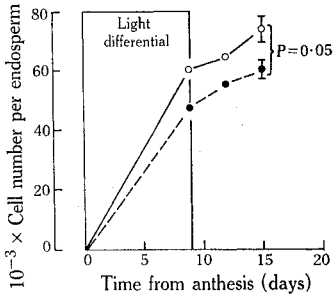

Fig. 6.—Effect of temperature on endosperm cell development. Each result is the mean of four replicates, each based on the analysis of four grains taken from the basal florets of the four central spikelets. Vertical lines indicate 2 × S.E.

Fig. 7.—Effect of light on endosperm cell development. Light differential 0–9 days after anthesis: 4300 f.c. (○); 580 f.c. (●). Vertical lines indicate 2 × S.E.

A period of low light (580 f.c.) following anthesis, in contrast to temperature, resulted in a reduction in the number of cells per endosperm (Fig. 7). A second experiment with a light differential of only 7 days following anthesis gave similar results, and cell counts 21 days after anthesis were 64,638±1528 for 3500 f.c. and 54,464±1765 for 600 f.c., with the difference significant at the 0·01 level.

IV. Discussion

There were two aspects of stem development associated with early grain growth. Firstly there was elongation of the top internode (peduncle), which under low light conditions competed with the young developing grain for a limited supply of assimilates. Secondly the lower internodes of the stem accumulated assimilates in excess of the growth requirements of the plant (storage), but did not compete for these

with the developing grain. Thus conditions that stimulated the growth of the ear, such as high temperatures, or reduced the available supply of assimilates, such as low light, resulted in a very much reduced accumulation, or even loss, of dry material by the lower internodes. The subsequent utilization of accumulated carbohydrate from the stem has been examined in detail in other studies (Stoy 1965; Wardlaw and Porter 1967; Rawson and Evans, unpublished data).

The rate of grain development in relation to temperature was reflected in the rate of cell division in the endosperm (cf. Pope 1943). However, the ultimate number of cells formed was apparently independent of temperature in the range examined (15/10°C–27/22°C). Similar observations were made earlier by Hoshikawa (1962), but he also recorded that endosperm cell size was reduced when high temperatures were maintained throughout cell development. In contrast to temperature, limiting the supply of substrate available to the grain, by subjecting the plant to low light conditions, resulted in a decrease in the number of endosperm cells formed.

In view of the observed interactions between stem and ear growth and environmental effects on the early stages of endosperm cell development, the comparison of light and temperature effects on grain development during the early stages of cell division and during rapid starch deposition was of interest. The effect of temperature on final grain weight was least when applied during the early stages of development. Heaviest grains were produced at the highest temperature, but this was associated with a reduction in seed set. However, in contrast to the early temperature response, high temperatures during starch deposition significantly reduced grain weight at maturity, which is in agreement with the field data obtained by Wattal (1965). Low light conditions at either stage of development resulted in a significant reduction in final grain weight, the effect being greatest at high temperatures. The low-light effect was particularly noticeable during the cell-division stage, but the effect of low light at high temperature during starch deposition was just as inhibitory to subsequent growth, when the weight of grains at the start of the treatment is considered.

The evidence presented here for a single variety of wheat does suggest that environmental conditions during the early stages of grain development were important in determining the ultimate size of individual grains. Since varieties differ in their rates of early grain development (Rawson and Evans, unpublished data), and the number of seed set per ear is partially controlled by an interaction between developing grains (Rawson and Evans 1970), further investigation of varietal aspects of the early cell-division stages of grain development may be important in yield analysis and improvement.

V. Acknowledgments

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