Physiological and Biochemical Responses to Elevated Temperatures Influencing Grain Weight in Wheat

by

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To my parents whose efforts,
in a substantial way, made
this moment possible.

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SUMMARY

1. The hypothesis - ~~whether or not~~ that the rate of grain growth at high temperature is limited by the availability of assimilate has been tested in wheat (*Triticum aestivum* L. cv. Sonora), and also an attempt has been made to examine some of the other factors which could reduce single grain weight at high temperature.
2. The information pertaining to the effects of temperature (and of other environmental factors) on grain yield and individual grain weight, on the pattern of starch and protein accumulation, on some of the characteristics of grain growth, and on the sources and transport of carbon and nitrogen into and within the grain has been reviewed.
3. The above hypothesis has been tested by warming only the ears so that the effects of temperature on other parts of the plant are likely to be minimal, and hence eliminating the possibility that high temperature may have affected grain growth indirectly through its effects on other parts of the plant. The assumption made in this investigation is that the effects of temperature on the rate of grain growth are mediated through the levels of assimilate (sucrose) within the grain. Accordingly, the concentrations of sucrose and of other soluble sugars (excluding sucrose) in the grains developing in the control and warmed ears have been determined, and the hypothesis has also been tested by culturing detached ears at various levels of sucrose both during and after a brief episode of high temperature. It appears that the rate of grain growth at high temperature is possibly not limited by the availability of substrate for grain growth rather it is more likely due to the factors operating within, or close to the grain.
4. Warming the ears alone or the whole plant for brief periods during the grain filling phase reduced total ear grain weight which was mainly due to an effect of temperature on the individual grain weight.
5. Warming the ears at either high or low humidity (or the whole plant) significantly reduced the water content of the grains, but this reduction in water content of the grain is not simply related to the changes in water - or osmotic potential of the grain, or of the bracts. Warming the ears at high humidity in two out of three experiments had less effect on the rate of grain growth as compared

to warming the ears at low humidity. Also the reductions in total ear grain weight and individual grain weight at maturity were smaller in the ears warmed at high compared to low humidity. Therefore, high humidity in the warm air seems to ameliorate the deleterious effects of high temperature on grain growth.

6. Four cultivars of wheat have been compared to see if there were any differences among them in their response to elevated temperature in terms of grain yield or in the patterns of starch and protein accumulation in the grain. High temperature significantly reduced both total ear grain weight and individual grain weight, and these reductions were relatively greater for cv. RC-81-429 as compared to cvv. Kite, Sonora, and WW15. High temperature significantly reduced the amount of starch per grain, but had little or no effect on the amount of protein per grain. The number of B-type starch granules was significantly reduced in the grains from the warmed plants whereas the number of A-type starch granules was hardly affected.
7. The effect of temperature on the sucrose-to-starch conversion process in the endosperms cultured *in vitro* has been examined by culturing isolated endosperms on osmotically adjusted solutions of sucrose at various temperatures between 15 to 35°C. The experiments indicate that the process(es) involved in the conversion of sucrose to starch has a broad optimum around 25°C, whereas respiration in these endosperms increased with a rise in temperature within the range 15 to 35°C.
8. It is concluded:
   (a) Both the rate and the duration of grain growth are affected by elevation in temperature during the grain filling phase. Final grain weight at high temperature is reduced because there is no compensatory increase in the rate of grain growth at high temperature to balance the reduction in the duration of grain growth.
   (b) Reduction in final grain weight at high temperature seems not to be mediated either through its effects on the water relations of the grain or the other parts of the ear, or through the effect of temperature on the availability of assimilates for grain growth. It is more likely due to the effects of temperature on the processes operating within, or close to the grain.

(c) The accumulation of starch and protein in the grain respond differently to a brief period of high temperature during grain filling. Protein deposition seems to have a higher temperature optimum than the accumulation of starch.

(d) Different proceses operating in the grain seem to have different temperature optima. For example, the uptake of sucrose by the endosperm cells and respiration have higher optima than the processes related to the conversion of sucrose into starch.

(e) High temperature during the grain filling phase not only reduced grain yield, but also affects the composition of the grain which determines the quality of the flour.

Statement

I hereby declare that the work reported in this thesis is my own, that it contains no material previously published, except where due reference is made in the text, and no part of it has been submitted for any other degree.

I consent to this thesis being made available for photocopying and loan if accepted for the award of the degree.

(Sukhdev Singh Bhullar)

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ABBREVIATIONS, ACRONYMS AND SYMBOLS USED IN THIS THESIS

ABA	abscisic acid
ACS	aqueous counting scintillant
ADP	adenosine 5'-pyrophosphate
ATP	adenosine 5'-triphosphate
cm	centimetre
Concn	concentration
cv.	cultivar
cvv.	cultivars
DNA	deoxyribonucleic acid
g	gram(s)
$g l^{-1}$	grams per litre
h	hour(s)
K	kelvin
kPa	kilo pascal
l	litre
L.S.D.	least significant difference
M	molar
m	metre
MPa	mega pascal
mCi	millicurie
mg	milligram
min	minute
ml	millilitre
mm	millimetre
µCi	microcurie
µg	microgram
µl	microlitre
µm	micrometre
N	nitrogen
NADP	nicotinamide-adenine dinucleotide phosphate, oxidised
NADPH	nicotinamide-adenine dinucleotide phosphate, reduced
nm	nanometre
n.s.	not significant
P	turgor pressure
%	per cent

Ψ_π	osmotic potential
Ψ_w	water potential
rpm	revolutions per minute
s	second(s)
S.E.	standard error of difference of means
t ha^{-1}	tonnes per hectare
v/v	volume/volume (concn)
V.P.D.	vapour pressure deficit

SECTION 1: GENERAL INTRODUCTION

1.1 INTRODUCTION

The distribution and yield of crop plants in the different parts of the world is determined largely by temperature and the availability of water (Bunting et al. 1981). Temperature in addition to having a direct effect on the growth and development of the crop may interact with numerous other factors like water availability, day length (photoperiod), nutrition and light intensity, thus making it difficult to evaluate the effect of temperature *per se* on crop yield. In spite of the interactive nature of environmental factors, Monteith (1981) has evaluated separately the effects of light, temperature, and rainfall on year to year variation in yield for major arable crops in the east Midlands of England (Table 1.1). Summarizing the essentials (of Table 1.1), light makes small contribution. Temperature is responsible for a variation of ±7% to ±10%, mainly through its influence on the length of growth period. The effect of rainfall ranges from negligible figures for cereals in heavy soils to over ±10% for more drought sensitive crops on light soils.

Wheat yield is highly sensitive to temperature, and a 1°C increase in temperature during the months of May, June, and July in the east Midlands of England reduces the grain yield by 6% (Fig. 1.1). Similarly, a 1°C increase in temperature for each month of the wheat growing season in the United States of America could mean a loss of US$ 136m per year (Bryson 1975). High temperature has been reported to reduce the yield of almost every crop used as food by human beings (Bunting et al. 1981). Reduction in the yield of wheat due to high temperature during grain-filling is common in Australia (cf. Nix 1975; Davidson and Birch 1978), and can also be a problem in many of the established wheat growing areas (McDonald et al. 1983).

Temperature not only reduces the quantity of seed produced, but may affect the quality of the seed during its formation. High temperatures and water stress during grain-filling in wheat result in an increase in grain protein per cent (cf. Nix 1975). It has been reported (Sosulski et al. 1963) that high temperature during grain development may result in a change in amino acid composition of the protein, which could be due to a change in the proportion of different proteins (gliadins and glutenins) or in the proportion of different ~~molecules~~ amino acid residues in the same protein.

TABLE 1.1 **Estimates of yield and variation for major arable crops in the east Midlands of England.

Crop Type	Soil Type	Effective growth period (days)	Total dry matter (tha^{-1})	Yield* (tha^{-1})	Components of variation (±%) light	temperature	rainfall
				grain			
Spring cereals	heavy	59 ±5	11.8±1.2	6.1±0.6	2	8	2
	light	55 ±7	11.0±1.5	5.7±0.8	2	9	9
				grain			
Winter cereals	heavy	89 ±8	17.8±1.9	9.3±1.0	2	9	1
	light	85 ±9	17.0±2.1	8.8±1.1	2	9	6
				tubers			
Potatoes	heavy	55 ±7	11.0±1.5	37 ±5	2	9	9
	light	52 ±9	10.4±2.0	35 ±7	2	10	15
				roots			
Sugar beet	heavy	83 ±6	16.6±1.5	30 ±3	2	4	8
	light	80 ±11	16.0±2.5	29 ±5	2	4	13

* Yield estimated from total dry weight using these factors: grain 0.52 ; tubers 3.4 ; beet roots 1.8
** Adapted from Monteith (1981)

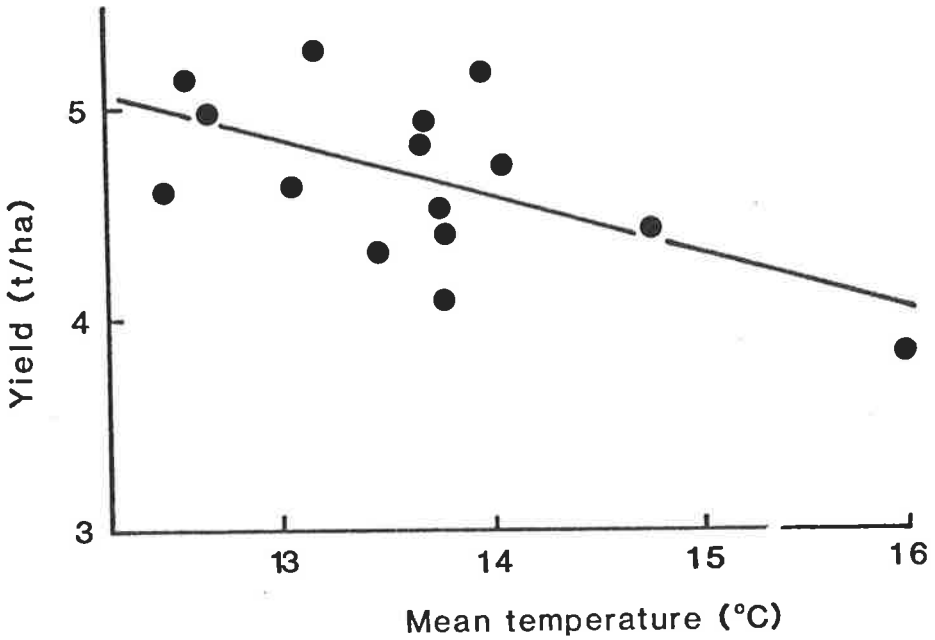


Fig. 1.1 Yield of wheat in the east Midlands (corrected for a time trend of 1% per year) as function of mean temperature for May, June, and July 1963-1978. Line of best fit has a slope of -6% K^{-1} at 14°C.

(Adapted from Monteith 1981)

Soyabean seeds that develop at temperatures above 33°C have a low germination percentage (Green et al. 1965; Siddique and Goodwin 1980). Physiological disorders such as, "hollow heart" in the pea, may be induced and enhanced by high temperature during the seed growth and development (Perry and Harrison 1973).

Much of Australia's wheat is grown in a Mediterranean-type climate. It is sown at the beginning of rains in the Autumn, grows through the mild winter and spring, a period of moderately reliable rainfall, and is harvested in late spring or early summer under rapidly rising temperature and drought stress. Some frost damage in the ear may occur in the early maturing cultivars (in the northern wheat belt), if sown too early. Nevertheless, the major limitation to wheat grain yield is due to hot and dry post-flowering conditions (Davidson and Birch 1978). As the main objective of this study is to determine the factors limiting grain weight at high temperture(s), grain growth under normal and above optimum temperature conditions only have been reviewed in the following sections.

1.2 YIELD COMPONENTS

Grain yield in cereals is a product of grain number and individual grain weight. Grain number is determined during the reproductive phase lasting from the formation of double ridges until several days after anthesis, while individual grain weight is affected most by prevailing environmental conditions after anthesis and throughout the grain filling phase.

1.2.1 GRAIN NUMBER

Grain number in wheat is determined by the number of spikelets per spike, the number of florets per spikelet and proportion of florets that actually set grains (Evans and Wardlaw 1976).

A greater number of spikelets is formed under high than under low light intensity (Friend 1965; Fischer 1975; Rahman et al. 1977). High levels of nitrogen (Beveridge et al. 1965; Holmes 1973; Langer and Liew 1973) and phosphorous (Rahman and Wilson 1977a) result in greater numbers than do low levels of these nutrients. Conditions, such as long days and high temperature, that accelerate floral development usually reduce spikelet number (Rawson 1970; Allison and Daynard 1976; Rahman and Wilson 1977b, 1978; Warrington et al. 1977; Kolderup 1979). In wheat, once the terminal spikelet has been formed, environmental conditions have no further influence on the spikelet number, although the number of florets within a spikelet may still be affected by environment.

Water stress during the reproductive phase causes a serious reduction in grain set and, hence, the yield of wheat (Salter and Goode 1967; Dubetz and Bole 1973; Saini and Aspinall 1981). The number of grains per spike of wheat is reduced when the plants are grown at temperatures above 20°C during the reproductive phase, mainly due to a reduction in grain set (Fischer and Maurer 1976; Warrington et al. 1977; Kolderup 1979; Rawson and Bagga 1979; Saini and Aspinall 1982). Wheat plants grown at 27°C during the day, and 22°C at night immediately following anthesis had a lower grain set than those grown at 15°C day and 10°C night temperature (Wardlaw 1970). High temperature coinciding with anthesis has adverse effects on pollen viability and stigma receptivity (Imrie 1966; Goss 1968; Saini et al. 1983).

1.2.2 INDIVIDUAL GRAIN WEIGHT

Final weight per grain is a function of the rate and the duration of grain growth (Brocklehurst 1977). Genotypic variation in grain weight has been attributed to differences in the duration of grain-filling (Asana and Bagga 1966; Syme 1967), or to variation in the rate of grain growth (Asana and Williams 1965; Bingham 1967; Brocklehurst 1977; Pinthus and Sar-Shalom 1978; Simmons and Crookston 1979), or to both sources (Sofield et al. 1977a – see Fig. 1.2; Stamp and Geisler 1976, Gebeyehou et al. 1982; Gleadow et al. 1982).

The growth rates and final weight per grain at different positions in a spike and within a spikelet differ (Rawson and Evans 1970). Grains in the middle spikelets of the ear grow faster than those in apical or basal spikelets, and grains developing in the second floret grow faster than those in positions distal or proximal to the second (Bremner 1972; Rawson and Ruwali 1972; Pinthus and Millet 1978; Simmons and Crookston 1979; Thornley et al. 1981; Singh and Jenner 1982a).

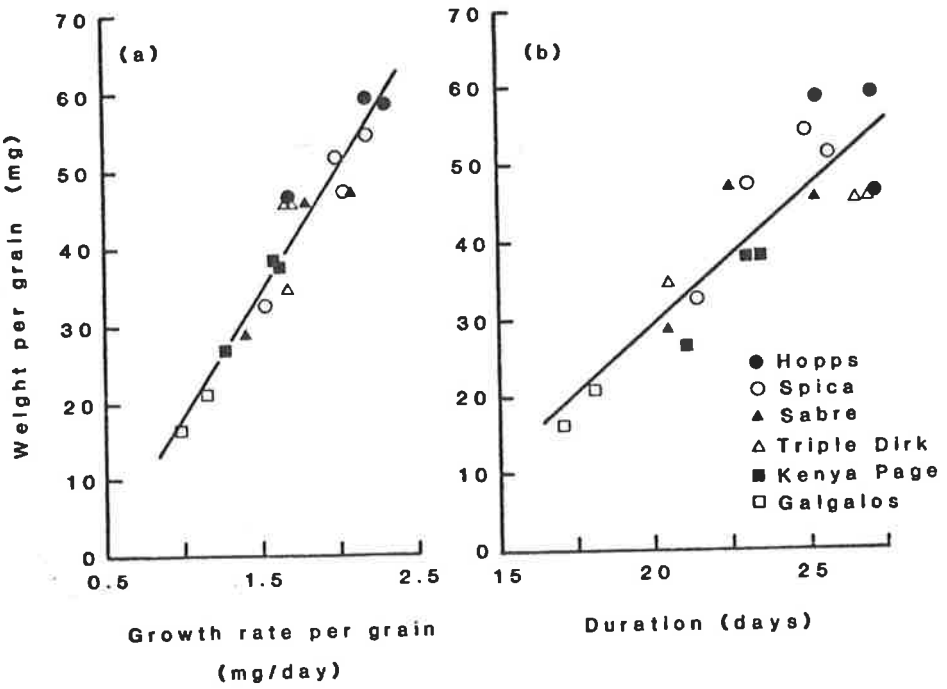


Fig. 1.2 Dependence of final dry weight per grain on (a) growth rate (mg per grain per day), and (b) growth duration (days) for all floret positions in the central spikelets of the six cultivars.

(Adapted from Sofield et al. 1977a)

Environmental conditions during grain growth have pronounced effects on the rate and the duration of grain filling. The rate of growth per kernel increases with a rise in temperature in rice (Nagato and Ebata 1966; Sato and Inaba 1973), and in wheat (Thorne 1970; Sofield et al. 1974, 1977a; Spiertz 1974, 1977; Chowdhury and Wardlaw 1978; Wardlaw et al. 1980). In wheat, between temperatures 15/10°C (day/night) to 21/16°C the rate of dry matter accumulation increases sharply, but there is little or no increase in rate with further increase in temperature. The duration of grain growth on the other hand decreases with increase in temperature (Fig. 1.3) throughout the whole range. Hence, the optimum temperature for maximum grain weight is about 18/13°C (Fig. 1.3).

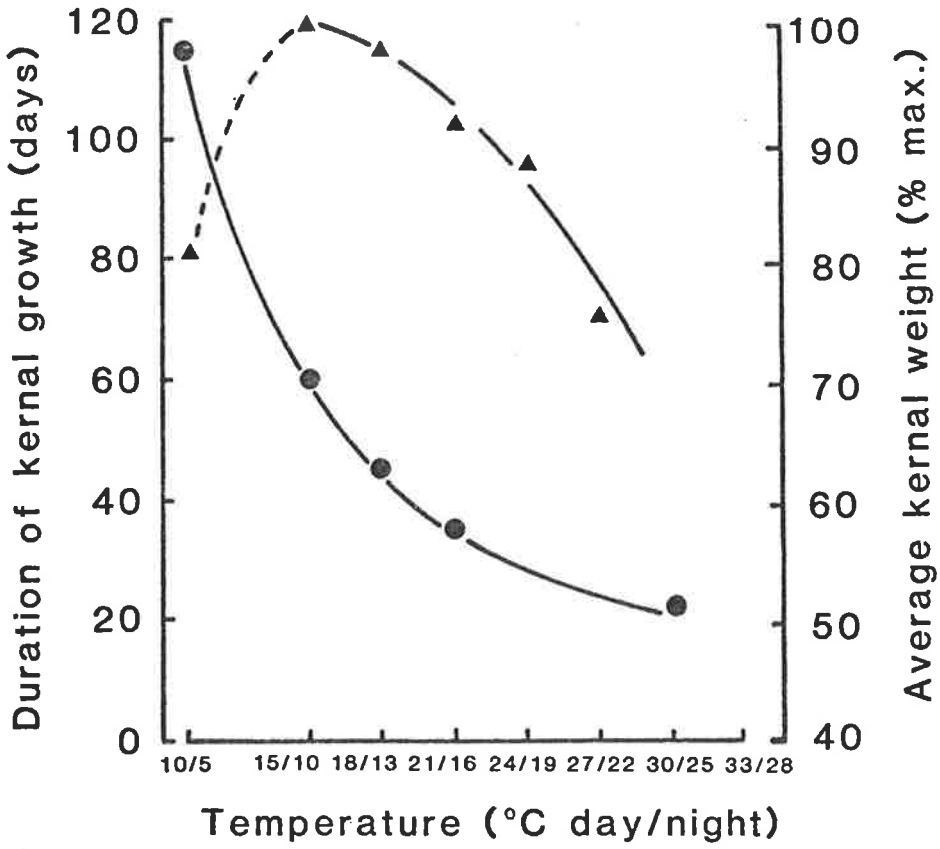


Fig. 1.3 Relationship between temperature, maximum grain weight (▲), and duration of grain growth (●) for wheat. (Adapted from Chowdhury and Wardlaw 1978)

Lower radiation reduces the accumulation of dry matter in wheat grains (Asana et al. 1969; Willey and Holliday 1971a, 1971b; Spiertz 1974, 1977; Fischer 1975; Ford and Thorne 1975; Sofield et al. 1977a; Jenner 1979; Martinez-Carrasco and Thorne 1979a). Water deficit during

the early stages of grain development or during grain filling depresses the final weight per grain (Asana et al. 1958; Robins and Domingo 1962; Aspinall 1965; Wardlaw 1971; Brocklehurst et al. 1978; Brooks et al. 1982). Mild water deficit has no effect on the accumulation of dry matter in wheat grain, but a severe water deficit reduces the rate of starch accumulation (Brocklehurst et al. 1978) and hastens the termination of grain filling in wheat (Wardlaw 1971; Brooks et al. 1982).

1.3 ANTHESIS, POLLINATION AND FERTILIZATION

Development of the stamens and gynoecium in the wheat flower is synchronous (Percival 1921). At the time of flowering the lodicules swell, forcing apart the lemma and palea (Peterson 1965; Goss 1968; De Vries 1971). Stamen filaments elongate, reaching three times their initial length, and extrude the anthers which split and shed their pollen. Concurrently, the stigmas quickly separate from each other and appear more feathery. The appearance of the first anther(s) from the spikelet(s) situated mostly about two-thirds of the way up from the base of the ear is often denoted the stage of *anthesis* and subsequent development of the grain is mostly related to this event. Pollination occurs just before or at the time of anthesis.

The fertility of the pollen and the ovary depends on the conditions during their development, both high temperature and water stress being unfavourable (Bingham 1966; Wardlaw 1970, 1971; Saini and Aspinall 1981, 1982). Optimum temperature for pollen germination and pollen tube growth is around 20°C (Hoshikawa 1960). A pollen tube containing the male gametes penetrates the embryo sac, one haploid male nucelus fuses with the egg nucleus to form the diploid zygote, while the second male nucleus fuses with the two polar nuclei forming a *triploid primary endosperm nucleus* from which the endosperm is derived. The time from pollination to fertilization in wheat varies from 3.5 hours at 30°C to as long as 8.5 hours at 10°C (Hoshikawa 1959).

1.4 GRAIN GROWTH

The course of grain growth in wheat may be divided into three main phases of growth.

(a) Early development with little accumulation of dry matter.

(b) The period of linear intensive growth.

(c) The maturation with small increases followed (possibly) by small decrease of dry matter.

1.4.1 EARLY DEVELOPMENT

Following fertilisation, initial growth is mainly in the pericarp (Rijven and Banbury 1960), while the endosperm nuclei undergo rapid divisions without cell wall formation. The sequence of the events for the transformation of acellular to cellular tissue of the endosperm are poorly understood, and there is disagreement on some details of the process. According to Brenchley (1909) the entire endosperm tissue arises by cellularisation of a syncytial mass, formed as a result of multiple free nuclear divisions of the primary endosperm nucleus (Section 1.3). Gordon (1922), on the other hand, showed that starchy endosperm tissue arises by serial tangential cell divisions of a cambial layer lining the embryo sac, this layer having been derived by multiple divisions of the first endosperm nucleus and persisting at maturity as the aleurone layer. Some workers (Sandstedt 1946; Buttrose 1963a; Evers 1970) have combined the two theories suggesting that the inner endosperm arises as described by Brenchley and the outer layers as described by Gordon.

Development of the embryo lags behind that of the endosperm and there is no appreciable increase in its weight until 10–12 days after anthesis (Jennings and Morton 1963a), but subsequently growth continues throughout the remaining period of grain development.

1.4.2 THE LINEAR PHASE

Together, starch and protein make up about 90% of the dry matter of the mature wheat grain (Section 1.5). Both starch and protein start accumulating about 6 to 8 days after anthesis and build up at a steady rate thereafter (Jennings and Morton 1963a; Bremner 1972; Sofield *et al.* 1977b).

1.4.2.1 ACCUMULATION OF STARCH

1.4.2.1.1 STORAGE FORM

Starch in the cereal grains is deposited in the form of organised structures called starch granules (Badenhuizen 1958). At maturity in the endosperm of wheat (Meredith 1981), barley (Buttrose 1960) and rye (Dronzek et al. 1974) there are two types of starch granules: the large lenticular or A-type and small spherical or B-type ranging from less than 1 to 10 microns. Small starch granules may account for up to 96% of total starch granule number (Duffus and Murdoch 1979; Gleadow et al. 1982) but because of their smaller size they may only make up as little as 10% (Buttrose 1958), but sometimes as much as 30 to 40%, of the total weight of starch (Hughes and Briarty 1976; Evers and Lindley 1977).

Variation in final grain weight and the appearance of the grain (e.g. large plump, small plump and shrivelled, Brocklehurst and Evers 1977), is associated with variation in the number of B-type granules. A reduction in final weight per grain due to water stress during grain filling is mainly due to a reduction in the number of B-type granules (Brocklehurst et al. 1978) and partly also due to a reduction in the size of the A-type starch granules (Brooks et al. 1982).

1.4.2.1.2 STARCH GRANULE INITIATION AND DEVELOPMENT

Minute starch granules are present in the wall of the unfertilized ovary. After fertilization starch granules present in the outer layer of the developing pericarp increase in size rapidly for 4 to 5 days. The enzymatic hydrolysis of these starch granules begins even before they have reached maximum size, and starch disappears from the outer pericarp just after the caryopsis has reached maximum length (Sandstedt 1946; May and Buttrose 1959).

In the endosperm, starch granules (A-type) first appear between days 4 to 14 after anthesis depending on the temperature (Brenchley 1909; Percival 1921; Sandstedt 1946; Hoshikawa 1962): the higher the temperature the earlier their appearance. The number of A-type amyloplasts per cell reaches a maximum around 6 days after anthesis, then drops, while the number per endosperm reaches a maximum around 8 days after anthesis, thereafter remaining constant (Fig. 1.4).

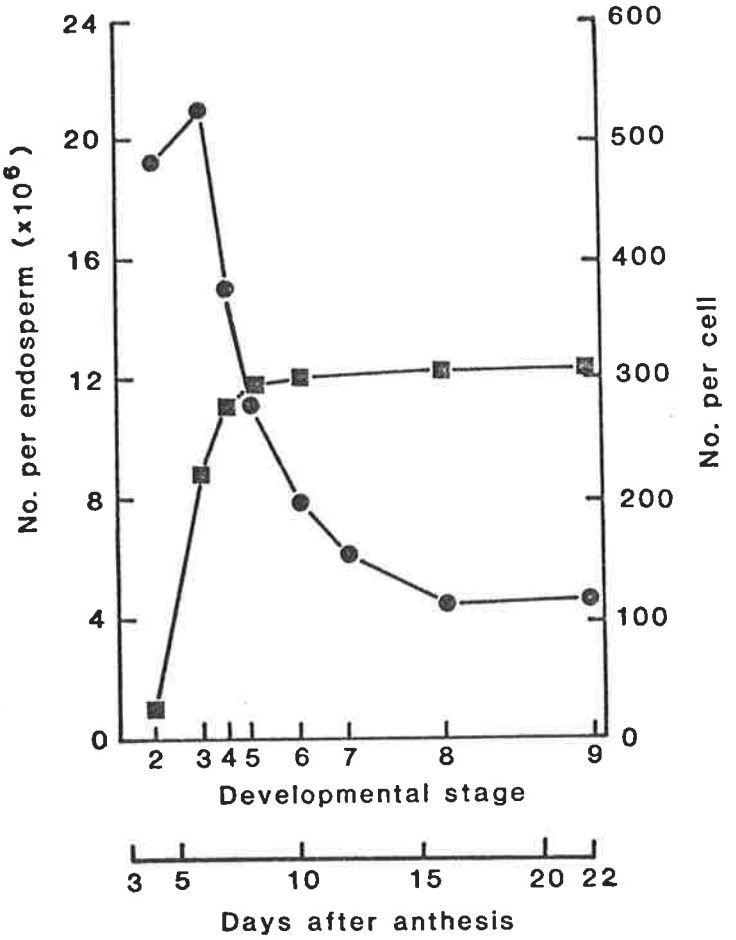


Fig. 1.4 Corrected numbers of A-type amyloplasts per cell (●) and per endosperm (■) during wheat endosperm development. (Adapted from Briarty et al. 1979).

The division of A-type amyloplasts ceases around 6 to 8 days before the cessation of endosperm cell division (Briarty et al. 1979), and so the cells of the outer layers (later formed) of the endosperm contain fewer A-type granules.

Smaller granules (B-type) begin to appear between days 12 to 20 after anthesis (Hoshikawa 1962). Small granules appear within A-type amyloplasts, the membrane of which then extrudes and constricts, releasing the small granules into the cytoplasm (Buttrose 1960).

Amylose content relative to the total amount of starch increases during grain development (Matheson 1971), and is due to an increase in the amylose content of the starch granules in all the size classes (Duffus and Murdoch 1979).

1.4.2.1.3 GROWTH OF THE STARCH GRANULE

Evidence from *in vivo* carbon-14 incorporation into granules of maize endosperm (Gafin and Badenhuizen 1959) and evidence from scanning electron microscopy on the development of the starch granule in wheat endosperm (Evers 1971) supports the postulate that the starch granule grows by apposition of material onto its surface. Within the plastid the starch granule occupies a fairly constant proportion (about 80 per cent) of the volume until 16 days after anthesis, and then the partial granule volume rises to 93 per cent at maturity. Granule growth rate appears to vary in different cell layers and at different stages of endosperm development, thus, resulting in a range of starch granule sizes in different parts of the endosperm (Briarty et al. 1979).

The composition within a starch granule varies from its core to its periphery, and presumably arises from the differing mechanisms for the initiation of the granule and its subsequent growth (cf. Jenner 1982a). In starch granules from wheat and barley, ring formation is controlled by environmental conditions (of light and temperature), whereas ring formation in the granules from potato tubers is not affected by environmental conditions (Buttrose 1963b).

1.4.2.2 PROTEIN ACCUMULATION

Proteins in the mature wheat grain can be divided into four groups (on a solubility basis; Osborne 1907) - albumins, globulins, gliadins (or prolamins) and glutenins. Albumins and globulins, which together comprise about 15% of grain protein, are chiefly found in the embryo and aleurone layer of the grain. The albumins may be chiefly metabolic proteins, while most of the globulins appear to be storage proteins that occur in protein bodies located in the cells of aleurone layer and in some of the cells of embryo (Buttrose 1963a). The albumins and globulins accumulate mainly during the first two weeks of grain growth and may continue to accumulate slowly for a further two weeks (Rijven and Cohen 1961; Jennings and Morton 1963a). The storage proteins (gliadins and glutenins) which are stored in protein bodies in the endosperm appear 6 to 12 days after anthesis and continue to accumulate until maturity (Graham and Morton 1963; Evers 1970; Campbell et al. 1981; Mecham et al. 1981; Bechtel et al. 1982). The synthesis of gliadins may begin later than glutenins (Bilinski and McConnel 1958).

There are considerable differences in the protein content (as a percentage of the dry weight) between cultivars (Haunold et al. 1962; McNeal et al. 1966; Johnson et al. 1967; Orth and Bushuk 1972; Wu and McDonald 1976; Donovan et al. 1977a,1977b; Sofield et al. 1977b; Pearman et al. 1978; McDonald et al. 1983). Also, a single cultivar grown under different conditions may vary in protein content (Swanson 1938; Rennie 1956; Hutcheon and Rennie 1960; Sosulski et al. 1963; Dubetz 1977), and there is variation in this attribute among different florets within a spikelet (McNeal and Davis 1954; Ali et al. 1969; Bremner 1972; Simmons and Moss 1978).

The rate of protein synthesis is enhanced more relative to starch at high temperatures (Campbell and Read 1968; Spiertz 1977) during grain development. Sosulski and co-workers (1963) have reported that high temeprature during grain development may change the composition of amino acids in the grain protein.

1.4.3 TERMINATION OF GRAIN GROWTH

The final stages of grain growth follow a curved course of gradually diminishing rate. Several changes occur in the plant and the grain more or less simultaneously, hence, making it difficult to ascertain whether termination of grain growth is a result of processes operating in the plant or in the grain itself, or to a combination of both. Green parts of the plant become senescent and there is a progressive loss of photosynthetic function. There is a rapid loss of water from the grain, during the final stages of grain growth (Meredith and Jenkins 1975). Whether the rapid fall in the water content is the cause or consequence of the cessation of grain growth is not clear. Work by Cerning and Guilbot (1973) indicates that water loss may precede the completion of dry matter, but this report is not supported by earlier workers (Harlan and Pope 1923; Jennings and Morton 1963a; Aspinall 1965) where it has been shown that the decrease in water content began only when maximum dry weight was attained. Radley (1976) suggested that the initiation of water loss from the grain at maturation might result from an increase in the permeability of the pericarp and might also involve the hormone ABA. King (1976) and Sofield et al. (1977b) did not find any evidence for Radley's suggestions, however.

The cessation of accumulation of starch is not attributable to a lack of availability of sucrose, but is rather due to a loss of capacity of endosperm to convert sucrose to starch (Jenner 1982a). The suggestion has been made that disintegration of the endosperm nuclei may initiate grain maturation (Alexandrov and Aleandrova 1954 cited in Frazier and Appalanidu 1965). However, such a suggestion is at variance with other reports in the literature. For example, there is no fall in DNA or RNA content in the endosperm during ripening (Jennings and Morton 1963b) and the number of nuclei appears to decrease after reaching a maximum value around 18 days after anthesis, which is much earlier in development than ripening (Hoshikawa 1962; Rijven and Wardlaw 1966; Singh and Jenner 1982b).

1.5 MATURE GRAIN COMPOSITION

The contribution of the individual parts of the grain of wheat towards its mature weight and the composition of different parts has been given by Peterson (1965):

Part of the grain	Per cent of total grain weight	Per cent of total amount in the grain: carbohydrate	protein (N x 5.7)
Embryo	2.5	1.5	8.0
Endosperm	84.0	87.9	72.0
Aleurone cell layer	6.5		16.0
Nucellus and seed coat	2.5	10.6	2.3
Pericarp	4.5		1.7

Endosperm is the main contributor towards mature grain weight and contains a major proportion of carbohydrates (mostly starch) and proteins. Starch in the endosperm is not evenly distributed: a cell in the centre of the endosperm contains about 8 times as much starch as a cell in the sub-aleurone layer (Jenner 1982a) while the quantity of protein per cell is about the same (14.7×10^{-5} mg, Evers 1970) in both regions.

1.6 CHANGES IN SOME OTHER GROWTH CHARACTERISTICS

1.6.1 WATER CONTENT AND WATER RELATIONS

During the first third of the period of grain growth, both water and dry matter accumulate in the grain, thereafter the water content of the grain remains constant until the grain ripens (Fig. 1.5), Sofield et al. 1977b; Brooks 1980; Jenner 1982b).

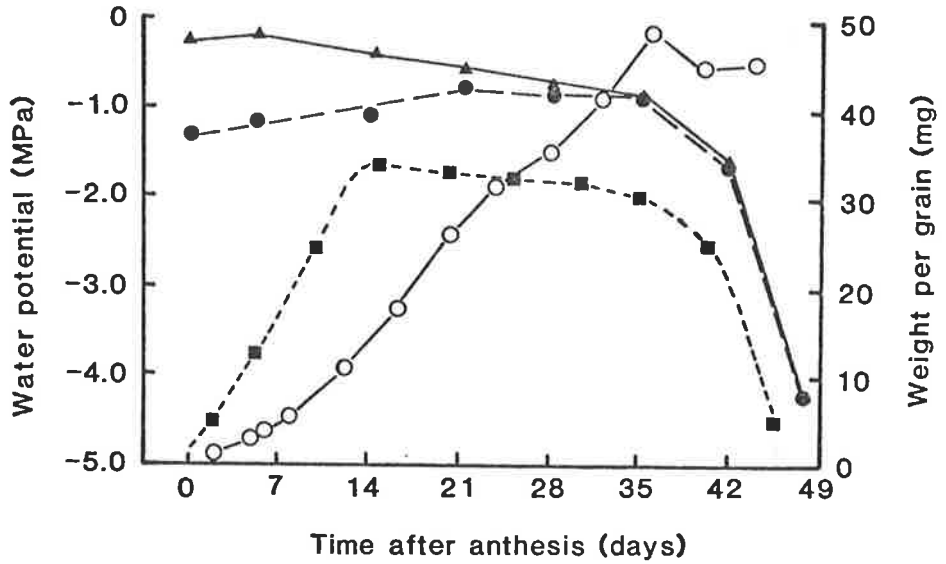


Fig.1.5 Grain dry weight (○), water content (■), and water potential of grain (●), and glumes (▲) during grain filling. (Adapted from Barlow et al. 1980)

During most of the grain-filling phase the water potential of the grain remains constant and decreases sharply as the grain matures (Fig. 1.5). The fluctuations in water and osmotic potentials in the grain are very small in comparison with the other parts of the plant both under normal (Kirkham 1979; Barlow et al. 1980) and water stress conditions (Barlow et al. 1980; Brooks et al. 1982; Aspinall et al. 1983; Barlow et al. 1983). This attenuation of water and osmotic potential of the grain, could possibly be due to its hydraulic isolation from the rest of the plant (the continuity of the xylem is interrupted at the base of the grain, Zee and O'Brien 1970a).

Little is known about the mechanism(s) of water transport into and within the grain, or of the resistances to the movement of water into and within the grain. Evidently, the accumulation of dry matter in the grain is independent of both the direction of flow and the net flux of water into the grain (Jenner 1982b).

1.6.2 CELL NUMBER AND CELL VOLUME

Cell number is the main determinant of final grain weight (Singh 1982), and strong emphasis has been laid on differences in cell number per endosperm to explain differences in grain weight among varieties (Brocklehurst 1977), between florets within a spikelet (Singh and Jenner 1982a) and to factors such as shading (Wardlaw 1970; Brocklehurst et al. 1978), grain removal (Brocklehurst 1977) and water stress (Wardlaw 1971; Brocklehurst et al. 1978). Evers (1970) reported that no cell division occurred in the endosperm of wheat later than 16 days after anthesis and other workers (Jennings and Morton 1963b; Wardlaw 1970; Briarty et al. 1979; Singh and Jenner 1982b) have come to similar conclusions using cell counts. However, biochemical analyses (Donovan 1979) have shown that the maximum DNA content of wheat endosperm is not reached until 25 days after anthesis, but this increase in DNA content per endosperm is presumably due to endoreduplication of nuclear DNA, rather than increase in cell number per endosperm (Donovan 1983). The rate and the duration of cell division in wheat endosperm is affected by temperature, the higher the temperature the earlier the cessation of cell division (Hoshikawa 1962; Wardlaw 1970; Radley 1976; Singh 1982). In barley, the rate of cell division in both the endosperm and the embryo increases with increasing temperature (Pope 1943). Once the cell number is determined, further increase in grain volume is mainly due to increase in cell volume (Fig. 1.6).

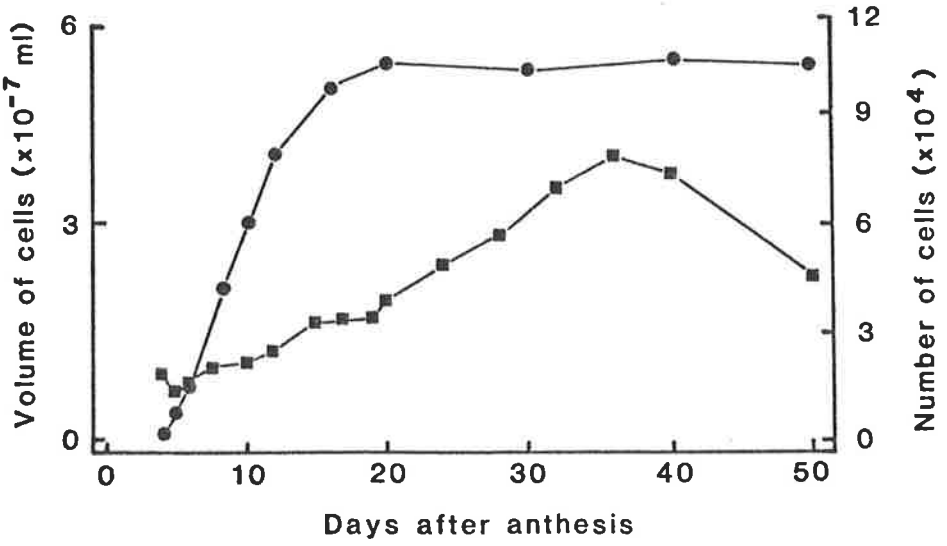


Fig. 1.6 Changes in the number of cells per endosperm (●) and volume per cell (■) during development of wheat grain. (From Hughes 1976)

Cell volume is also affected by temperature; wheat endosperm developing at high temperature has smaller cell size compared to that developing at ~~high temperature~~ lower temperature (Radley 1976). Final cell number on the other hand seems to be little affected by temperature during grain growth (Hoshikawa 1962; Wardlaw 1970).

1.6.3 SOLUBLE SUGARS AND AMINO ACIDS

Considerable information is available on the qualitative (Abou-Guendia and D'Appolonia 1972, 1973; Cerning and Guilbot 1973) and quantitative (Jennings and Morton 1963a; Cerning and Guilbot 1973; Jenner and Rathjen 1972a, 1972b; Kapoor and Heiner 1976; Meredith et al. 1980; Kumar and Singh 1981; Chevalier and Lingle 1983) changes in the free sugars of the wheat grain during grain growth. The most abundant sugars in the grain are sucrose, glucose, fructose and fructans, while glucosans and maltose occur in small quantities. Raffinose which is uniquely located in the embryo (Duffus 1979) appears only during the later stages of grain development. Absolute amounts and proportions of various sugars differ considerably among reports. However, most of the reports agree that sucrose and other soluble sugars (on per grain basis) peak around 10-15 days after anthesis and decline to steady level thereafter.

Non-protein nitrogen forms a high proportion (about 48%) of total nitrogen during the early stages of grain development; the proportion declines markedly to about 15% during the rapid grain-filling period, then declines more slowly to 3% at maturity. The amount of amino acids per grain is almost constant during grain development (Jennings and Morton 1963a).

1.6.4 HORMONES

The role of phytohormones in yield formation has been reviewed by Michael and Beringer (1980). Developing grains of wheat contain several growth substances the levels of which change substantially during the course of development (Wheeler 1972). Cytokinin activity shortly after fertilization is particularly high (Fig. 1.7), and its maximum level seems to coincide with the maximum rate of mitosis. Large grained varieties of barley (Michael and Seiler-Kelbitsch 1972) and wheat (Herzog and Geisler 1977) show a significantly higher level of cytokinin

activity than small grained ones, indicating that cytokinin may affect grain weight through an effect on cell division. Soon after the maximum level of cytokinin is observed, gibberellin content increases and this peak coincides with the cell expansion phase (Radley 1976). Auxin content increases somewhat later than do the gibberellins and it is present in maximum quantity just before maximum fresh weight, when starch and protein is accumulating rapidly in the endosperm. A fairly close relationship has been reported between grain growth rate and the levels of auxin and gibberellin in wheat (Wheeler 1972) and barley (Mounla and Michael 1973). ABA activity in the cereal grain increases during grain growth and its activity is at a maximum when grain growth stops and the decline in water content begins (Wheeler 1972; McWha 1975; King 1976; Radley 1976).

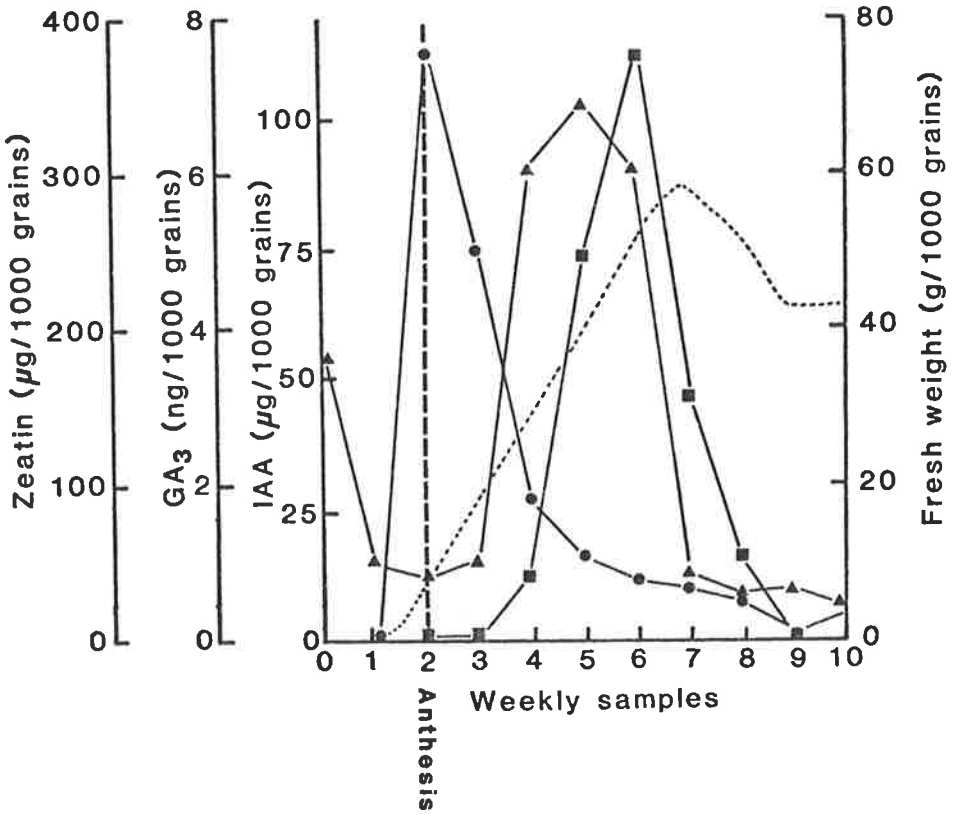


Fig. 1.7 'Hormonal' levels in ears and developing grain of wheat (cv Kloka). Cytokinin —●—; auxin —■—; gibberellin —▲—, and fresh weight --------.
(After Wheeler, 1972)

The grains in wheat ears grown at 25°C compared to those grown at 15°C mature sooner and reach their peak of gibberellin and ABA activity earlier (Radley 1976).

1.7 THE SOURCES OF GRAIN CARBON AND NITROGEN

1.7.1 CARBON

Sucrose is generally considered to be the substrate for starch biosynthesis in the developing cereal grains (Duffus 1979; Jenner 1982a), and is mainly derived from concurrent photosynthesis during grain filling (Archbold 1945; Porter 1962; Rawson and Hofstra 1969). The flag leaf and the ear itself are the main sources of carbon for the developing grain (Stoy 1965; Wardlaw 1968; Rawson and Hofstra 1969).

All parts of the wheat ear are capable of CO_2 fixation (Carr and Wardlaw 1965; Kriedemann 1966; Evans and Rawson 1970), and the contribution of ear photosynthesis to grain filling has been reported to vary from 10% (Lupton 1969) to 60% of dry matter (Frey-Wyssling and Buttrose 1959; Saghir et al. 1968, Rawson and Evans 1970). The awns can make a significant contribution to grain weight (Grundbacher 1963; Apel 1966; Evans and Dunstone 1970; Evans and Rawson 1970; Evans et al. 1972; Olugbemi et al. 1976).

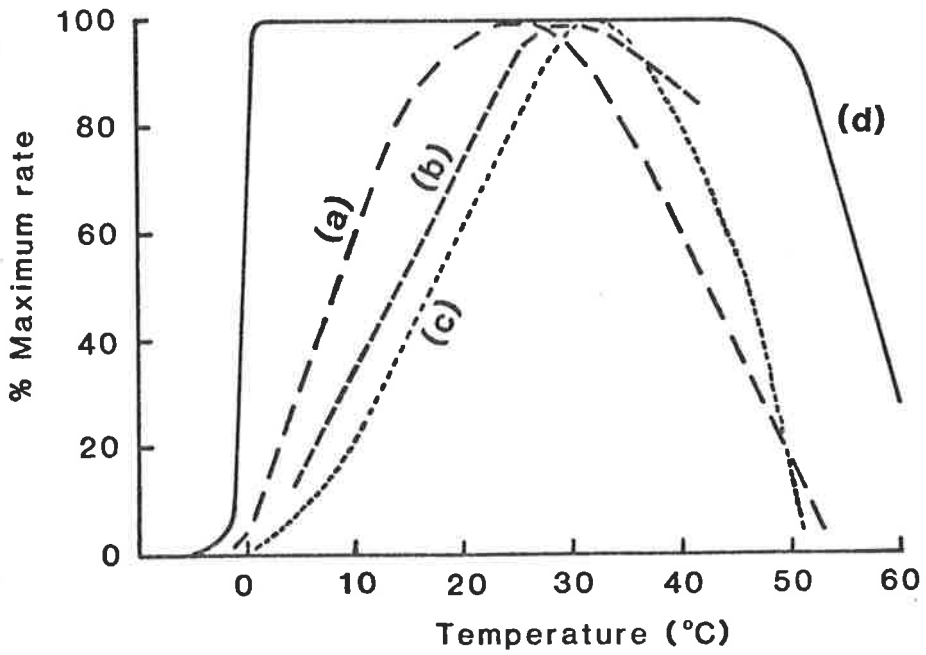


Fig. 1.8 The effect of temperature on net photosynthesis (a), vein loading (^{14}C-export) (b), translocation (the movement of ^{14}C-photosynthate through a 10 cm length of peduncle) (c), and growth (the rate of ^{14}C import by the grain) (d), in wheat. (Adapted from Wardlaw 1976)

Temperature and other environmental factors have a substantial effect on assimilate production and its distribution pattern. Flag leaf photosynthesis has a broad optimum around 25°C, declining sharply at temperatures below 15°C and above 30°C (Fig. 1.8). Increase in temperature of the whole plant, or the ear alone, enhances the movement of assimilates from the flag leaf to the ear (Wardlaw 1974; Ford et al. 1976; Wardlaw et al. 1980). The various processes involved in translocation may differ in their degree of temperature dependence, the loading of assimilate into the phloem, and its unloading into the grain are (possibly) coupled with cellular metabolism and, therefore, strongly dependent on temperature (Fig. 1.8). The mass flow of assimilate in the vessels, on the other hand, is scarcely dependent upon temperature (Fig. 1.8).

1.7.2 NITROGEN

There are two possible sources of grain nitrogen in wheat: soil nitrate absorbed and reduced during grain filling and transported directly to the grain, and remobilization of nitrogen from the senescing leaves and stem. Remobilization of nitrogen from other organs is highly dependent on environmental conditions during grain filling. Under conditions of adequate soil nitrogen, low temperature and adequate moisture levels, senescence and remobilization of nitrogen from leaves is slow, and much of the grain protein may be derived from N taken up during grain filling (Pavlov 1969). On the other hand, under less favourable conditions (high temperature, water stress and low soil nitrogen) almost all grain nitrogen may be derived from remobilization of nitrogen from other sources within the plant (Wardlaw 1975).

1.8 TRANSPORT OF CARBON AND NITROGEN INTO AND WITHIN THE GRAIN

Dry matter deposited as starch and protein in the developing wheat grain is derived from substrates produced elsewhere in the plant. Both carbon (sucrose) and nitrogen (amides and amino acids) are transported (along the longitudinal axis of the wheat grain) in the vascular bundle running in the furrow (Frazier and Appalanaidu 1965; Sakri and Shannon 1975; Donovan et al. 1983). The continuity of the xylem as it enters the pericarp of wheat caryopsis is interrupted by modified thick-walled

tracheary elements (Zee and O'Brien 1970a) hence, the phloem provides the main conduit for the transport of carbon and nitrogen (Donovan et al. 1983). There is minimal metabolism of the sucrose during its transport through the grain phloem (Sakri and Shannon 1975), metabolism of amino acids may occur (Donovan et al. 1983), however.

From the phloem sucrose is thought to move across the tissues of chalaza, pigment strand and nucellular projection (Frazier and Appalanaidu 1965; Jenner 1974a) into the endosperm cavity (Bradbury et al. 1956). The route of sucrose from the endosperm cavity to the cells of the endosperm is not certain. However, the balance of evidence favours the possibility that sucrose takes an apoplastic route (Jenner 1974a; Rijven and Gifford 1983). The movement of sucrose from the phloem to the endosperm cells is not dependent upon hydrolysis of sucrose (Jenner 1974b; Sakri and Shannon 1975).

There is a downhill sucrose concentration gradient from the phloem to the endosperm cells (Jenner 1974a) and gradients appears to be steeper at two places. One of these operates between the phloem and the endosperm cavity, and the other at the boundary of the cellular compartment and extracellular space. The physiological nature of these resistances is unknown, but the first might be a manifestation of unloading and/or transport across the pigment strand and nucellar projection, while the second may reflect trans-membrane resistances to the flux of sucrose into the cells. Transport of sucrose across the cell membrane is either by diffusion (Jenner 1980a) and/or "facilitated diffusion" (Rijven and Gifford 1983).

So far, there is no published information on the transport of amino acids within the endosperm. However, transport by the same route and/or mechanism as described for sucrose is not unlikely.

1.9 CARBOHYDRATE METABOLISM IN THE PERICARP

Little is known of the contribution to grain filling of the layers which surround the endosperm. During most of the grain filling period the pericarp is a bright emerald green tissue surrounded by a translucent outer layer. The green chlorophyllous layer of the pericarp is active in carbon dioxide fixation (Carr and Wardlaw 1965; Evans and Rawson 1970) and has a number of biochemical characteristics similar to that found in the leaves of C_4 plants (Nutbeam and Duffus 1976). The

tissue contains a number of enzymes characteristic of C_4 photosynthesis including high activities of phosphoenolpyruvate carboxylase and orthophosphate dikinase (Duffus and Rosie 1973; Wirth et al. 1977).

Sucrose derived from pericarp photosynthesis may be available to the endosperm for starch synthesis (Nutbeam and Duffus 1976). Furthermore, the photosynthetic activity of the cross cells may supply oxygen for endosperm respiratory processes, and the carbon dioxide released by endosperm respiration may be refixed during this photosynthesis (Cochrane and Duffus 1979). Hence, the pericarp may play a vital role in supplying carbohydrate precursors, reducing losses and even controlling overall endosperm activity by regulating the oxygen supply (Duffus and Cochrane 1982).

1.10 TEMPERATURE, METABOLIC PROCESSES AND MEMBRANE STRUCTURE

Temperature conditions encountered by crop plants may broadly be divided into three ranges: a lower range around zero to 5°C, where there is little or no growth or development, increasing the temperature from 5°C up to about 30°C accelerates growth and development, but at temperatures above this optimum, growth and development decline reaching zero at an upper limiting temperature. Inhibition of growth and/or development at both extremes may be due to the effect of temperature on the membrane structure and biological integrity of the system, and has been reviewed in detail by Levitt (1980). The acceleration in rate of development between 5°C and 30°C is mainly through the effects of temperature on the rate of metabolic processes which govern cellular differentiation (cf. Monteith 1981). Different physiological processes may differ greatly in their sensitivity to temperature; even the same process may differ in its sensitivity at different physiological stages of development.

The wheat grain during its growth passes through a sequence of developmental stages (Rogers and Quatrano 1983), and the duration of each stage is presumably determined by temperature. Although there are many reports on the effect temperature on the duration of grain growth (Section 1.2.2), reports on the effects of temperature on the individual developmental stages are lacking, however. Each developmental stage possibly requires a certain number of degree days for its completion, which Monteith (1981) prefers to call "thermal time", stressing that

time as perceived by the plant, is a function of temperature whereas, in conventional physical terms, temperature is a function of time. Plants appear to gauge the rate of developmental processes by reference to a biochemical clock which ticks faster as temperature increases. Thus, the plant is maintaining a constant rate of development (in reference to the biochemical clock), but it is developing faster by reference to the physical clock.

The biological activity of cells is, to greater or lesser extent, dependent on temperature. This is due to the temperature dependence of thermodynamic and kinetic constants that determine directions and rates of chemical reactions, conformational transition of biological macromolecules, phase transition of lipids etc. Any temperature shift invariably results in alteration of a metabolic rate and distorts existing relationships between reaction rates of numerous constituents.

The cold-induced shift in the equilibrium between starch and sugars in potatoes, is a classical example of an effect of temperature on carbohydrate interconversion. At temperatures between +1°C and +5°C sugars, especially sucrose, accumulate in potato at the expense of starch, and the process can be reversed by raising the temperature (Burton 1972; Isherwood 1973, 1976). The rate of sucrose to starch conversion in the grain of detached ears of wheat at 30°C (supplied with sucrose), has been shown to be 4.4 times greater compared to that at 15.5°C (Jenner 1968). The rate of respiration (determined on isolated grains) is a linear function of temperature (Fig. 1.9)

Many physiological processes of the plant are inhibited at high temperature. It is well known that proteins are denatured at high temperature (Alexandrov 1977) and there is evidence that high temperature might also disrupt membrane structure. For example, the irreversible inhibition of photosynthesis at high temperature correlates with the loss of photosystem II activity (Pearcy et al. 1977), the loss of photophosphorylation (Mukohata et al. 1973) and a change in the organization of chlorophyll in the membrane (Schreiber and Berry 1977; Schrieber and Armond 1978). The disruption of all these membrane-associated processes occurs simultaneously. It seems reasonable, therefore, to postulate that all these high temperature induced effects are related to a perturbation of membrane structure and function by heat.

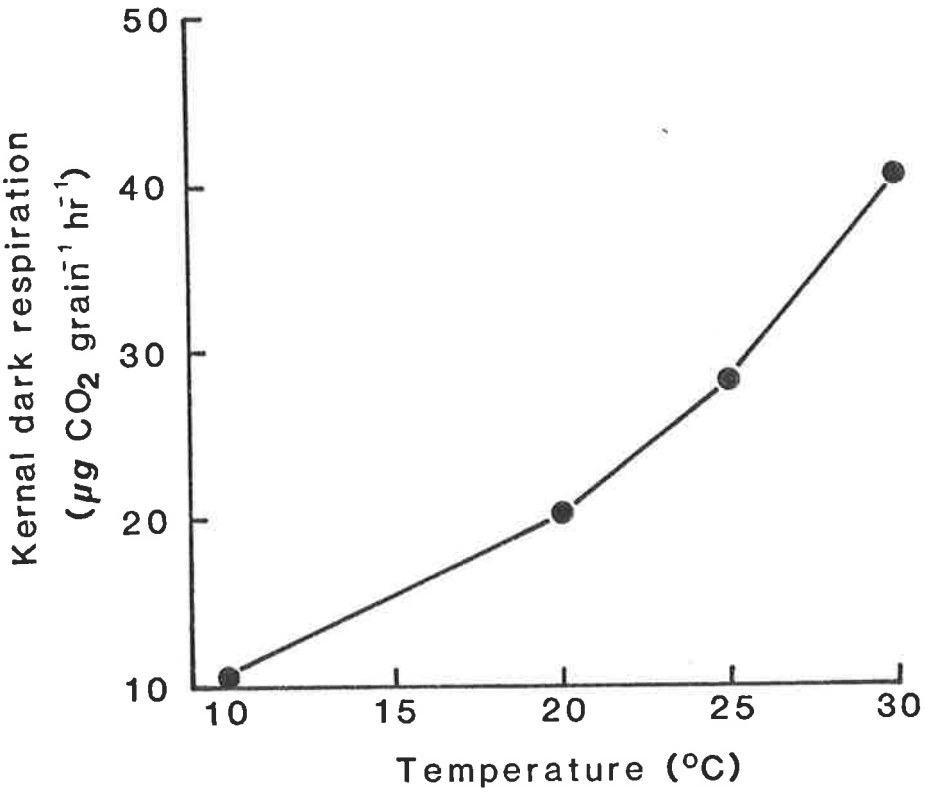


Fig. 1.9 Effect of temperature on dark respiration of isolated wheat grains.
(Adapted from Chowdhury and Wardlaw 1978)

In corn mitochondria, coupling between oxidation and phosphorylation began to decrease at 30-35° and at 40°C uncoupling was complete (Kurkova and Andreeva 1966). Thermal uncoupling in chloroplasts occurs at temperatures lower than that of mitochondria, which in turn is lower than that leading to heat-inactivation or perturbation of the plasma membrane. The simplest explanation for such a difference in sensitivity to high temperature may be due to differences in membrane composition. It is generally accepted that the chloroplast membrane is the most sensitive to high temperature, the mitochondria less so, and plasma membrane least sensitive (Levitt 1980).

1.11 SUMMARY AND OUTLINE OF THIS INVESTIGATION

Yield formation in wheat is a complex process, resulting from the interaction of many plant processes with each other and the environment. Temperature begins to affect crop yield when the seed from the previous crop is being formed and continues to have an affect through all the growth stages of the plant until the day of final harvest. Although, the

potential grain yield in wheat is determined by the time of anthesis, the actual yield at harvest is highly dependent on the environmental conditions during the period of grain growth. Temperatures above about 20°C during grain filling result in a reduction in the final weight per grain (Section 1.2.2). Exposing the ears alone to temperatures above 25°C during grain-filling is effective in reducing the final weight per grain.

Starch is a major component of cereal grain dry matter, and heat stress or water stress induced reductions in grain dry matter are accompanied by a reduced starch content (Section 1.4.2.1.1). Starch occurs in wheat endosperm as two distinct types of granule: A-type and B-type (Section 1.4.2.1.1). Although high temperature during grain-filling reduced starch deposition, little is known about the effect of temperature on the production of starch granules. Hoshikawa (1962) reported that grains from wheat plants grown at 30°C seemed to have smaller A-type and fewer B-type granules than those from the plants grown at 20°C, but no critical measurements of granule number were made. So, a part of this investigation deals with the effect of a brief period of an elevated temperature on the numbers of both types of granule.

Under conditions of high temperature senescence of the green tissue (especially the leaves) is enhanced and there is a significant increase in the respiration of the grain (Stoy 1965; Spiertz 1974; Chowdhury and Wardlaw 1978; Vos 1981). These responses may possibly lead to a shortage of carbohydrates for grain growth (Thornley 1971). However, no critical evaluation of the availability of substrate for grain growth under conditions of high-temperature has been made. Neither the physiological nor the biochemical reasons for the observed reductions in the grain weight as a result of high temperature have been resolved.

The following possibilities have been investigated in this study:

(a) changes in the water relations of the ear and/or the grain (high-temperature induced water-stress due to excessive evaportion); or

(b) alteration in the availability of soluble carbohydrates for grain-filling; or

(c) impairment of the synthetic capacity of the grain.

The quantitatively most important component of grain dry matter, apart from starch, is protein. Several studies have found that protein, as a per cent of dry matter, increases under stress conditions (Section

1.4.2.2), which might suggest that protein and starch accumulation in the grain might have a differential response to stress conditions. To test this hypothesis four cultivars of wheat have been compared for their sensitivity to a brief period of heat-stress during grain-filling for the pattern of starch and protein accumulation.

Temperature possibly has a direct effect on the metabolic processes of the grain, and these processes may differ greatly in their sensitivity to temperature. Effects of temperature on uptake and incorporation of ^{14}C-sucrose by isolated wheat endosperm has, therefore, been examined.

SECTION 2: MATERIALS AND METHODS

MATERIALS AND METHODS

2.1 THE SOURCES OF MATERIALS

2.1.1 THE SEED SOURCE

Wheat (*Triticum aestivum* L.) Cv. Sonora, which was the main material for this investigation, was supplied by Dr. A.J. Rathjen, Agronomy Department, Waite Agricultural Research Institute. The seed for the other three cultivars of wheat (Kite, WW15, RC-81-429) were kindly supplied by Mr. Robin Wilson of Roseworthy Agricultural College, South Australia.

2.1.2 CHEMICALS AND REAGENTS

All the chemicals and reagents used for this study were of analytical grade or at least laboratory grade. The source of the important chemicals is given where they appear first. Glass distilled water or low conductivity deionised water was used for making solutions.

2.2 PLANT CULTURE CONDITIONS

2.2.1 PLANT CULTURE

Five to six, healthy, uniform seeds were sown either in plastic bags arranged in wooden boxes (six bags per box), or in plastic pots, containing 1.2 litres of steam-sterilized soil. Soon after germination the seedlings were thinned to 3 per container. All tillers were removed periodically to restrict the plants to the main culm. An appropriate amount of liquid fertilizer (Supagro, Agchem. Pty. Ltd., Parafield Gardens, S.A.) was added to supply 30 mg nitrogen per plant after four weeks of sowing. Chemical analysis of the fertilizer was as follows:

Major Elements

5.24% N as Urea Nitrogen
1.66% N as Ammonia
2.02% N as Nitrate
8.92% Total Nitrogen

3.70% P as water soluble Phosphorous
7.65% K as Nitrate of potassium

Trace elements

0.004% Zinc
0.01% Copper
0.02% Iron
0.02% Magnesium
0.02% Magnese
0.003% Cobalt
0.002% Molybdenum
0.001% Boron

2.2.2 GROWTH CONDITIONS

Plants were raised for 2 to 4 weeks, (unless specified otherwise) in a glass house maintained between 15 - 25°C, and then transferred to a growth room. The glass house was white washed during the hot summer months and cooling was provided by evaporative coolers. Winter heating by steam pipes was supplemented with a sealed oil element unit.

The growth room had a plant platform of 2.9 x 1.0 m with a light bank of 8, Lu 400/EB high pressure sodium lamps (Lumalux, Sylvania) and 10, 65 watt white fluorescent tubes (Osram, Australia) providing a photon flux density of about 560 µmol $m^{-2}s^{-1}$ (400-700nm) at ear level. The photoperiod was 14 h in all experiments and the day/night temperature was held at 21/16°C for control plants. The relative humidity in the growth room, determined by wet and dry bulb thermometers, varied between 60% and 80% during the day (average 70%, vapour pressure deficit, KPa 0.75) and between 75% and 85% during the night (average 80%, KPa 0.50).

2.3 TREATMENTS

2.3.1 ARRANGEMENT OF PLANTS

Ears were individually labelled with the date of anthesis (Introduction - Section 1.3), and timing of all subsequent operations was related to this date. About one week after anthesis bags having at least two ears of the same date of anthesis were arranged in boxes to form uniform sets of the plants.

2.3.2 IMPOSITION OF TREATMENTS

Whole plants or ears only were exposed to high temperature at various developmental stages. Plants were exposed to high temperature by transferring them to a controlled environment growth cabinet maintained at the required temperature for a desired length of time. The growth cabinet had a plant platform of 1.2 x 1.0 m with a light bank of 4, Lu 400/EB sodium lamps (Lumalux, Sylvania) and 5, 65 watt white fluorescent tubes (Osram Australia) providing a photon flux density of about 560 µmol $m^{-2}s^{-1}$ at ear level. The relative humidity in the growth cabinet was maintained within the range 60-80% (determined by wet and dry bulb thermometers) during the day and the night.

The relationship between the various high temperature treatments and stage of grain development (used for imposition of a treatment) are detailed in the sections describing the specific experiments.

2.3.3 EAR WARMING

The ears were warmed in transparent perspex boxes (76 x 28 x 26 cm) positioned at the level of the ears (Fig. 2.1). Ears were held in position by thin sheets of perspex (thickness 3 mm) and gaps around every penduncle was sealed using a small piece of Blu-Tack (Emhart Australia Pty. Ltd.). A single box contained up to 72 ears. The stream of air entering the box was heated by a thermostatically controlled heating coil, and the total volume of air in the box was displaced in 1.4 min. by the incoming air. There was a slight temperature gradient ($< 1^{o}C$ between the inlet and outlet of the box. Blocks of ears were arranged in such a way that any effect of this gradient would be eliminated as a block effect during sampling. Light intensity inside the box was slightly reduced by the perspex top (500 µmol $m^{-2}s^{-1}$ as compared to 560 µmol $m^{-2}s^{-1}$); light intensity at the flag leaf level (below the box) was decreased slightly more, to 400 µmol $m^{-2}s^{-1}$ as compared to 500 µmol $m^{-2}s^{-1}$ at the same level in other parts of the room. The temperature of the air surrounding the flag leaf was essentially the same as the rest of the room (Section 2.2.2). Temperature of the air inside the box and the air outside was continually monitored using thermocouple probes. When necessary, additional measurements of temperature were made (at specific places) by using copper-constantan thermocouples using melting ice as a reference.

Fig. 2.1 An ear warming assembly.

Part A

a. Fan
b. Thermostatically controlled heating coil
c. Flexible reinforced ducting (50 mm diameter)
d. Water tank
e. Humidifier
f. Mixing chamber with or without humidifier

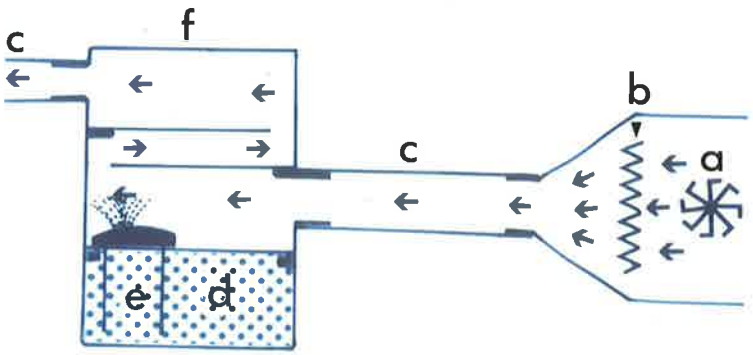
Part B

g. Perforated air inlet
h. Night temperature thermostat
i. Day temperature thermostat
j. Thermocouple probe for recording the temperature
k. Vent for manipulation of the ears

Part C

l. Temperature control board
m. Air outlet
n. Wheat ears cultured on sucrose
o. Refrigerated water bath
p. Change over clock

A



2.3.4 HUMIDIFICATION

Higher levels of humidity in the air were achieved by passing it through a humidifying box which contained a humidifier (De Vilbiss vaporizer, Pennsylvania, U.S.A.). The voltage supply to the humidifier was controlled by voltage controllers (Berco Rotary Regavolt, England) which were operated by a change over clock coinciding with the photo period of the growth room, thus, keeping the relative humidity of the air passing through the ear warming box constant during the day and the night. Relative humidity in the boxes was determined by using wet and dry bulb thermometers.

2.4 LIQUID CULTURE OF WHEAT EARS

Wheat ears were cultured in the nutrient medium according to the modified method of Singh and Jenner (1983). Plants were cut close to the base of the culm and immediately placed in a flask containing distilled water. All further operations were carried out in a laminar flow cabinet to minimize microbial contamination. Leaf blades were removed and the stem was surface sterilized by wiping with a diluted solution (1 to 4) of a commercial bleach (White King, Kiwi Australia Ltd., Melbourne). The stem was first cut below the second node from the top and then it was cut again 2 cm below the peduncular node under sterile deionised water. The penduncles were fitted through a cotton plug in an autoclaved 90 ml glass tube containing 40 to 60 ml of filter sterilized culture medium. The tubes containing the detached ears were maintained at 1-2^{o}C by immersing them in a refrigerated water bath while keeping the ears at desired temperatures.

Constituents and preparation of culture medium stock solutions:

		$g l^{-1}$
Major Elements Solution A :	$CaCl_2$ –	8.80
Major Elements Solution B :	KH_2PO_4 –	24.00
:	$MgSO_4.7H_2O$ –	7.57

Minor elements stock solution:

H_3BO_3	-	6.20
$MnSO_4.4H_2O$	-	22.30
$ZnSO_4.4H_2O$	-	8.60
KI	-	0.83
$Na_2MoO_4.2H_2O$	-	0.25
$CuSO_4.5H_2O$	-	0.026
$CoCl_2.6H_2O$	-	0.026

Iron solution:

$FeSO_4.7H_2O$	-	5.57
Na_2EDTA	-	7.45

Vitamin solution:

Thiamine HCl	-	0.04
myo - Inositol	-	10.00

(All solutions were prepared as described by Linsmaier and Skoog 1965).

The culture media were prepared by combining the above solutions as follows: major elements solution A, 50 ml; major elements solution B, 50 ml; minor elements solution, 1 ml; iron solution, 5 ml; vitamin solution, 10 ml; sucrose (20, 40 or 60 gl^{-1}) and L-glutamine (0.5 g N l^{-1}, BDH chemicals Ltd., England). All the solutions prepared were adjusted to pH 5.0 and made to 1 litre and then sterilized by filtering through 0.22 µm Millipore filters (Millipore Corp. Bedford, Massachusetts).

2.5 METHODS OF DETERMINATION

2.5.1 FRESH AND DRY WEIGHTS

Immediately after harvesting, the sample's fresh weight was recorded and it was then dried in an oven for 48 h, at 80°C and reweighed after equilibration at laboratory temperature in a desiccator over self-indicating silica gel (about 6 - 20 mesh). The differences between the fresh and dry weights was taken as the water content of the sample. The samples (of grains, rachis or floral organs) used for extraction of sugars or for nitrogen determinations were freeze-dried after taking the fresh weight. Freeze-dried samples were weighed after allowing them to come to laboratory temperature in a desiccator over silica gel.

2.5.2 SPIKELETS AND GRAIN NUMBER

A uniform system of specifying the position of the sampled florets was used. Spikelets were numbered from the base of the ear towards its tip, number 1 being taken as the lowest fertile spikelet. Within each spikelet, florets were designated a, b, c etc. in acropetal succession.

The total number of spiklets on an ear, excluding sterile spikelet(s), at the base of ear was recorded.

For grain number estimations, the grains were separated individually from their enclosing bracts and counted. An aborted grain, one that appeared more like an ovary rather than a typical grain, was discarded and not included for grain number estimation.

2.5.3 WATER POTENTIAL (Ψ_w)

Two types of psychrometers were used in this investigation. A Spanner Psychrometer was used for large samples (8 grains or their enclosing bracts - glume, palea and lemma without awns), while a Wescor (HR 33T) instrument was used for single grain samples or its enclosing bracts. Grains or bracts were removed from the ears, and immediately sealed in steel chambers. After 3 - 3.5 h equilibration, water potential was recorded automatically (for Spanner Psychrometer) or manually (Wescor Psychrometer). For the Wescor instrument, the value for each sample was taken as an average of 3 readings at 30 min intervals of both psychrometric and dew point modes.

2.5.4 OSMOTIC POTENTIAL (Ψ_π)

Osmotic potentials were measured after cell membranes had been disrupted by freezing and thawing - samples were sealed in 2.2 ml plastic tubes and placed immediately in liquid nitrogen. After freezing samples were allowed to equilibrate with room temperature (25 $\pm 1^{o}C$), while sealed in the tubes, then transferred to a psychrometer chamber and osmotic potential determined as water potential.

2.5.5 TURGOR POTENTIAL (P)

Turgor potential was taken as the difference between Ψ_w and Ψ_π of samples from opposite sides of the same ear.

2.5.6 STARCH GRANULE NUMBER AND SIZE

Four endosperms per sample were fixed in acetic-alcohol (1:3;v/v) for 24 h and then stored in 70% aqueous ethanol. Fixed material was transferred through a series of mixtures of ethanol and water, and finally to water. The material in 2 ml of deionised water was incubated with 1.0 ml of 1% cellulase (EC 3.2.1.4, from Trichonderma viride, Sigma Chem. Co.) containing 0.01% of Chloramphenicol (Parke, Davies and Co., Sydney) and 0.01% Streptomycin (Glaxo Australia, Pty. Ltd.) in 25 mM potassium phosphate buffer, pH 7.0, at 35°C for 6 h with occasional shaking. At the end of incubation, the endosperm remains were macerated with a glass rod, and the suspension was passed through a 14 gauge hypodermic needle, and the syringe was rinsed with above potassium phosphate buffer to wash out any remaining endosperm. The suspension was made to 10 ml, and centrifuged at 2000 g for 20 min and the supernatant was discarded. The starch granule pellet was suspended in 5 ml of buffer (25 mM potassium phosphate, pH 7.0) and starch granules were stained by adding 100 µl of iodine solution (0.05% iodine in 0.5% KI - Brooks et al. 1982). Starch granule suspension was made upto a known volume (10 or 25 ml) with deionised water.

Starch granule number was determined using a portion of the starch granule suspension (20 to 40 µl) by method of Singh and Jenner (1982b) for nuclei counting using Olympus BH system microscope with calibrated eye piece graticule. Classification of starch granules into larger (A-type) > 10 µm and smaller (B-type) < 10 µm was carried out by using an ocular micrometer.

2.5.7 NET FLUXES OF DRY MATTER AND OF WATER THROUGH THE STALK OF THE GRAIN

The net fluxes of dry matter and of water through the stalk of the grain were determined by using the method of Jenner (1982b), and details of the method are as follows:

2.5.7.1 BASIS OF THE METHOD

Fluxes of water and dry matter were measured gravimetrically. Briefly, grains were stripped of their enclosing bracts (glumes, lemmas and paleas) and sealed within capsules which contained water or

solutions of known water potential. Fluxes were measured as differences between initial and final weights of the grains and the contents of the capsules.

2.5.7.2 PREPARATION OF EARS

Experiments were started on day 20 after anthesis on control ears (held at 21/16°C throughout) or on ears warmed at 33/25°C (day/night) for five days (between days 15 and 20 after anthesis) at high or low humidity (Section 2.5.7.7.2). The ears were secured to a curved frame exposing the row of spikelets on its convex upper surface to manipulation. From a set of three spikelets, the a florets (most basal within a spikelet) were removed and their grains were weighed. Florets distal to the b grain were removed, and the palea, the lemma and the glume were carefully removed, in that order, from the b floret, leaving the grain attached to the rachis by its slender stalk. Three florets were prepared in this way on any one ear, and ears on which any exposed grains appeared damaged (their stalks broken) were rejected.

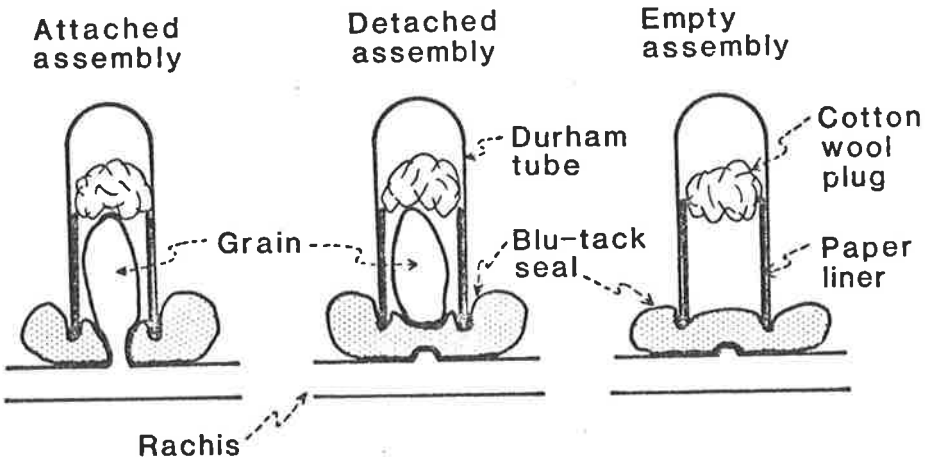


Fig. 2.2 The three types of assembly. The capsules contain a small plug of cotton wool, and a filter paper liner (14 x 6 mm) at the open end. The saturated plug (soaked with water or solution of known water potential) is pulled down until it contacts the liner immediately before the capsule is placed over the grain. Attached and detached assemblies are idential to each other except for lack of continuity between the grain and the rachis in the detached assembly. Exchanges of matter between the Blu-Tack seal and the rachis and the atmosphere are measured with the empty assembly.

2.5.7.3 ENCAPSULATION

The purpose of encapsulation was twofold: to enclose the grain within a system allowing the evaluation of net fluxes of matter between the grain and the plant, and to regulate the water potential gradient between the plant and the atmosphere surrounding the grain.

About 400 mg of Blu-Tack was weighed to the nearest 0.1 mg (weighing was done on a balance with a repeatability of ±0.05 mg). A small piece of Blu-Tack was moulded into a little collar around the stalk and pressed on the rachis, sealing broken surfaces resulting from the removal of bracts. The collar also provided support for the grain. A larger piece of Blu-Tack was moulded around the base of the grain, forming a seal firmly attached to the rachis. About one quarter of the grain was embedded in the seal, the seal completely surrounded the grain, giving a separation of 2 - 3 mm between the grain and the rachis.

Capsules were prepared from small plastic Durham tubes (0.35 ml volume). The cotton plug was injected with 0.1 ml of water or a solution of polyethylene glycol (PEG 4000, BDH reagent grade), and the capsule was weighed, the cotton plug was pulled down so that it was positioned 4-6 mm from the mouth of Durham tube and immediately inverted over the grain and pressed firmly into the Blu-Tack. As the capsule was placed over the grain the paper liner slid into the tube, making contact with the plug, and the plug touched the distal end of the grain. Thus, at least the distal end of the grain, and the liner surrounding it and touching it in parts were wetted with solution. The grain was not totally immersed in liquid, and small air-spaces around it were connected with the reservoir of air at the distal end of the tube through the porous plug. A ridge of Blu-Tack was raised around the capsule and tamped to form a seal. One floret on the other side of the ear was left intact within its bracts for comparison with the encapsulated grains. Plants with encapsulated ears remained undisturbed for 4 days in the growth room (Section 2.2.2)

2.5.7.4 FINAL WEIGHING

At the end of the experimental period the whole assembly (Blu-Tack, grain and capsule) was removed from the rachis and weighed. The three components of the assembly were separated from each other and weighed, and the grains were dried.

2.5.7.5 DRYING THE GRAINS

Individual grains were inserted into wells drilled in metal blocks and dried in an oven at 80°C for 2 days. After cooling in a desiccator over self-indicating silica gel, the grains were reweighed. The difference between the fresh weight and the dry weight was taken as the weight of water in the grain.

2.5.7.6 TESTING FOR BROKEN STALKS

Manipulation of the grain while embedding it in the seal sometimes resulted in breaking the stalk. As there was no simple way of establishing whether or not the stalk was intact at the time of encapsulation, broken stalks were detected at the end of experimental period. Ears were cut from the culm and cultured for 1 h in ^{14}C-sucrose solution (20 mg ml^{-1}, 0.5 mCi g^{-1} sucrose). After final weighing and drying (Section 2.5.7.5) the grains were secured, furrow uppermost, to a metal planchet in a small pad of Blu-Tack. The planchets were counted with an low-background gas-flow counter [Nuclear - Chicago, Model C115] with a thin window.

2.5.7.7 ESTIMATIONS OF FRESH WEIGHT, DRY WEIGHT AND WEIGHT OF WATER IN THE b GRAIN

As the initial fresh and dry weights of the b grains in the attached assemblies could not be measured directly, these parameters were estimated as follows.

2.5.7.7.1 SELECTION OF SPIKELET NUMBERS

Grains from both a and b florets were taken, separately, from spikelets 4 to 14 (numbered from the base of the ear, Section 2.5.2) of a group of 8 ears on day 20 after anthesis. Both grains were weighed (separately) fresh and again after drying. The difference between the fresh weight and the dry weight was taken as their water content. Analyses of variance for fresh weight, dry weight and water content for floret a or b revealed that spikelets 6 to 11 did not differ significantly for any of the above parameters for either floret, and

hence, grains from these spikelets were selected for determination of the relationship between the a and the b grains.

2.5.7.7.2 PREDICTING THE WEIGHTS OF THE b GRAIN

Grains from both a and b florets were taken from 6 spikelet positions (6 to 11) for determination of the relationship between a and b, on day 20 after anthesis, for the following sets of ears:

Treatment	No. of ears used	Treatment period (between days after anthesis)
(a) Control-held at 21/16°C throughout	25	no warming
(b) High humidity-ears warmed at 33/25°C	16	15–20
(c) Low humidity-ears warmed at 33/25°C	24	15–20

Spikelet by spikelet regression analyses of grain b on grain a were performed for fresh weight, dry weight and water content, within a treatment. Usually, grains b and a, within a spikelet for all the treatments, were closely related. However, many values of the coefficients differed substantially from unity (Table 2.1).

Within-spikelet differences between a and b were analysed by the analysis of variance for all the three treatments and they are plotted in Fig. 2.3. Although there were significant differences ($P \leq 0.05$) between spikelets (all three parameters in low humidity and fresh weight in control ears), nevertheless these differences were small.

The numbering system for spikelets was arbitrary, so odd and even sides of the ear were not distinguishable unequivocally from each other. Moreover the total number of spikelets varied from ear to ear (between 18 - 21). Thus, there would have been no justification for using correction factors for individual spikelets, so the mean values for the difference between a and b grains were used for correcting the weight of the a grain to obtain the estimate of the corresponding initial b grain

Table 2.1

Relationship between a and b grains by spikelet positions. Values are Regression Coefficients for b on a. Details in the text.

Spikelet Number	Ears warmed at:								
	*Control Ears			*High Humidity			*Low Humidity		
	Fresh weight	Dry weight	Water content	Fresh weight	Dry weight	Water content	Fresh weight	Dry weight	Water content
6	1.004	1.020	1.002	1.040	1.023	1.137	1.013	0.991	1.022
7	0.984	0.813	1.034	0.971	1.085	0.896	0.970	0.973	0.985
8	1.020	0.990	1.046	1.007	0.971	1.107	1.001	0.973	1.000
9	0.946	0.878	0.968	0.934	0.960	0.984	1.004	0.982	0.991
10	0.992	0.931	1.033	1.013	0.974	0.967	0.983	0.985	0.984
11	0.985	0.797	1.089	1.003	0.953	0.977	0.976	0.995	0.993

* 25, 16 or 24 ears were used, for Regression Coefficient determination, for control, for ears warmed at high humidity or low humidity respectively.

parameter. The actual differences used are set out in the tabulation below.

Parameter	Treatment		
		Ears warmed at:	
	Control	High humidity	Low humidity
Fresh weight (mg)	-2.6	-1.6	-1.7
Dry weight (mg)	-0.2	-0.2	-0.2
Water content (mg)	-2.4	-1.4	-1.5

Treatments were allocated systematically in a Latin Square design (3 x 3) to each spikelet position in turn starting with spikelet number 6. Thus possible differences in response to treatments between spikelets (Fig. 2.3) were not confounded with any treatment.

2.5.7.8 THEORETICAL

The transfer of mass from the rachis into the grain through its stalk is described by:

$$F = F_{dm} + F_w, \qquad (1)$$

where F is the net flux of matter into the grain, and F_{dm} and F_w are fluxes of dry matter and water respectively. Negative values of F, F_{dm} or F_w signify flux in the reverse direction i.e. from the grains into the rachis.

The value of F is given by:

$$F = \Delta A - \Delta D, \qquad (2)$$

where ΔA and ΔD are, respectively, the differences between the initial weights and final weights of the attached (A) and detached (D)

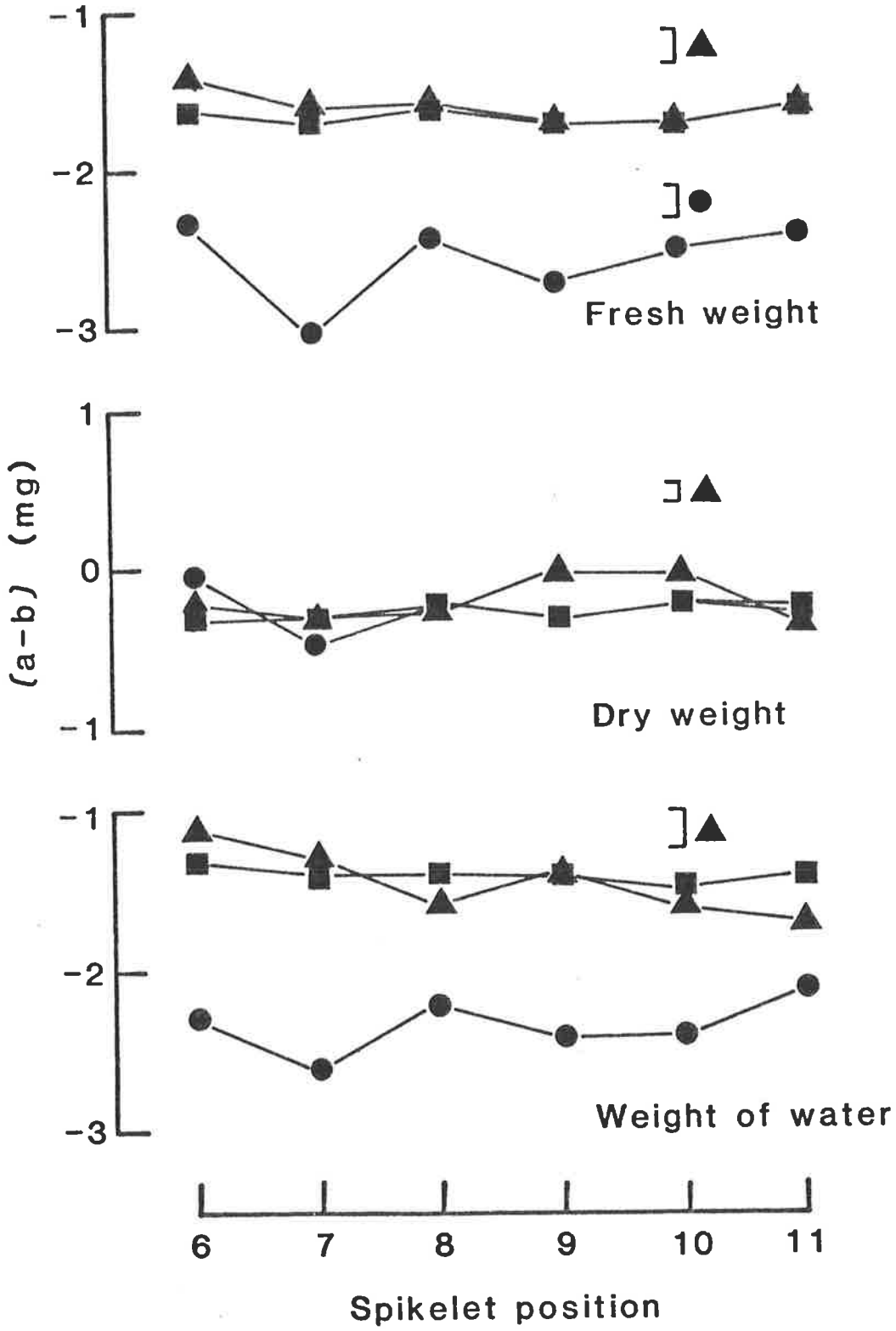


Fig. 2.3 Spikelet by spikelet differences between a and b grains. For control (●), ears warmed at high humidity (■), and ears warmed at low humidity (▲).

assemblies (Fig. 2.2). Detached assemblies were included to evaluate all changes in weight (evaporation, etc.) which were not attributable to the transfer through the stalk. It is assumed that all losses or gains in weight of the components of attached and detached assemblies are

identical except for those resulting from transfers through the stalk in the attached assembly.

The intial weight of A is taken as the sum of the components at the time of encapsulation, the weight of the b grain being estimated from the weight of the a grain taken from the same spikelet.

Given that A_{dm} and D_{dm} are the weights of dry matter in the b grains of the attached and detached assemblies respectively, F_{dm} is measured using:

$$F_{dm} = \Delta A_{dm} - \Delta D_{dm}, \quad (3)$$

were ΔA_{dm} and ΔD_{dm} are the differences between the initial and final b grain dry weights in the attached and detached assemblies. Again, initial values of the b grain dry weights are estimated from the corresponding a grains.

F_w can be estimated from equation (1) by difference, thus:

$$F_w = F - F_{dm} \quad (4)$$

F_w can also be estimated by addition as follows:

$$F_w = \Delta AG_w + \Delta AC - AL + WL. \quad (5)$$

Here, ΔAG_w is the change in the weight of water in the b grain of the attached assembly, and ΔAC is the change in the weight of the capsule of an attached assembly. AL is the loss of water by evaporation (or by other means) from the attached assembly, and WL is the loss of water by evaporation, when the components of the assemblies are being finally weighed. As AL cannot be measured directly, DL is measured instead. DL is the loss of water (as in AL above) from the detached assembly, and it is assumed that DL and AL are equal.

$$DL = \Delta D - BA, \quad (6)$$

Where BA is the weight of matter exchanged between the Blu-Tack seal of the attached assembly and the rachis and/or the atmosphere. It is assumed that transfer of matter between the seal and the rachis, and the atmosphere outside the capsule are the same in all assemblies. BA is

thus estimated by weighing a Blu-Tack seal before and after sealing an empty capsule (Fig. 2.2) on the rachis.

Finally,

$$F_w = \Delta AG_w + \Delta AC - DL + WL \qquad (7)$$

Both methods of estimating F_w have been used in expt. 10 (reported in Section 3.4). The validity of each method is dependent upon a different set of assumptions, so close agreement between the two methods lends credence to the estimates. In addition, data gathered for the additive method in equation (7) provide information on the exchange of water between the grain and its surroundings.

2.6 ANALYTICAL TECHNIQUES

2.6.1.1 EXTRACTION OF SOLUBLE CARBOHYDRATES

Samples of freeze-dried grains, rachis or floral organs were boiled in about 20 ml of 80% (v/v) aqueous ethanol for 20 min. Usually after the first extraction, samples were stored in the same extractant at 4°C. After the first extraction, samples (grains only) were ground using a polytron (Kinematica GmbH, Switzerland), and the samples were boiled and extracted with two successive portions of 80% ethanol. All the three extracts were combined and then vacuum dried at 45°C. The dried extracts were redissolved in 5 or 10 ml of deionised water, and in these extracts the amount of soluble carbohydrates were determined.

2.6.1.2 ESTIMATION OF TOTAL SOLUBLE SUGARS, SUCROSE, GLUCOSE, AND FRUCTOSE

Anthrone was used to determine total soluble sugars (Yemm and Willis 1954).

Measurements of sucrose, glucose, and fructose were made by using enzymic method of Jones et al. (1977). This method may be used to determine glucose, fructose, and sucrose by sequential addition of various enzymes in a single cuvette assay by using the compromise pH 6.9, or individually by adding only required enzymes to complete the reaction(s) from any of the above sugars to 6-phosphogluconate. The sequence of reactions is as follows:

$$\text{Sucrose} \xrightarrow{\text{Invertase}} \text{Fructose} + \text{Glucose}$$

$$\text{Glucose} + \text{Fructose} + 2\text{ATP} \xrightarrow{\text{Hexokinase}} \text{G-6-P} + \text{F-6-P} + 2\text{ADP}$$

$$\text{F-6-P} \xrightarrow[\text{Isomerase}]{\text{Phosphoglucose}} \text{G-6-P}$$

$$2 * \text{G-6-P} + 2\text{NADP} \xrightarrow[\text{Dehydrogenase}]{\text{Glucose-6-P}} 2 * \text{6-P-Gluconate} + 2\text{NADPH}$$

Sucrose was determined by omitting the enzyme phosphoglucose isomerase from the reaction mixture using sucrose as a standard. Amounts of glucose released from starch hydrolysis were determined by excluding the enzymes invertase and phosphoglucose isomerase from the reaction mixture and using glucose as a standard. Glucose and fructose in the endosperm cavity sap were determined by sequential addition of enzymes glucose-6-dehydrogenase, hexokinase and phosphoglucose isomerase in the same cuvette. The amounts of glucose and fructose were calculated using the following equations:

$$C = \frac{\Delta E \times V}{\varepsilon \times d \times v} \qquad \mu\text{mole ml}^{-1} \text{ in the sample}$$

or

$$C = \frac{\Delta E \times V \times MW}{\varepsilon \times d \times v} \qquad \mu\text{g ml}^{-1} \text{ in the sample}$$

where:

- ε – Extinction coefficient for NADPH 6.22 cm^2 $\mu mole^{-1}$ at 340 nm
- ΔE – Change in extinction ($O.D_{340}$)
- V – Total assay volume (2.010 and 2.020 ml for glucose and fructose respectively)
- v – Volume of sample used (1 ml)
- d – Path length (1 cm)
- C – Concentration mole ml^{-1} or $\mu g\ ml^{-1}$
- MW – Molecular weight mole or μg

All the enzymes used in the determination of sugars were of the highest purity available as insufficient purity of the reagents used, particularly of the enzymes, would result in false values. The amounts of glucose and fructose may include the amounts of glucose-6-phosphate and fructose-6-phosphate respectively.

2.6.2 CHLOROPHYLL MEASUREMENTS

Chlorophyll from pericarps (four per sample) was extracted with cold 80% (v/v) alkaline aqueous acetone (containing 0.02% sodium carbonate) as described by Bruinsma (1963) and measurements of chlorophylls, a, a + b and b were made at 645, 652 and 663 nm respectively with a spectrophotometer.

2.6.3 NITROGEN DETERMINATIONS

To samples of four grains, one selenium catalyst tablet (BDH Chemicals, England) and 4 ml of concentrated H_2SO_4 (A.R. grade) were added, and the samples were digested at 400°C for half an hour and then at 290°C for a further 20 min. The digests were allowed to cool and diluted to 30, 40 or 50 ml (depending on expected amount of nitrogen) with deionised water. Total nitrogen was determined by colorimetric measurement based on the reaction of ammonia with sodium phenate using a Technicon Auto analyser. Glycine was used as a standard, the recovery was 88 ±1.7%, and all the sample values were corrected for 100% recovery.

2.7 DETERMINATION OF SOME ENDOSPERM CHARACTERISTICS

2.7.1 COLLECTION OF ENDOSPERM CAVITY SAP

Grains of wheat (16 ±1 days after anthesis) were stripped of their enclosing bracts, and less than 1 mm of the basal portion of the grain was sliced off to expose the endosperm cavity. Twelve to 15 grains prepared in this way were stood cut face down on the top of a steel mesh disc (0.5 mm thick, 14 mm diameter), supported at the bottom of a plastic barrel (15 mm in diameter, 13 mm in height) made with sawn-off 10 ml plastic syringe (Terumo Australia Pty. Ltd.) which was secured in a perspex adaptor. A microcentrifuge tube (500 l) was secured at the end of the barrel for collection of endosperm cavity sap (Fig. 2.4).

Once packed, the grains were maintained in a vertical position, brush end uppermost, except during centrifugation. To reduce the metabolism of the grain, and evaporative losses from its cut surface, all operations were carried out in a cold room (2°C) and the barrels were covered with 'parafilm' immediately after the grains had been inserted.

Sap was collected by spinning the assemblies (6 in a set; Rotor type SW27) at 0°C in L2-65B ultracentrifuge. Details for time and spin speed are given in the related section (Section 3.6).

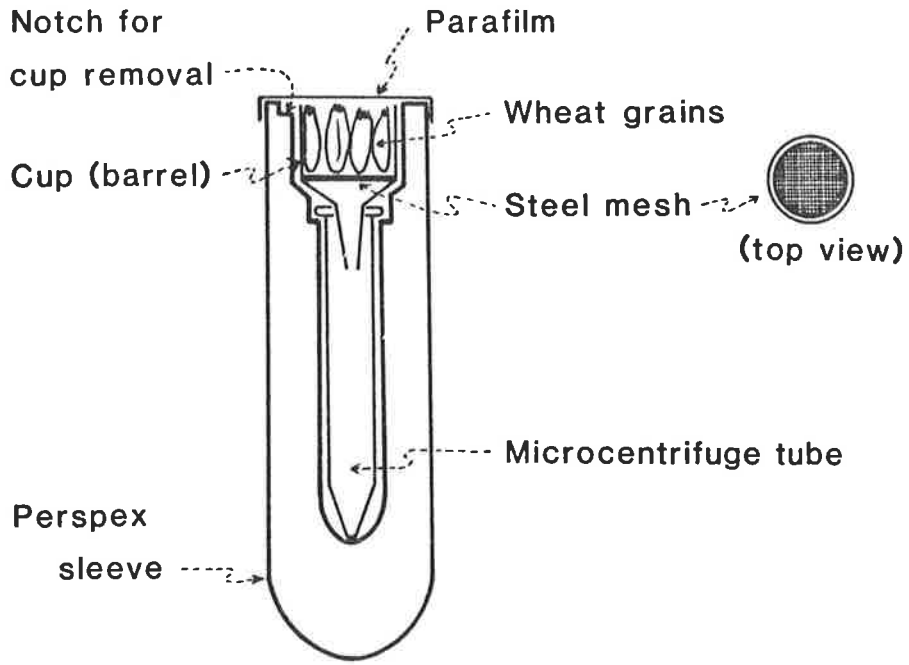


Fig. 2.4 Endosperm cavity liquid collection assembly.

2.7.2. DIALYSIS OF POLYETHYLENE GLYCOL AND PREPARATION OF OSMOTICALLY ADJUSTED SUCROSE SOLUTIONS.

Polyethylene glycol (Mol.wt. 4000; BDH Chemicals Australia) was dialysed (Dialysis tubing Benzoylated, Sigma Chemical Co.) against deionised water for 5 weeks with 7 changes of water at 4°C. At the end of dialysis PEG 4000 solution was freeze dried. A 24% (w/v) stock solution of PEG 4000 was prepared and this solution was used for the preparation of osmotically adjusted solutions. Assuming that the osmotic concentration of sucrose and that of PEG 4000 in a solution are additive for various ratios of the two solutes, it is possible to calculate amounts of PEG 4000 required for a number of sucrose concentrations to obtain a constant osmotic potential. The amounts of dialysed PEG 4000 required to prepare solutions of sucrose having osmotic potential of

Table 2.2

Amounts of sucrose and PEG 4000 required to prepared osmotically adjusted solutions of sucrose (Osmotic potential -0.82MPa).

Sucrose		*Ψ_π due to sucrose	Ψ_π from PEG 4000	**Amount of PEG 4000 required	Amount of water with PEG	Amount of deionised water to be added	**Expected total volume of the solution
mg ml^{-1}	mMolal	MPa		(mg)	(ml)	(ml)	(ml)
0	0	0.0	-0.82	226.1	0.942	0.048	1.21
20.3	59.4	-0.15	-0.67	196.0	0.817	0.183	1.19
30.6	89.5	-0.22	-0.60	184.0	0.767	0.233	1.16
41.1	120.2	-0.30	-0.52	170.0	0.708	0.292	1.13
51.8	151.5	-0.38	-0.44	153.0	0.637	0.363	1.12
62.5	182.7	-0.46	-0.36	136.0	0.567	0.433	1.11
84.4	246.8	-0.64	-0.18	80.0	0.330	0.667	1.10
107.0	312.9	-0.82	-	-	-	1.000	1.07

* Calculated from the freezing point depression for sucrose solutions (from Table 88, p. D-270 in CRC Handbook of Physics and Chemistry, 60th Edition, 1979-80).
** Stock solution of dialysed PEG 4000 = 6 gm/25 ml water.
*** Determined for each solution before millipore filtration and addition of ^{14}C-Sucrose.

+0.82 MPa are given in Table 2.2. To check the above assumption, osmotic potentials of a number of solutions prepared by using values from Table 2.2, were determined by Wescor Psychrometer and are given in the following tabulation:

Sucrose Concn (mMolal)	Expected Osmotic Potential (MPa)	Determined Osmotic Potential
59.4	-0.82	-0.81
120.2	-0.82	-0.83
182.7	-0.82	-0.93
246.8	-0.82	-1.14

with the exception of osmotic potential for 246.8 mMolal sucrose solution, the determined values for the sucrose solutions are very close to the theoretical values.

2.7.3 ESTIMATES OF FREE SPACE VOLUME

Radioactive ^{14}C-PEG glycol 4000 was purchased from Amersham Australia Pty. Ltd. [(specific activity 80 mCi/mmole (approx)]. A small amount of radioactive PEG 4000 (20 µCi) was mixed with 100 mg of non-radioactive PEG 4000 and dialysed for a week with 4 changes of deionized water at 4°C (as above), and this solution was used to prepare osmotically adjusted ^{14}C-PEG 4000 solution.

Dissected wheat grains (Section 2.8.1) were incubated individually in 150 µl of osmotically adjusted solution (-0.82 MPa), containing dialysed ^{14}C-PEG and sucrose at 20 gl^{-1} (59.4 mMdal), in 2.2 ml Eppendorf tubes. After incubation the grains were washed for 10 - 20 s in a large volume of water, gently blotted and weighed again. ^{14}C-PEG left in the grain was estimated as described in Section 2.10.2 after drying the grain.

2.8 ISOLATED GRAIN CULTURE

2.8.1 DISSECTION OF GRAIN

In the laboratory (maintained at 21 ±1°C) or in the laminar flow cabinet, grains were taken from b florets of the 10 spikelets forming the central section of the ear. The outer transparent layer of pericarp was peeled from the grain and discarded. The pericarp tissue in the furrow (containing the vascular bundle and other tissues of maternal origin) was stripped out by inserting the tip of one jaw of forceps into the endosperm cavity at one end of the grain and then sliding it along the length of the grain. This action tore out most of the tissue and exposed the endosperm surrounding the endosperm cavity (Bradbury et al 1956), and the flanks of the furrow. Variation from this procedure is dealt with in the relevant section (Section 3.6).

2.8.2 CULTURE OF GRAIN

Three methods of endosperm culture were used depending on the aim of experimentation, and are outlined as follows:

2.8.2.1 METHOD 1

Single grains (with or without dissection) were placed in a 0.2 ml glass capsules, containing 50 µl of ^{14}C-sucrose solution (stock ^{14}C-sucrose from Amersham Australia Pty. Ltd., specific activity 434.6 mCi/mmole) and the capsules were covered with small pieces of 'parafilm'. All capsules prepared in this manner were allowed to stand in a fume hood at ambient temperature (21 ±1°C) in the laboratory or shifted to the laminar flow cabinet. Details of individual experiments are given in section 3.6.

2.8.2.2 METHOD II

2.8.2.2.1 CULTURE

Isolated wheat grains (20 ±1 daa) were surface sterilized using sodium hypochlorite solution (1% available Cl) for 30-60 s and washed for 2 min in a large volume of deionised water. Grains were dissected as described in Section 2.8.1 and placed in 100 µl of ^{14}C-sucrose solutions (filter sterilized, osmotically adjusted to -0.82 MPa with dialysed PEG

4000 - Section 2.7.2) in pre-sterilized assemblies made from 2.2 ml Eppendorf tubes (Fig. 2.5) in a laminar flow cabinet. The concertina shaped filter paper (Whatman No. 50; 50 x 10 mm), secured in a barrel (5 x 7 mm) attached to the Eppendorf's lid with a small amount of Blu-Tack, was soaked with 0.075 ml of 30 gl^{-1} solution KOH and the lid was immediately closed. All the assemblies prepared in this way were clamped tightly, and attached to the modified arm of flask-shaker (Dynamax, Townson and Mercer, N.S.W.) in such a manner that the assemblies

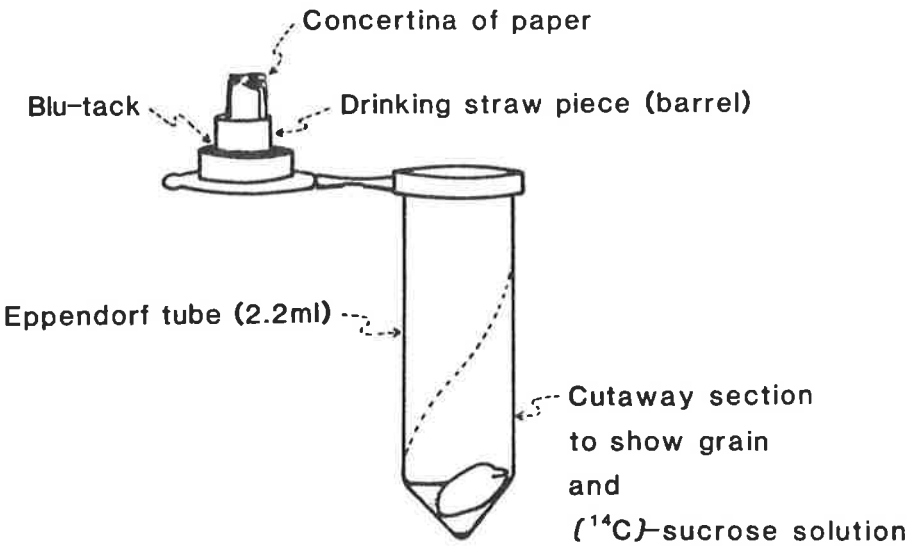


Fig. 2.5 Grain culture assembly

assemblies were dipping in a water bath set at the desired temperature. The grains were incubated for the desired length of time and at a set oscillation rate.

2.8.2.2.2 RECOVERY OF $^{14}CO_2$ DISSOLVED IN THE SOLUTION

At the end of incubation period, the lid of the assembly was opened carefully. The grain was picked out with a needle and its surface washed with 400 µl of deionised water. After the removal of the grain, 10 µl of 0.1 N HCl was added to the culture solution and the assemblies were clamped and heated for 10 min at 70°C in a water bath.

2.8.2.2.3 COUNTING OF $^{14}CO_2$ ENTRAPPED ON THE PAPER

The assemblies were allowed to cool, and then the paper which had been soaked in KOH solution was put in a 20 ml counting glass vial, and

1.35 ml of NCS tissue solubilizer, 0.04 ml of glacial acetic acid and 15 ml of scintillant (Ready Solv NA) were added. Samples were counted using the same procedure as in Section 2.10.2.

2.8.2.3 METHOD III

Five dissected grains (Section 2.8.1) were shaken gently in a water bath set at a desired temperature in 2.4 ml of ^{14}C-sucrose solution (osmotically adjusted to -0.82 MPa; filter sterilized). After four hours of incubation the solutions were replaced with fresh 2.4 ml solution and incubation allowed to continue for a further 4 h. The concentration of sucrose in the solutions used for culture is given in the related experiments.

2.9 DETERMINATION OF FREE SPACE SUGARS

Sugars in the apparent free space (see Briggs *et al* 1956) are defined as the amount released during 1.5 h into cold deionized water in which the grains are suspended. Grains either freshly prepared (Section 2.8.1) or after incubation in ^{14}C-sucrose, were rinsed for 10-20 s in a large volume of deionized water and blotted. Single-grain samples were placed in an Eppendorf tube (2.2 ml) containing 1 ml of cold deionized water, and 5-grain samples were placed in a 25 ml conical flask containing 5 ml of cold deionized water. The containers were embedded in crushed ice and shaken gently for 20-30 min. The water was collected, and replaced with a second water wash which after a further 60 min was withdrawn and combined with the first. Aliquots of combined samples were used to determine sugars (as described in Section 2.6.1.2) or levels of radioactivity (as described in Section 2.11.3).

2.10 DETERMINATION OF RADIOACTIVITY

2.10.1 ETHANOL SOLUBLE RADIOACTIVITY

Individual grains, before or after free space material removal, were put in 2.2 ml Eppendorf tubes and 1 ml of 80% (v/v) aqueous ethanol was added. The tubes were capped, clamped and heated for 20 min at about 85^{o}C. The extract was transferred to a volumetric flask (5 ml) and the grain was extracted two more times in 80% ethanol. The extracts were combined and the flask was filled to the mark with 80% ethanol. Portions

(0.2 ml) of ethanol extract were added to 1.8 ml scintillant (ACS: toluene 2:1; v/v) in small plastic tubes and counted in a Beckman LS 7500 scintillation counter programmed for ^{14}C - labelled samples and corrected for quenching.

The procedure for extraction and counting of ^{14}C in ethanol soluble material for 5 grain samples was exactly the same, except that a larger volume of ethanol (3 ml) per sample was used and extraction was carried out in 15 ml glass tubes, and the final volume was made to 10 ml.

2.10.2 TOTAL ETHANOL INSOLUBLE RADIOACTIVITY

After extraction of ethanol soluble material, grains were dried in an oven at 80°C for 24 h. After drying, the samples were allowed to equilibrate with laboratory temperature in a desiccator and their weights recorded. Grains were clamped individually in Eppendorf tubes (2.2 ml) after addition of 0.1 ml of water and autoclaved for 160 min at 121°C to gelatinize starch. When cool, 1.35 ml of NCS tissue solubilizer (0.6 N solution, Amersham Australia Pty. Ltd.) was added. The tubes were clamped and incubated in a water bath set at 50°C with occasional shaking for 24 h, or until the tissue had disintegrated leaving visible only a few transparent fragments of pericarp. The tubes were opened and the tube along with its contents was pushed into a 20 ml counting glass vial. Fifteen ml of scintillant (Beckman Ready Solv NA) and 0.04 ml of glacial acetic acid were added to the vial. The vial was capped tightly, shaken vigorously and stored in the dark for at least 24 h to allow for the decay of chemiluminescence. The samples were counted using an appropriate programme.

2.11.1 EXTRACTION OF STARCH

The residues after ethanol extraction (Section 2.10.1), were autoclaved after the addition of deionised water (0.3 or 1.5 ml depending on sample size; 0.3 ml grain^{-1}) for 160 min at 121°C to gelatinise starch. After this step, different methods for extracting starch from single grain and large (5 grains) samples were used.

For a single grain, samples were macerated with a glass rod and the volume was made to 4 ml with deionised water. To each tube 1 ml of enzyme mixture, containing 1.5 mg each of α-amylase (EC3.2.1.1, HT1000, Miles Laboratories, U.S.A.) and amyloglucosidase (EC3.2.1.3, from

Rhizopus Mold, Sigma Chemicals Co.) in 0.1 M acetate buffer (pH 4.8) was added and samples incubated at 60°C for 3h. After the incubation samples were centrifuged at 2100g for 10 min. The supernatant was decanted into a flask, and 5 ml of 80% ethanol was added to each tube. Tubes were heated at 80°C for 10 min, allowed to cool, and centrifuged as described earlier. The second extract was combined with the first. Extracts were dried under vacuum at 40°C. Each dried sample was dissolved in 2 ml of 20% aqueous ethanol. Amounts of radioactivity in the samples were determined as described in Section 2.11.3. Radioactivity in the residue left after solubilization of starch (insoluble material other than starch) was determined by digesting the residue in 1.35 ml of NCS tissue solubilizer for 6h at 50°C, and counting by the procedure described in Section 2.10.2.

For 5-grain samples radioactivity in starch was determined as follows. Samples after gelatinisation were allowed to cool to laboratory temperature, and were dispersed in 3 ml of 6.15 M perchloric acid. An aliquot (equivalent of one grain) was precipitated with iodine by the method of Pucher, Leavenworth and Vickery (1948). After decomposing the iodine complex the precipitate was dissolved in 1 ml of 0.5 NaOH by heating in boiling water for 2 min (McCready and Hassid 1943). The starch solution was neutralized by adding 1 ml of 0.5 N HCl, and the total volume of solution made to 5 ml with deionised water. Sub-samples of this solution were used to determine radioactivity in starch (Section 2.11.3) and the amount of starch in the samples (Section 2.11.2).

2.11.2 DETERMINATION OF AMOUNT OF STARCH

An aliquot of starch solution (Section 2.11.1) was hydrolyzed by adding 1 ml of an enzyme mixture, containing 1.5 mg each of α-amylase and amyloglucosidase in 0.1 M acetate buffer, pH 4.8, and incubating the samples at 60°C for 3 h.

The amount of glucose released was determined by the method of Jones *et al* (1977) modified by omitting the invertase and P-glucose isomerase enzymes (Section 2.6.1.2). The amount of starch was calculated from the amount of glucose by multiplying the values for glucose by 0.947.

2.11.3 DETERMINATION OF ^{14}C IN STARCH AND FREE SPACE SUGARS

Portions (400-600 µl) of starch solution or glucose solution obtained after enzymic hydrolysis of starch (Section 2.11.1) or solutions obtained for apparent free space sugars (Section 2.9) were mixed with 1.2 to 1.5 ml of scintillant (Ready Solv EP) in 2.0 ml plastic tubes to form a clear gel. The plastic tubes were inserted into 20 ml glass counting vials and counted in a Beckmann LS 7500 Scintillation counter programmed for a gel system for ^{14}C-labelled samples and corrected for quenching.

2.12 STATISTICAL ANALYSIS

In all experiments a minimum of four replicates were used. Conventional analyses of variance and linear regression analyses were performed. Least significant differences were calculated for all significant ($P \leq 0.05$) main effects and interactions, and are given in tables or plotted in the figures as bars.

SECTION 3: RESULTS

3.1 EAR WARMING AND GRAIN YIELD COMPONENTS

Introduction

High temperature during the post anthesis period adversely affects grain yield mainly through its effect on individual grain weight (McDonald et al. 1983). Individual grain weight is a function of the rate and the duration of grain filling (Evans 1975; Brocklehurst 1977), and a reduction in dry weight per grain at high temperature is mainly due to a reduction in the duration of grain filling (Chinoy and Nanda 1958; Asana and Williams 1965; Wardlaw 1970; Sofield et al. 1974, 1977a; Spiertz 1974, 1977; Warrington et al. 1977; Rawson and Bagga 1977; Wardlaw et al. 1980). Reduction in the rate of grain growth has also been reported (Asana and Williams 1965).

In most of the reports, the effects of high temperature on grain growth have been observed either in the field (Fischer and Maurer 1976; Marcellos and Single 1972; Gallagher et al. 1976; McDonald et al. 1983; Davidson and Campbell 1983) where a number of other factors may interact to give a final response, or in control environments by raising the temperature of the whole plant for a part of the grain filling period (Wardlaw 1970) or throughout the grain filling phase (Sofield et al. 1974, 1977a; Spiertz 1977; Wardlaw et al. 1980).

Raising the temperature of the plant may well give rise to a complex of responses in different parts of the plant which subsequently may interact to affect grain growth. At high temperature, toxic substances like gramine have been reported to accumulate in leaves of barley (Hanson et al. 1981, 1983) and accelerated senescence of leaves is a common observation. Considering the above reports, warming the whole plant may present difficulties in ascertaining whether the observed reduction in single grain weight is a consequence of an effect of high temperature on the processes operating within the grain or through its effect on the provision of substrate or other factors from the other parts of the plant. Hence, it may be important to have some other means of investigating the effect of temperature on grain growth, somewhat more independent of effects of temperature on other parts of the plant.

Localized warming or cooling of plant parts *in situ* has been used to investigate the effects of temperature on transport and translocation of assimilates (Moorby et al. 1974; Wardlaw 1974; Wardlaw and Moncur 1976; Walker and Ho 1977). Warming of the ears alone or the rest of the

plant seperately or keeping the ears and the rest of the plant at different temperatures have been used by some workers (Sato and Inaba 1973; Ford et al. 1976; Radley 1976) to investigate the factors affecting grain growth at high temperatures. However, only limited information is available on effects of a brief period of high temperature on grain growth and grain yield. The first objectives of this study were:

(a) To see whether a brief period of ear warming during the grain filling phase does result in a reduction in final dry weight per grain (as reported by Ford et al. 1976). If so, what number of days and how great a temperature differential (day/night) are necessary to observe a significant reduction in dry weight per grain.

(b) To see whether there are any macroscopic differences in grain growth characteristics, when the whole plant or ears only were warmed for a brief period during the grain filling phase.

Ears (only) were exposed to moderate to high temperatures for limited periods (1 to 10 days) during the grain filling phase with the intention of affecting grain growth. Treatments were started on day 10 after anthesis in an attempt to avoid any interference with grain set and early stages of endosperm development.

Materials and Methods

Experiment 1

Four batches of plants were raised in succession in the growth room, and about a week after anthesis were arranged as described in Section 2.3.1 for imposition of ear warming (Section 2.3.3). Ears only, from 10 days after anthesis, were exposed (within a batch) to a common temperature differential (day/night), keeping the relative humidity in the box constant (above about 70%, day and night), for various lengths of time, while keeping the rest of plant at ambient temperature (21/16^{o}C). The summary of treatments is as follows:

Plant batch no.	Ear temperature day/night (°C)	Vapour pressure deficit day/night (kPa)	Period of exposure to high temperature (days):				
1.	30/25	1.27/0.95	2	4	6	8	10
2.	33/25	1.51/0.95	2	4	6		
3.	36/25	1.78/0.95	2	4			
4.	39/25	2.10/0.95	1	2			

Afterwards the ears were removed from the box, and allowed to mature at control conditions (21/16°C). At maturity five ears from each treatment were randomly selected and sampled as follows. The spikelets within an ear were numbered, starting from the base of the ear towards its tip, and the ear was divided into four zones, with four spikelets each, starting with second spikelet (i.e. zone I: spikelets 2-5; zone II: spikelets 6-9; zone III: spikelets 10-13; zone IV: spikelets 14-17). Within each zone, grains from florets a, b and c (designated acropetally in a spikelet) were sampled separately and then all grains from the same floret position were combined. All other grains (outside selected zones) of the ear were pooled, and dry weights of all the samples were determined individually (Section 2.5.1). To obtain the single grain dry weight for each floret position within a zone, the aggregate weight for all the grains in that floret position was divided by the number of grains in that zone.

Experiment 2

Plants were raised in the glasshouse for four weeks and then transferred to the growth room. About a week after anthesis plants were arranged as described in experiment 1 and divided into two groups, ears in one group of plants were enclosed in the box for 6 days starting from 15 days after anthesis, while the plants in the other group were allowed to stay at ambient conditions (21/16°C). Eight grains from the a and b florets of four central spikelets from four separate ears were harvested on days 15, 20, 25, 31 and 37 after anthesis, and when the ears had ripened. At maturity 10 ears for each treatment were harvested for total ear grain weight determinations.

Experiment 3

Plants were raised and arranged as described in experiment 2. Plants were divided into four groups, and on day 15 after anthesis, plants were given one of the following treatments: (a) allowed to remain at ambient conditions (21/16°C); (b) shifted to a growth cabinet set at 31/25°C; (c) shifted to a growth room set at 26/24°C; (d) the ears were enclosed in the box at 31/25°C while the rest of the plant was exposed to 26/24°C. At day 21 after anthesis plants were shifted to control conditions (21/16°C). Eight grains from florets a and b, from four central spikelets from five separate plants, were harvested on days 15, 17, 19, 21, 23, 34 after anthesis. At maturity five ears for each treatment were harvested to determine individual grain weight and yield components.

Results

Experiment 1

The effects of various ear warming treatments on grain yield components are summarized in Table 3.1.1. The control values for the four batches of plants used for different ear warming treatments were not identical. For example, total ear grain weight was lower for the 39/25°C treatment than for the other three treatments. This difference in total ear grain weight between different batches of plants was due mainly to differences in grain number per ear.

With the exception of ears warmed for only one day at 39/25°C, grain weight per ear, within a treatment, decreased progressively with an increase in duration of ear warming (Fig. 3.1.1a). When comparing reductions in total ear grain weight between different ear warming treatments, a one day increase in ear temperature from 21/16°C to 30/25°C, to 33/25°C, to 36/25°C, or to 39/25°C resulted in a 4.4%, a 6.4%, a 6.1%, or a 10% decrease in grain weight per ear respectively. Values were calculated by regression analysis on the original data and the equations are given in the following tabulation.

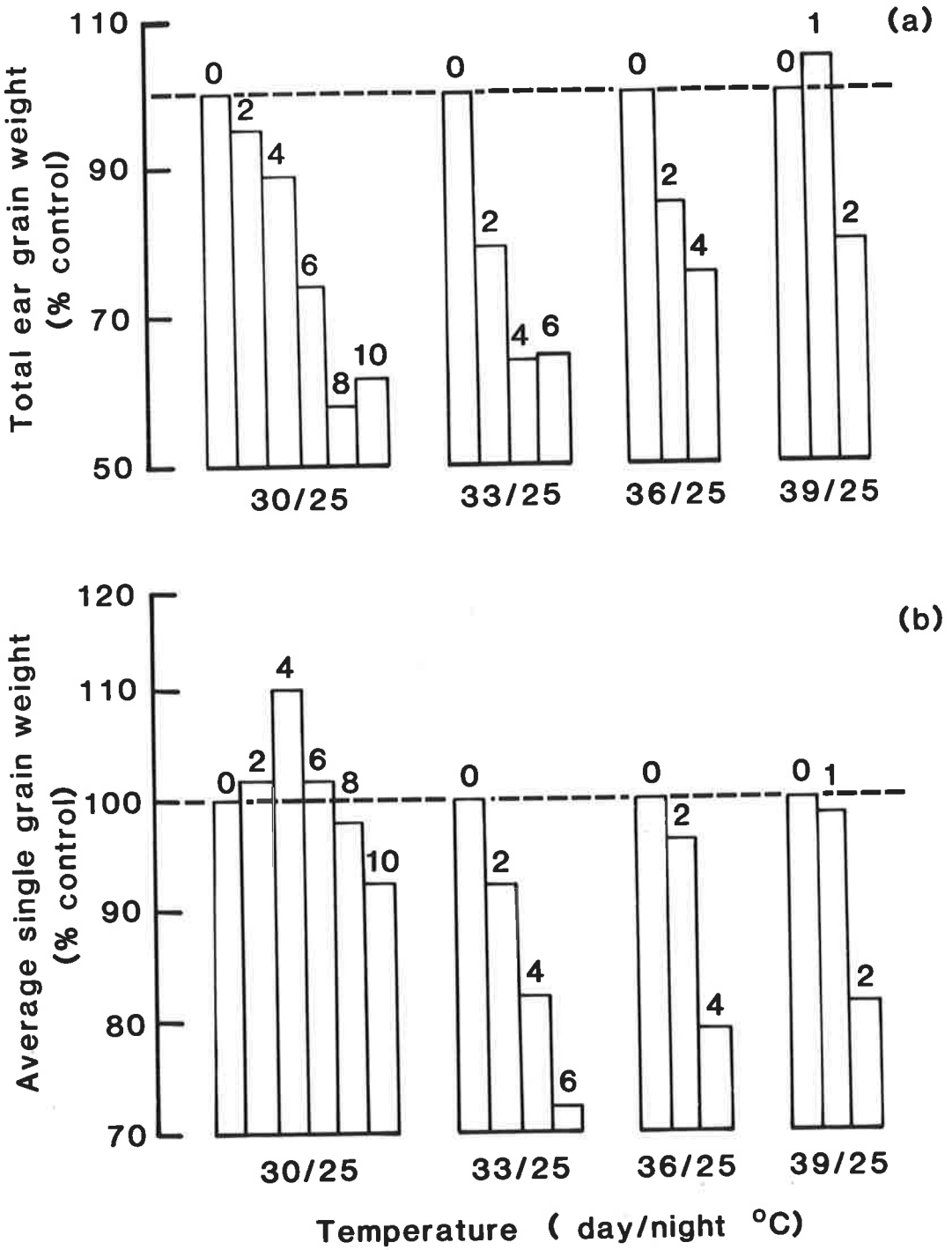
Table 3.1.1

Effects on yield components of brief periods ear warming at different day temperatures (night temperature was kept constant at 25°C) for various lengths of time from day 10 after anthesis. After ear warming treatments were over, ears were allowed to mature at 21/16°C (day/night). Values in parentheses are per cent differences from plants maintained at 21/16°C throughout. (Expt.1)

Temperature day/night (°C)	Parameter	Control 21/16°C	Period of exposure to high temperature (days)						L.S.D.
			1	2	4	6	8	10	
30/25	No. of spikelets per ear	20.6	-	20.0	19.0	19.0	18.8	18.8	n.s.
	No. of grains per ear	67.6	-	62.6	52.2	49.4	40.0	45.6	6.7
	Total ear grain wt (mg)	2357	-	2243 (-4.8)	2005 (-10.6)	1744 (-22.2)	1376 (-41.6)	1458 (-38.1)	250
	Average single grain wt (mg)	35.0	-	35.8 (+2.3)	38.7 (+10.6)	35.7 (+2.0)	34.4 (-1.7)	32.4 (-7.4)	n.s.
33/25	No. of spikelets per ear	21.0	-	19.4	21.2	21.6			n.s.
	No. of grains per ear	66.6	-	56.0	50.8	57.2			4.7
	Total ear grain wt (mg)	2437	-	1931 (-22.8)	1559 (-36.0)	1568 (-35.7)			221
	Average single grain wt (mg)	36.7	-	34.2 (-6.8)	30.6 (-16.6)	27.4 (-25.3)			3.3
36/25	No. of spikelets per ear	20.4	-	21.0	21.0				n.s.
	No. of grains per ear	61.8	-	54.8	59.2				3.9
	Total ear grain wt (mg)	2298	-	1952 (-15.1)	1743 (-24.2)				225
	Average single grain wt (mg)	37.2	-	35.7 (-4.0)	29.5 (-20.7)				4.2
39/25	No. of spikelets per ear	19.6	19.0	18.0					n.s.
	No. of grains per ear	55.2	58.2	53.4					n.s.
	Total ear grain wt (mg)	1930	2016 (+4.5)	1525 (-20.9)					207
	Average single grain wt (mg)	35.1	34.6 (-1.4)	28.6 (-18.5)					1.5

Fig. 3.1.1

Total ear grain weight (a), and average single grain weight (b) for control ears and ears warmed at high temperature. Ears were warmed for various lengths of time (in days), starting 10 days after anthesis, at different day temperatures; the night temperature was kept constant at 25°C. Numbers on the top of bars (mean of five replicates) refer to the number days for which ears stayed at high temperature, while 0 (zero) refers to ears which stayed at 21/16°C throughout. (Expt. 1).


(a)
Total ear grain weight (% control)
110
90
70
50
0
2
4
6
8
10
0
2
4
6
0
2
4
0
1
2
30/25
33/25
36/25
39/25
(b)
Average single grain weight (% control)
120
110
100
90
80
70
0
2
4
6
8
10
0
2
4
6
0
2
4
0
1
2
30/25
33/25
36/25
39/25
Temperature (day/night °C)

Temperature differential day/night	The Regression equation	Correlation coefficient
(°C)		
30/25	y = 2389 - 105x	-0.844** (n = 30)
33/25	y = 2320 - 149x	-0.831** (n = 20)
36/25	y = 2275 - 139x	-0.835** (n = 15)
39/25	y = 2026 - 203x	-0.645** (n = 15)

(** Significant at 1% level of significance)

The regression coefficients confirm the inverse relationship between the temperature differential (used for ear warming) and total ear grain weight at maturity. When these results were calculated on the basis of accumulated degree-days (cf. Gilmore and Rogers 1958; Cavin and Yao 1967; Monteith 1981; Davidson and Campbell 1983; Johnson and Kanemasu 1983; Russelle et al. 1984) above the ambient temperature (21/16°C), by the following equation:

$$\text{Degree - days} = (\Delta T_d.14 + \Delta T_n.10).ND/24$$

where,

ΔT_d - increase °C above ambient during the day (14 h)

ΔT_n - increase °C above ambient during the night (10 h)

ND - no. of days for which ears were warmed at high temperature.

There were no significant differences in the regression coefficients between ear warming treatments, and overall there was a 10.3 mg ($r = -0.747$, significant at $P < 0.01$) decrease in total ear grain weight for each 1°C-day increase.

Reduction in total ear grain weight was attributed to a reduction in grain number as well as average grain weight (Fig. 3.1.1b). Reduction in grain number was more pronounced in the 30/25°C and 33/25°C treatments, whereas there was no reduction in grain number in the 39/25°C treatment. The contribution of the reduction in grain number to reduction in ear grain weight ranged from 73.5% after 2 days at 36/25°C to 80% after 10 days at 30/25°C. In treatment 30/25°C, grain number was significantly reduced by four days of ear warming, and there was a

Fig. 3.1.2

Grain set in different floret positions for 30/25°C (a), and 33/25°C (b) ear warming treatments from Expt. 1. Subscript number for a bar refers to the number of days for which ears stayed at high temperature. Bars not marked with the same letter (superscript) within a set of bars are significantly different from each other.

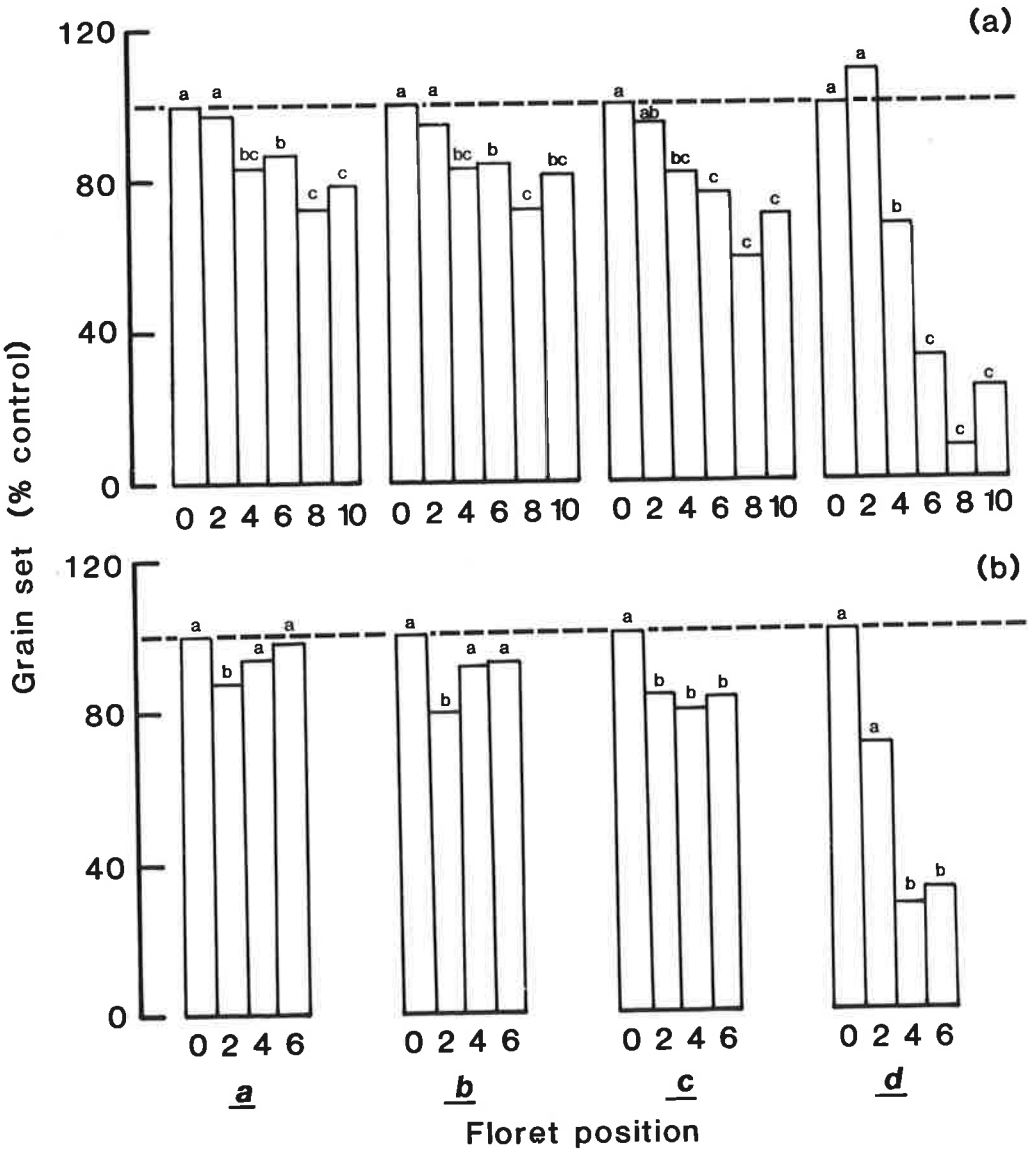

(a)
120
80
40
0
a a bc b c c
0 2 4 6 8 10
a a bc b c bc
0 2 4 6 8 10
a ab bc c c c
0 2 4 6 8 10
a a b c c c
0 2 4 6 8 10
(b)
120
80
40
0
a b a a
0 2 4 6
a b a a
0 2 4 6
a b b b
0 2 4 6
a a b b
0 2 4 6
a b c d
Floret position
Grain set (% control)

Fig. 3.1.3

Dry weight for grain b in different parts of the ear for 30/25°C (a), 33/25°C (b), 36/25°C (c), and 39/25°C (d) ear warming treatments from Expt. 1. For control (●), or ears warmed for

★ 1 day
▲ 2 days
■ 4 days
○ 6 days
△ 8 days
□ 10 days

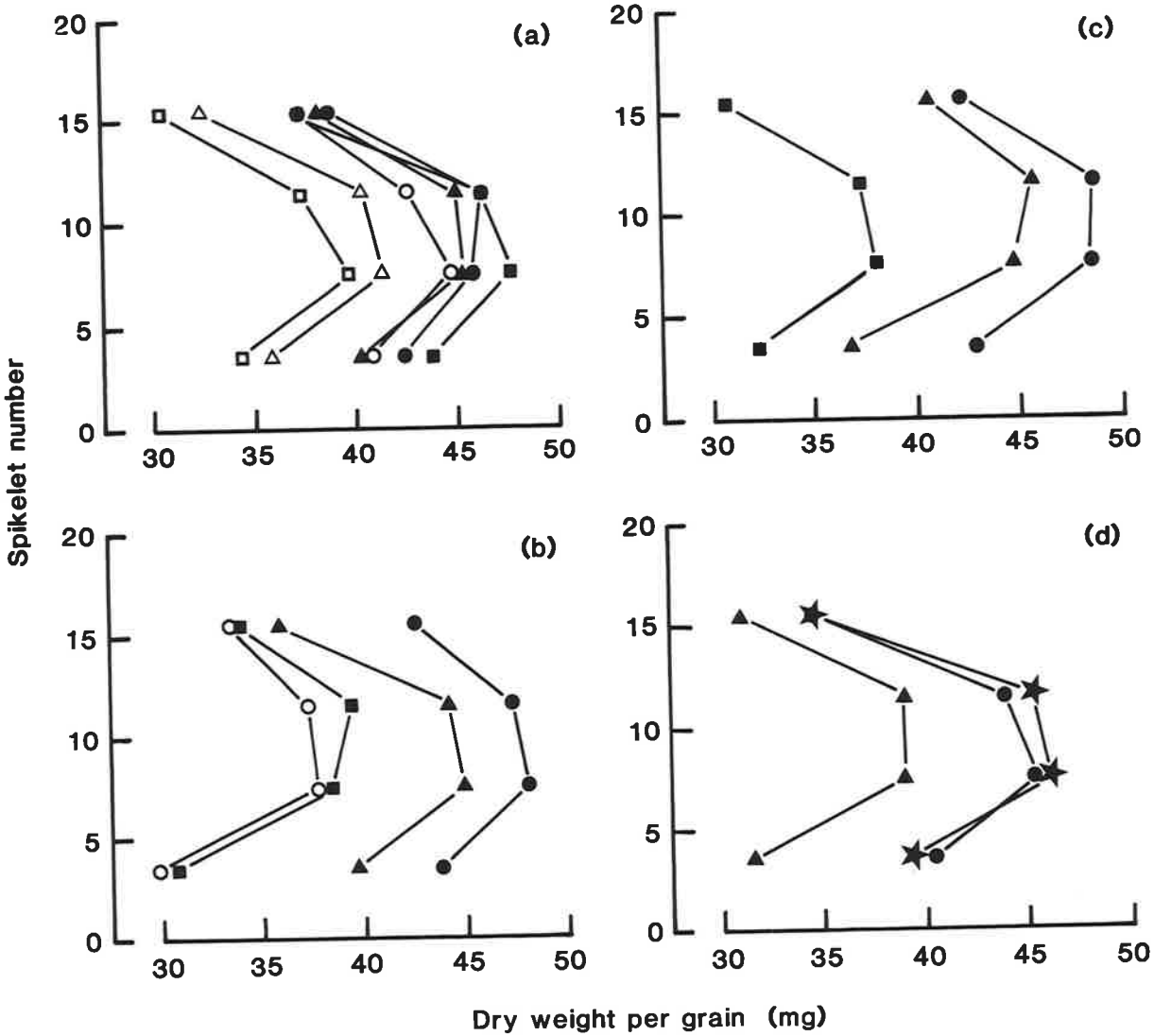

(a)
(b)
(c)
(d)
Spikelet number
Dry weight per grain (mg)
0
5
10
15
20
30
35
40
45
50

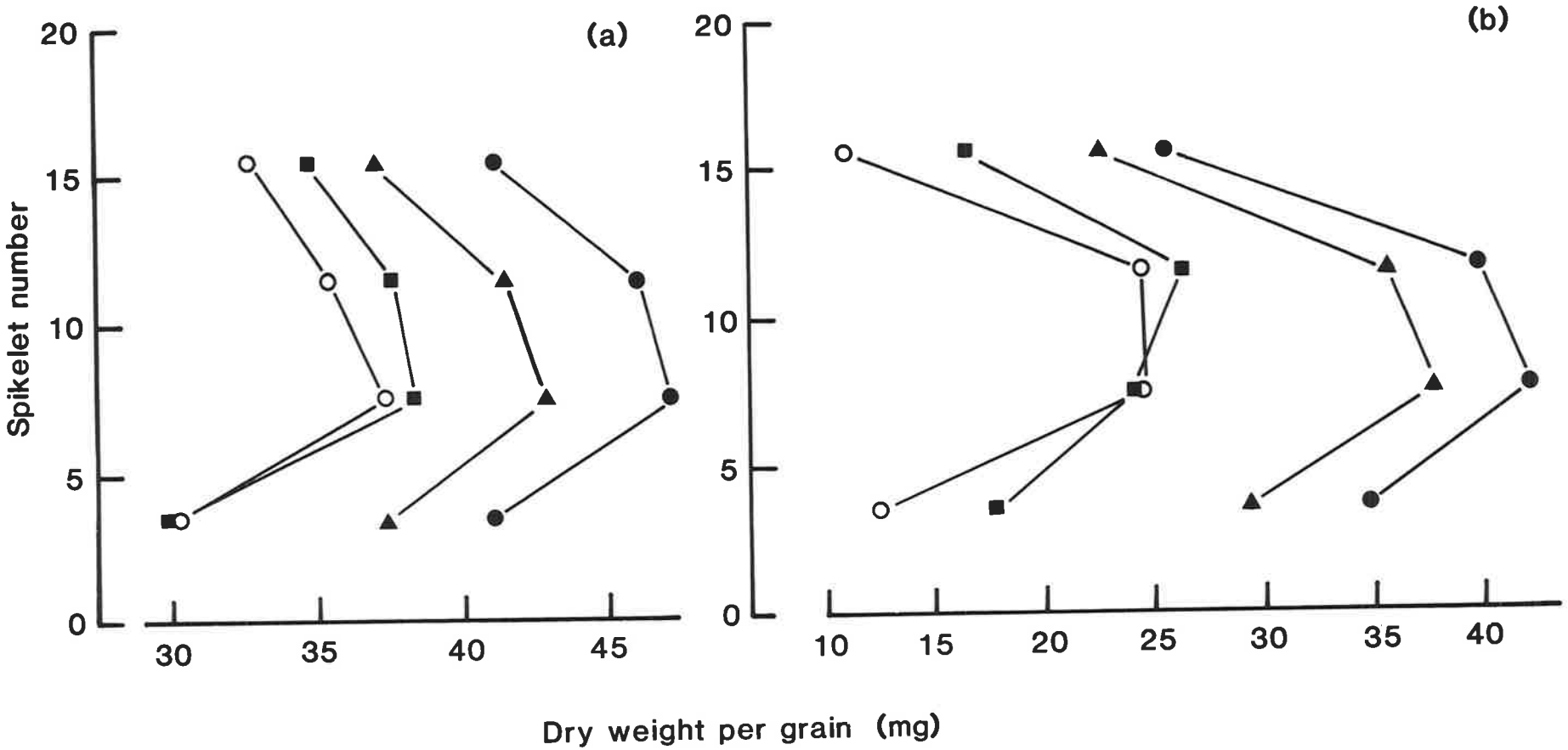
Table 3.1.2

Average dry weight for different floret positions over a selected portion (spikelets 2-17 inclusive) of the ear for various ear warming treatment from Expt. 1. Values in parentheses are per cent decrease or increase over control.

Temperature day/night (°C)	Floret position	Control 21/16°C	Period of exposure to high temperature (days)						L.S.D.
			1	2	4	6	8	10	
30/25	a	41.7	-	41.7 (0.0)	42.6 (+2.2)	40.0 (-4.0)	37.2 (-10.8)	35.4 (-15.1)	n.s.
	b	43.2	-	42.2 (-2.3)	43.7 (+1.0)	41.3 (-4.4)	37.1 (-14.1)	35.4 (-18.1)	n.s.
	c	32.9	-	33.3 (+1.2)	32.2 (-2.1)	28.3 (-14.0)	27.1 (-17.6)	26.0 (-20.9)	n.s.
	Mean	39.3		39.1 (-0.5)	39.5 (+0.5)	36.5 (-7.1)	33.8 (-14.0)	32.3 (-17.8)	n.s.
33/25	a	43.8	-	39.6 (-9.6)	35.1 (-19.7)	33.9 (-22.6)			4.9
	b	45.4	-	41.2 (-9.3)	35.7 (-21.4)	34.7 (-23.6)			4.8
	c	35.4	-	31.0 (-12.4)	21.1 (-40.4)	18.1 (-48.9)			6.5
	Mean	41.5		37.3 (-10.1)	30.6 (-26.3)	28.9 (-30.4)			4.4
36/25	a	44.0	-	40.3 (-8.4)	34.4 (-21.8)				4.5
	b	45.5	-	42.0 (-7.7)	34.6 (-24.0)				3.4
	c	33.9	-	31.2 (-8.0)	26.2 (-22.7)				4.7
	Mean	41.1		37.8 (-8.0)	31.7 (-22.9)				4.2
39/25	a	40.6	41.3 (+1.7)	34.9 (-14.0)					3.2
	b	41.0	41.3 (+1.0)	35.1 (-14.4)					3.1
	c	29.5	30.4 (+3.1)	22.5 (-23.7)					3.8
	Mean	37.0	37.7 (+1.9)	30.8 (-16.8)					1.5

Fig. 3.1.4

Dry weight for grain a(a) and grain c(b) for 33/25°C ear warming treatment from Expt. 1. For control (●), and ears warmed for ▲ 2 days, ■ 4 days, ○ 6 days. Note the differences in scale for x-axis for grains a and c.


(a)
(b)
Spikelet number
20
15
10
5
0
30
35
40
45
10
15
20
25
30
35
40
Dry weight per grain (mg)

slight increase in average grain weight by 2, 4 and 6 days of ears warming (Fig. 3.1.1b). There was no significant reduction in average single grain weight even after 10 days of ear warming. An analysis of reduction in grain number on individual floret positions revealed that abortion of grains was more prevalent in distal floret positions than proximal ones (Fig. 3.1.2).

A reduction in average grain weight due to ear warming, in all treatments, was attributable to reductions in grain weight at all floret positions, and almost the same per cent reductions were observed in different zones of the ears (data for b floret, Fig. 3.1.3). When comparing reductions among different floret positions, within a zone of the ear, floret c was more responsive as compared to floret a or b in three out of four treatments (Table 3.1.2 and Fig. 3.1.4).

Experiment 2

In the first experiment, ears considered as controls were not enclosed in the box at 21/16°C. Therefore, it was assumed that observed differences in the total ear grain weight or individual grain weight between control and ear warming treatments were due only to an increase in air temperature inside the box, and not due to a change in light intensity, light quality or air movement for the ears enclosed in the box. The validity of these assumptions was tested in this experiment.

When the air passing through the box was not supplemented with heat, the temperature of air inside the box was 3°C higher during the day (possibly due to the positioning of the box in the proximity of the light bank) compared to the temperature of air outside the box.

Fresh weight, dry weight, or water content of the grains developing in the box did not differ significantly from the grains developing in the control environment at any stage of grain development (Table 3.1.3 and Fig. 3.1.5). A 3°C increase of temperature during the day for 6 days (between day 15 and 21) did not result in an observable acceleration in the rate of grain growth. This observation is in agreement with earlier reports (Sofield et al. 1977a; Chowdhury and Wardlaw 1978), where there were no detectable differences in the rate or the final dry weight per grain, when the plants were grown in the temperature range of 18/13°C to 24/19°C. However, it is conceivable that any possible inhibitory effects of the box were counterbalanced by slight promotive effects of a 3°C increase in temperature.

Table 3.1.3

Fresh weight, dry weight and water content for a + b grains taken from four central spikelets from control ears (C), or ears enclosed in the box (E) at 24/16°C (between days 15 and 21 after anthesis). (Expt. 2)

Days after anthesis (DAA)		Treatment	Weight per grain (mg): Fresh weight	Dry weight	Water content
*15		C	49.1	17.7	31.3
20		C	52.4	24.5	27.9
		E	52.0	25.0	26.9
	Mean		52.2	24.8	27.4
25		C	58.1	30.6	27.5
		E	57.7	31.1	26.6
	Mean		57.9	30.9	27.1
31		C	68.2	39.5	28.7
		E	69.0	39.5	29.5
	Mean		68.6	39.5	29.1
37		C	65.6	39.8	25.9
		E	66.9	39.9	27.0
	Mean		66.3	39.9	26.5
L.S.D.	DAA		5.8	3.5	n.s.

* day 15 values not included in the analyses.

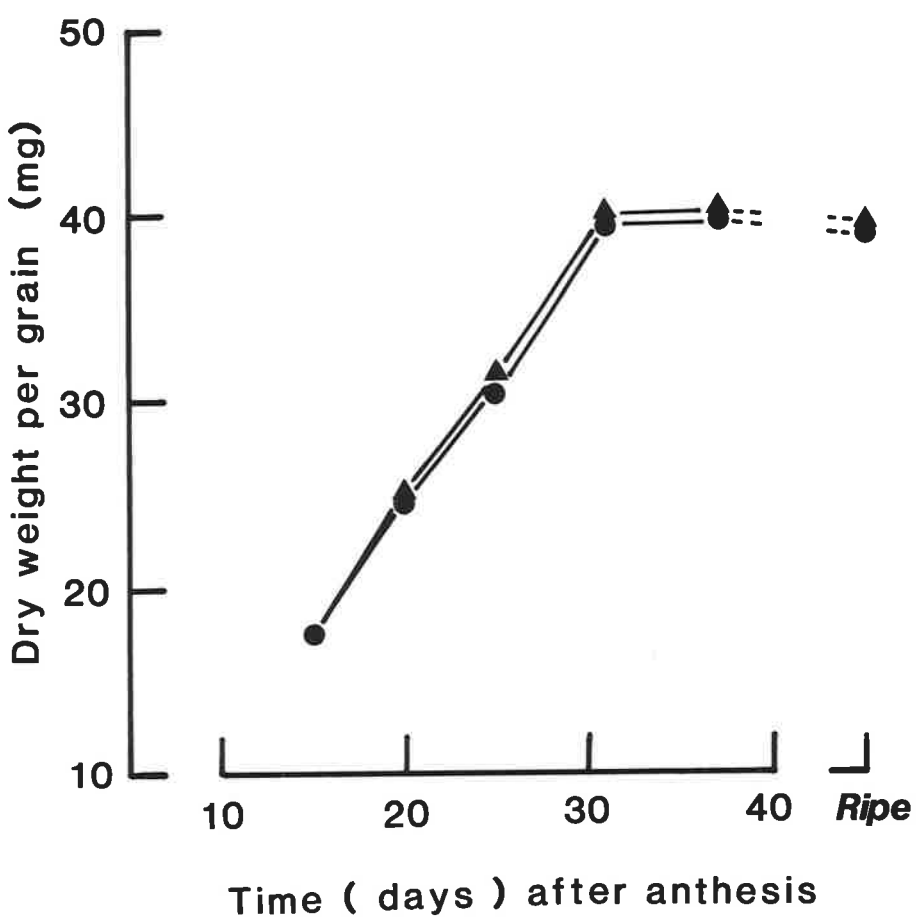
Fig. 3.1.5

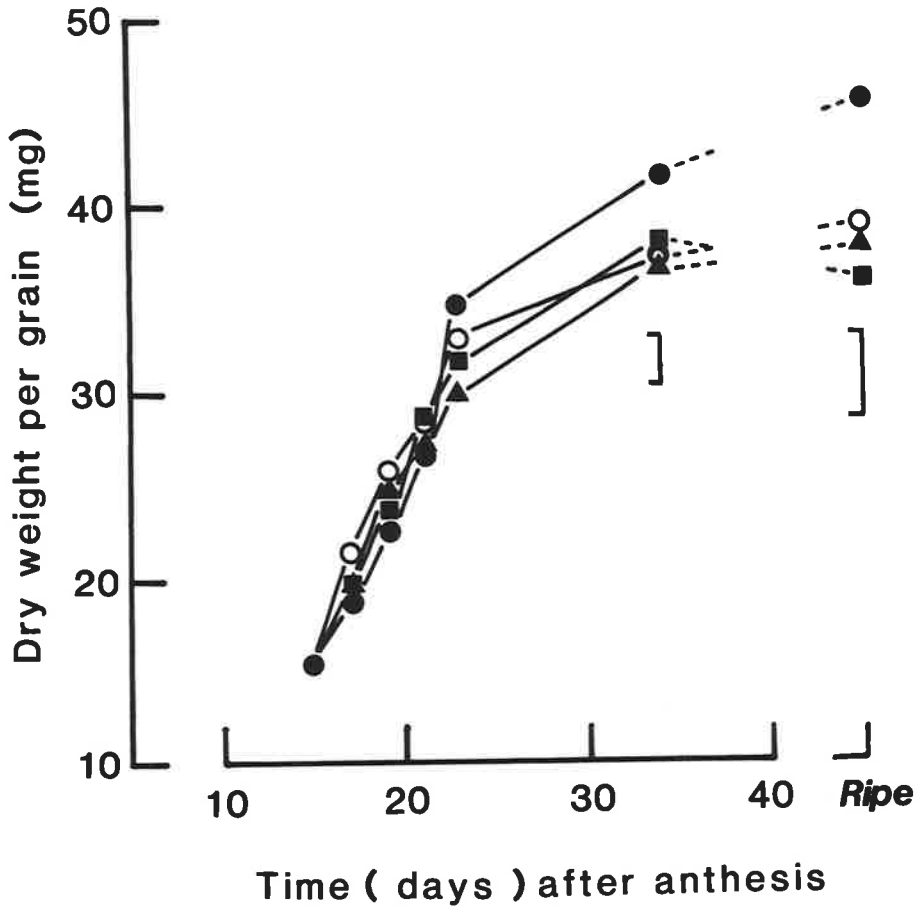
Dry weight data for a + b grains taken from central four spikelets from control ears, 21/16°C (●) and ears enclosed in the box (▲) at 21/16°C. Ears were enclosed in the box between days 15 and 21 after anthesis. (Expt. 2).

Fig. 3.1.6

Dry weight data for a + b grains taken from central four spikelets for various treatments in Expt. 3. Symbols are

(●) Control
(▲) Ears at 31/25°C while the rest of the plant was at 26/24°C
(○) Whole plant at 26/24°C
(■) Whole plant at 31/25°C


Dry weight per grain (mg)
50
40
30
20
10
10
20
30
40
Ripe
Time (days) after anthesis


Dry weight per grain (mg)
50
40
30
20
10
10
20
30
40
Ripe
Time (days) after anthesis

Experiment 3

Raising the temperature of the whole plant or the ears (only), temporarily accelerated dry matter accumulation in the grain between days 15 and 21 after anthesis (Fig. 3.1.6). Two days after the heat stress treatments were over (on day 23 after anthesis) dry matter per grain was reduced in those heat stress treatments involving warming of the whole plant or the ears only at 31/25°C (Table 3.1.4) as compared to grains developing at the cooler temperature (21/16°C). At a later stage of grain growth, on day 34 after anthesis, reductions in dry weight per grain were more pronounced for the whole plant (31/25°C) or ear warming treatments (2.9 mg and 4.6 mg respectively) as compared to warming the whole plant at 26/24°C (1.6 mg). However, at maturity, dry weight per grain was significantly reduced in all heat stress treatments (Fig. 3.1.6), and there were no significant differences between any of the heat stress treatments.

Water content in the grain was reduced in all the heat stress treatments two days after the beginning of the treatments (day 17 after anthesis) as compared to grains developing in control ears, and it stayed lower in the grains which were heat stressed at all the subsequent stages of grain growth .

Total ear grain weight at maturity was significantly reduced in all the three heat stress treatments (Table 3.1.5) and was mainly due to a reduction in individual grain weight rather than a reduction in grain number as observed in some treatments of experiment 1.

Discussion

Total grain weight per ear is a product of grain number and individual grain weight, and both these components seem to be affected by a brief period of ear warming during grain filling (Tables 3.1.1 and 3.1.5). Reduction in grain number, observed mainly in the two ear warming treatments (30/25°C and 33/25°C), was due mainly to a reduction in grain set at terminal florets (florets c and d, Fig. 3.1.2), however, some reduction in grain number was also observed at proximal (florets a and b) positions. Different florets within a spikelet or the same floret in different spikelets are asynchronous in development (Saini 1982; Singh 1982). For instance, floret d is 3-4 days slower in development than floret a within the same spikelet. Similarly, there is 1-2 days difference in flowering between the florets in the middle of the ear and the ones in the basal or the terminal spikelets. The developmental stage

Table 3.1.4

Fresh weight, dry weight and water content for a + b grains from the central four spikelets of the ears from: control plants (C), or plants held at 26/24°C (C1), or when ears were held at 31/25°C while the rest of the plant was at 26/24°C (E), or when whole plant was held at 31/25°C (T) between days 15 and 21 after anthesis. (Expt. 3)

Days after anthesis	Treatment	Weight per grain (mg): Fresh weight	Dry weight	Water content
*15	C	49.3	15.1	34.2
17	C	55.4	18.9	36.5
	C1	52.8	21.3	31.5
	E	50.9	19.4	31.5
	T	51.3	19.3	32.0
19	C	55.0	22.3	32.8
	C1	54.1	25.5	28.5
	E	53.5	24.7	28.7
	T	52.4	23.1	29.3
21	C	59.3	26.8	32.5
	C1	56.6	28.4	28.2
	E	52.4	26.7	25.7
	T	57.0	28.5	28.5
23	C	71.1	34.6	36.5
	C1	62.1	33.0	29.2
	E	57.1	30.0	27.1
	T	60.0	31.7	28.4
34	C	72.6	41.7	30.8
	C1	63.7	37.8	25.9
	E	62.9	36.8	26.1
	T	64.8	38.0	26.9
L.S.D.	interaction	n.s.	2.6	n.s.

* day 15 values were not included in the analyses.

Table 3.1.5

Effects on final grain yield components of raising the temperature of the whole plant or the ears only for a brief period (between days 15 and 21 after anthesis) during the grain filling phase. Values in parentheses are per cent of control values. (Expt. 3)

Treatment	Number per ear		Total ear grain weight (mg)	Average single grain weight (mg)
	Spikelets	Grains		
Control at 21/16°C	15.2	33.6	1329	39.6
Whole plant at 26/24°C	13.8	28.8	1001 (75.3)	34.8 (87.9)
Ears at 31/25°C the rest of plant at 26/24°C	14.6	32.8	1079 (81.1)	32.9 (83.1)
Whole plant at 31/25°C	15.0	33.8	1094 (82.3)	32.3 (81.6)
L.S.D. treatment	n.s.	2.3	100	3.5

of ears used for the imposition of ear warming treatments was judged on the basis of the most advanced florets. Therefore, the abortion of grains, as a result of ear warming may be due to differences in the physiological stage of development assuming that there are developmental differences in sensitivity to heat stress.

Reduction in grain weight was observable at all floret positions and in all the zones of the ear (Table 3.1.2, and Figs. 3.1.3 and 3.1.4). Nevertheless, a significant reduction in average grain weight was not observed in all ear warming treatments. For instance, warming ears for 4 or more days at 30/25°C did not result in a significant reduction in average single grain weight. However, this treatment caused abortion of grains, and furthermore, a higher proportion of the potentially small grains (florets c and d, Fig. 3.1.2) were aborted than those potentially growing larger. Many workers have previously reported an increase in the weight of remaining grains, when numbers of grains on a spike are reduced artificially (Stoy 1965; Konovalov 1966; Bingham 1967, 1969; Brocklehurst 1977; Bremner and Rawson 1978; Martinez-Carrasco and Thorne 1979a, 1979b; Radley and Thorne 1981; Thorne 1981; Saini 1982). Thus, the aberrant response to ear warming at 30/25°C could be explained by assuming that (a) the distribution of grain weight was shifted in the direction of higher average weight per grain by the change in the distribution of floret fertility, and (b) that any reduction in grain weight due to heating was partly or wholly counterbalanced by the promotive effect of reduction in grain number. However, no simple explanation for a greater reduction in grain number at lower temperatures compared to higher temperatures could be given. But it may be possible that persistent high temperature for longer periods may interfere with the development of endosperm, but an early return to a lower temperature after an exposure to extremely high temperature permits eventual development of endosperm to a mature grain.

There were no differences apparent in the observed grain growth characteristics, when the whole plant or the ears alone were warmed (Table 3.1.5). Obviously, enclosing ears in the box, when air at about ambient temperature (24/16°C) was passing through it, had no discernible effects on characteristics (fresh weight, dry weight or water content) of grain growth compared to grains in the ears growing at ambient conditions (21/16°C, Fig. 3.1.5). Therefore, it is assumed (in the sections to follow) that grain growth in ears enclosed in the box is a response to a temperature differential, and not to differences in light

quality (not determined), or light intensity (Section 2.3.3), or air movement.

3.2 RESPONSES OF GRAIN GROWTH TO EAR WARMING

Introduction

High temperature during grain growth results in an accelerated senescence of green tissue (especially leaves) and leads to a significant increase in the respiration of the grain (Stoy 1965; Spiertz 1974; Wardlaw et al. 1980; Vos 1981). These responses may lead to a shortage of carbohydrate (assimilate) available for grain growth (Thornley 1971; Spiertz 1977). Some workers (Chowdhury and Wardlaw 1978; Wardlaw et al. 1980) have suggested, however, that a reduction in grain weight is not a result of reduced availability of assimilate.

These conclusions referred to above have been drawn from photosynthetic and respiratory measurements of the ear or the plant and actual amounts of carbohydrate available for distribution to the grain or amounts of soluble sugars in the grain have not been evaluated. A reduction in dry weight per grain due to a brief period of elevated temperature during the grain filling phase (Section 3.1, Wardlaw 1970; Ford et al. 1976; Radley 1976) may be a result of factors within the grain and/or factors operating in the other parts of the plant.

Raising the temperature of the whole plant or the ear alone, results in a reduction in water content of the grain (Table 3.1.4), which could result in a change in the water relations of the grain. The aims of the experiments described in this section were:

(a) To see whether a brief period of ear warming during grain filling results in a change of water relations of the grain or in the other parts of the ear.

(b) To determine whether a reduction in dry weight per grain, as a result of brief period of ear warming, is due to a change in the availability of carbohydrate for dry matter accumulation in the ear or the grain itself.

Materials and Methods

For each experiment, plants were raised in the glasshouse for 2 weeks and then transferred to the growth room (Section 2.2.2). Ear warming treatments were carried out in the same growth room, and that is where the plants stayed until maturity.

Experiment 4

Ears were warmed at 33/25°C (day/night) for 6 days, between days 10 and 16 after anthesis. Warm air passing through the ear warming box was not supplemented with additional water vapour to maintain high levels of relative humidity, so mean vapour pressure deficit was 2.5 (kPa) during the day (14 h) and 0.95 (kPa) during the night. This ear warming treatment would be referred to as low humidity in the text and in the legend to a table or a figure.

The growth of b and c florets was followed by taking samples from the four control spikelets from one side of the ear (from four separate ears) on days 10, 14, 18, 22, and 26 after anthesis and when ears had ripened. From the other side of the same ears, b and c grains were sampled simultaneously on days 10, 14, 18, 22, and 26 after anthesis for chlorophyll determinations (Section 2.6.2) in the pericarp.

Experiment 5

Ears were warmed for 7 days at 30/25°C from day 10 to day 17 after anthesis either at low humidity [mean V.P.D. 1.7 kPa (day), 0.95 kPa (night)] or at high humidity [mean V.P.D. 0.64 kPa (day) and 0.48 kPa (night)]. The a and b grains from spikelets 3-9 and 11-16 inclusive were harvested on day 10, 12, 14, 18, and 24 after anthesis and when the ears had ripened. Florets a and b from spikelet 10 on days 12, 14, 16, 18, 22, and 24 after anthesis were separated into grains and bracts, and were used for determination of water potential (Section 2.5.3).

Experiments 6a and 6b

Ears were warmed at 33/25°C, between days 15 and 21 after anthesis, at low humidity [V.P.D. 2.5 kPa (day) and 0.95 kPa (night)] or at high humidity [V.P.D. 0.75 kPa (day) and 0.48 kPa (night)].

Grains from the b florets and their enclosing bracts from the central four spikelets from one side of each of the four ears, and the portion of the rachis subtending them, were used for fresh weight, total soluble sugars and sucrose determinations (Sections 2.6.1.1 and 2.6.1.2). Sampling was done on days 15, 20, 25, and 30 after anthesis and when the ears had ripened. From the other side of the same ears similar samples were used for water content determinations.

From another set of the ears treated in the same way, three sets of 8 grains (a + b) from the control four spikelets from one side of the

Fig. 3.2.1

Effects of warming ears on change in (a) dry matter accumulation (solid symbols), and water content (open symbols); (b) sucrose content; and (c) other soluble sugars in the whole grains. Data for b grains of the central four spikelets are plotted. Bars indicate L.S.D. for treatments x time. Ears kept at 21/16°C (day/night through grain development (●, ○), or warmed at 33/25°C (day/night) for 6 days (▲, △). Solid horizontal bars refer to the treatment period in this and other figures in this section. (Expt. 4)

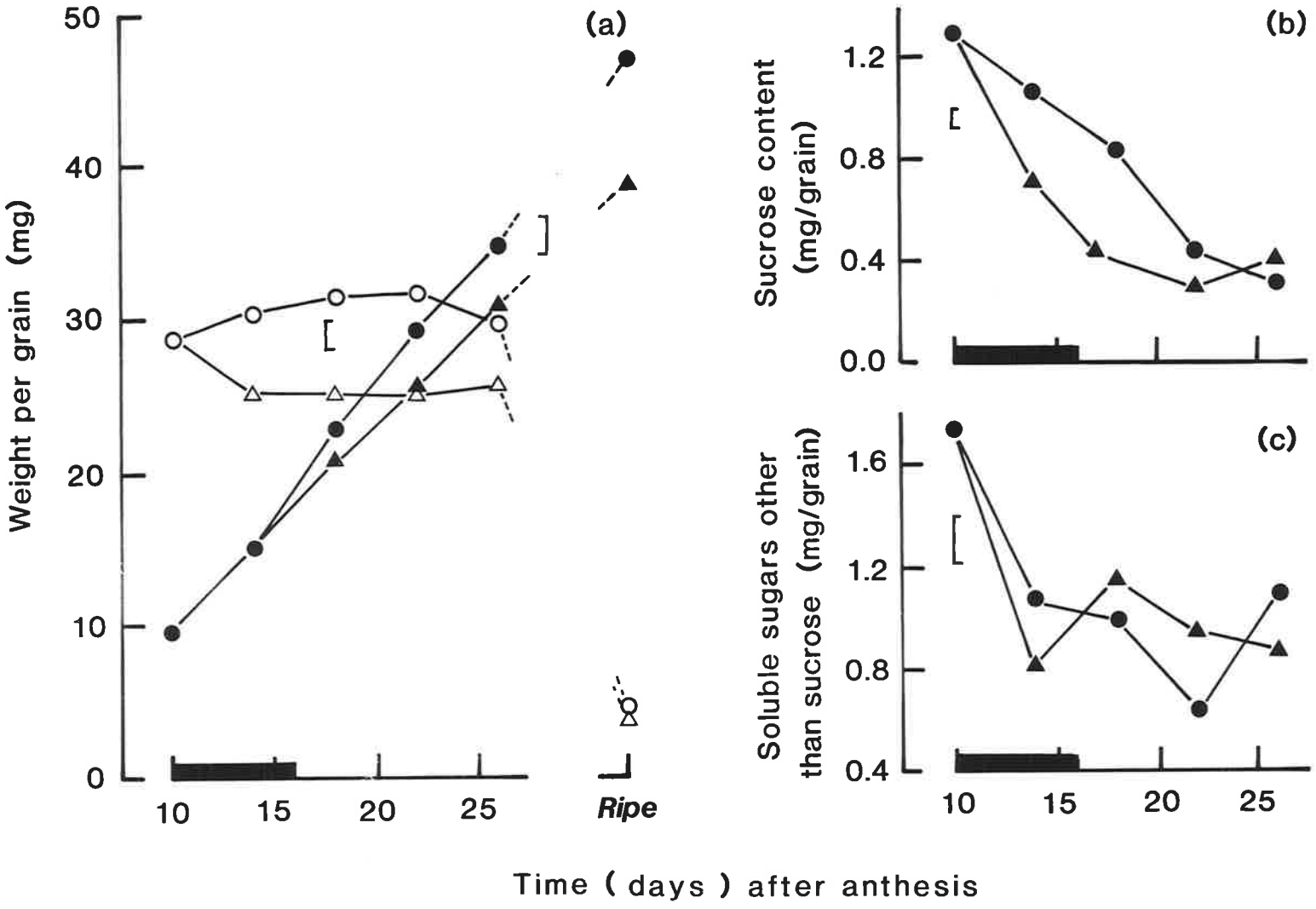

(a)
(b)
(c)
Weight per grain (mg)
Sucrose content (mg/grain)
Soluble sugars other than sucrose (mg/grain)
Ripe
Time (days) after anthesis

Table 3.2.1

Fresh weight, dry weight, and amounts of sucrose and other soluble sugars in the b grains taken from the central four spikelets from one side of the control (C) or warmed ears (T). Fresh weight and amounts of chlorophyll a(Ca) and chlorophyll b(Cb) of the pericarp of grain samples taken from the other side of the same ears. Ears were warmed at 33/25°C (day/night) between days 10 and 16 after anthesis. (Expt. 4)

Days after anthesis	Treatment	Weight per grain				Weight or amount per pericarp		
		Fresh (mg)	Dry (mg)	Sucrose (µg)	Other soluble sugars (µg)	Fresh (mg)	Ca (µg)	Cb (µg)
10*	C	38.4	9.6	1302	1741	14.4	5.06	2.44
14	C	45.5	14.9	1067	1060	13.3	5.15	1.81
	T	40.2	15.2	704	819	9.7	5.27	1.81
18	C	54.5	23.0	825	994	10.3	5.42	1.81
	T	46.0	20.9	425	1153	8.0	4.71	1.68
22	C	61.1	29.2	419	629	9.8	4.52	1.69
	T	50.6	25.6	283	948	7.6	3.29	1.26
26	C	64.7	34.7	301	1098	8.6	3.39	1.25
	T	56.7	30.9	400	860	8.1	2.33	0.91
Ripe	C	51.8	47.1	**N.E.	N.E.	N.E.	N.E.	N.E.
	E	42.7	38.8	N.E.	N.E.	N.E.	N.E.	N.E.
L.S.D. Interaction		n.s.	1.8	67	196	0.8	0.43	0.21

* Day 10 values not included in the analysis.
** Not estimated.

ear, and their enclosing bracts (the glume, the lemma and the palea) without awns were used for water potential (Ψ_w) measurements (Section 2.5.3), and similar samples from the other side were used for evaluation of osmotic potential (Ψ_π, Section 2.5.4).

Experiment 6b was a replicate of experiment 6a (as described above) except that the allocation of humidified air and low humidity air to the ear warming boxes was reversed to eliminate any possibility that the effects of humidity were confounded with unintentional differences between the two types of boxes.

The data for both experiments were analysed separately and as a whole. There were no significantly different responses between the two experiments in any of the parameters measured except for the amounts of sucrose in the grain, and there were no significant interactions between treatments and experiments. Grains in experiment 6b grew faster and became heavier during the early phase of grain growth than those in experiment 6a, but final weight per grain was much the same in both (45.6 mg in experiment 6a and 45.0 mg in experiment 6b). So, the results presented are the mean values of both experiments 6a and 6b.

Results

Experiment 4

Grains developing in floret b in warmed ears at 33/25°C accumulated dry matter at a slower rate (1.32 mg per day, calculated by regression between days 10 and 26 after anthesis) than those which stayed at 21/16°C (1.60 mg per day), and ripe grains from the warmed ears weighed 17.6% less than those in control ears (Fig. 3.2.1a). Grains from c florets (Table 3.2.2) displayed a similar response to warming by accumulating dry matter at the rate of 1.14 mg per grain per day as compared to 1.42 mg per day in the control ears. Ripe c grains from warmed ears weighed 29.8% less than those from the ears kept at 21/16°C.

Water content in both b (Fig. 3.2.1a) and c grains was significantly reduced after four days of ear warming and stayed lower than that in the cool ears during rest of the grain filling phase.

Sucrose content in the b grain (Fig. 3.2.1b) continuously declined with development at both temperatures, but ear warming resulted in a significantly faster drop in sucrose content. The response to warming was evident four days after the beginning of the treatment, and warming appears to have advanced the course of decline in sucrose content by

Table 3.2.2

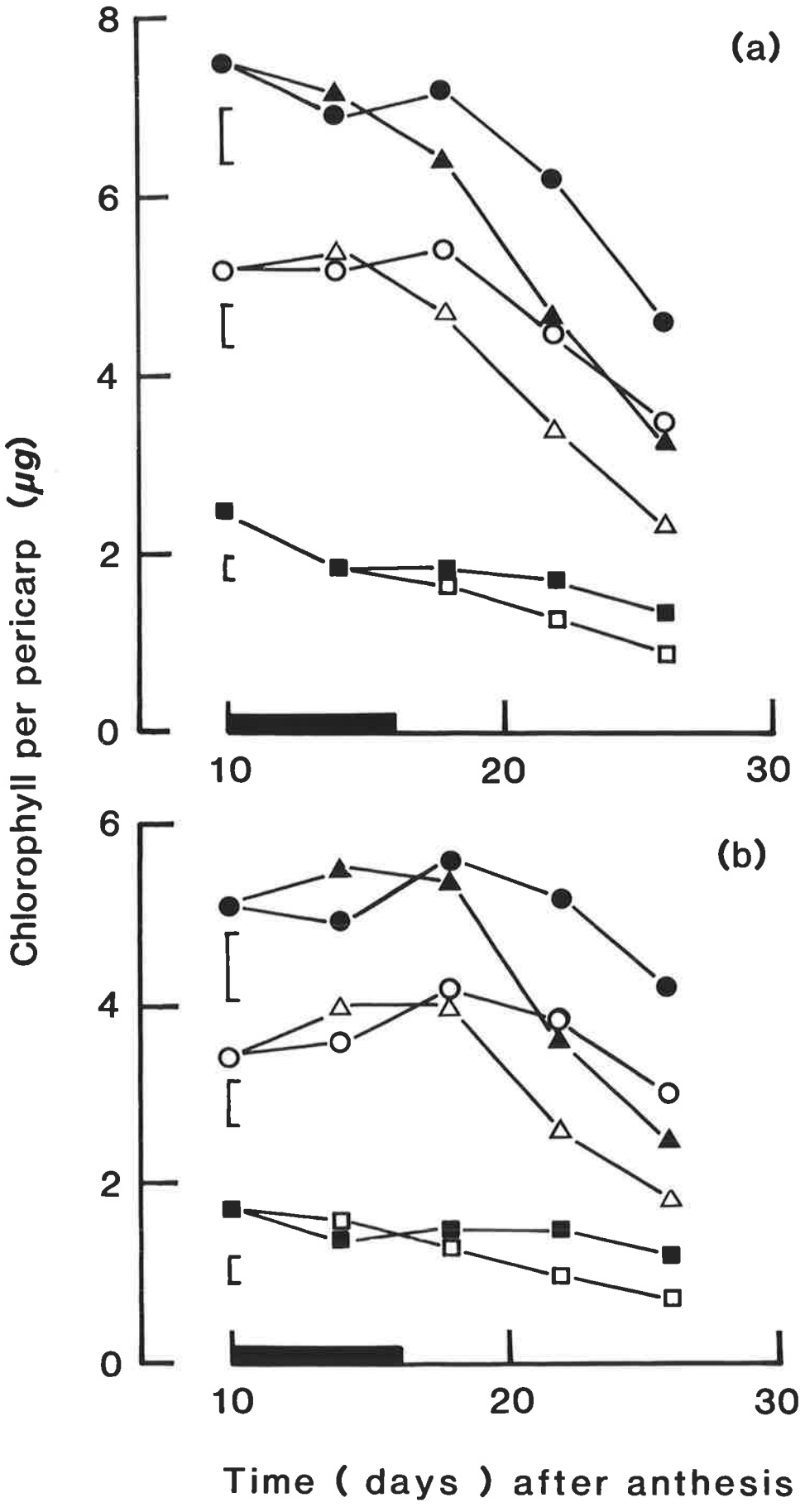
Fresh weight, dry weight, and amounts of sucrose and other soluble sugars in the c grain taken from the central four spikelets from one side of the control (C) or warmed ears (T). Fresh weight and amounts of chlorophyll a(Ca) and chlorophyll b(Cb) of the pericarp of grain samples taken from the other side of the same ears. Treatment details as for Table 3.2.1.

Days after anthesis	Treatment	Weight per grain				Weight or amount per pericarp		
		Fresh (mg)	Dry (mg)	Sucrose (µg)	Other soluble sugars (µg)	Fresh (mg)	Ca (µg)	Cb (µg)
10*	C	23.3	6.4	1077	773	13.0	3.41	1.72
14	C	32.8	10.5	954	1038	11.4	3.62	1.39
	T	27.2	9.7	693	600	7.5	4.06	1.53
18	C	43.8	18.5	662	717	9.2	4.22	1.46
	T	35.7	15.8	382	412	7.3	4.08	1.35
22	C	48.0	23.1	402	636	8.3	3.77	1.44
	T	36.5	18.6	195	574	5.9	2.62	0.97
26	C	53.0	28.4	387	772	7.1	2.97	1.23
	T	44.6	24.8	384	577	6.5	1.80	0.69
Ripe	C	43.5	39.6	**N.E.	N.E.	N.E.	N.E.	N.E.
	T	30.5	27.8	N.E.	N.E.	N.E.	N.E.	N.E.
L.S.D. Interaction		1.3	2.1	76	n.s.	0.9	0.50	0.23

* Day 10 values not included in the analysis.
** Not estimated.

Fig. 3.2.2

Total chlorophyll (●, ▲), chlorophyll a (○, △), and chlorophyll b (■, □) in the pericarp of b grains (a), and c grains (b). Data is for the grains from the central four spikelets from the control (●, ○, ■), or warmed (▲, △, □) ears. Bars indicate L.S.D. for times x treatments. (Expt. 4)


(a)
(b)
8
6
4
2
0
10
20
30
Chlorophyll per pericarp (μg)
Time (days) after anthesis

about five days. The soluble sugars fraction (excluding sucrose) also declined with development (Fig. 3.2.1c and Table 3.2.1) and was significantly lower on day 14 in the grains from the warmed ears compared to those which stayed at 21/16°C, but later on (day 22), the opposite effect was observed.

The amounts of sucrose and soluble sugars (excluding sucrose) in the c grain (Table 3.2.2) exhibited a similar pattern to the b grain. Although the amounts of sucrose and other soluble sugars (excluding sucrose) when expressed on a per grain basis were lower for the c grain (due to smaller size) than for the b grain, when these values were expressed on a dry weight or water content basis the values for c were either comparable or higher than those in the b grain.

Chlorophyll content in the pericarp

For much of the developmental period of the grain, the pericarp is a bright emerald green tissue surrounded by a transparent outer layer, and turns to a pale golden colour as the grain ripens. Chlorophyll content in the pericarp can, therefore, be taken as an indicator of developmental stage (cf. Rogers and Quatrano 1983).

About 72% of chlorophyll extracted from the pericarp of grain b or c was chlorophyll a (Fig. 3.2.2, and Tables 3.2.1 and 3.2.2), and changes in chlorophyll a paralleled changes in total chlorophyll. Chlorophyll content, in the pericarp of the b grain (Fig. 3.2.2a), changed little between days 10 and 18 after anthesis but thereafter started to decline, whereas such a decline in the chlorophyll content of the pericarp of the c grain was not apparent until day 22 after anthesis (Fig. 3.2.2b). Judging by the displacement of the curves on the abscissa, warming appeared to have accelerated chlorophyll degradation by about 4-5 days.

Experiment 5

Warming ears at high humidity for a brief period slightly accelerated dry matter accumulation (Fig. 3.2.3) compared to those grains in the ears warmed at low humidity. This difference was evident up to the sixth day after the beginning of the treatment. However, 8 days later dry matter accumulation was depressed in both treatments (by 2.2 mg and 2.3 mg for high and low humidity respectively) as compared to the grains in the ears which stayed at 21/16°C. Final weight per grain was greatest in the unwarmed ears (39.3 mg) followed by the grains in

Fig. 3.2.3

Effects of warming ears at 30/25°C (day/night) at high humidity or at low humidity on dry matter (solid symbols) and water content (open symbols) of the grain. Data for a+b grains of spikelets 3-9 and 11-16 inclusive are plotted. Bars indicate L.S.D. for treatments x times. (Expt. 5)

●,○ Ears kept at 21/16°C throughout grain development.
■,□ Ears warmed at high humidity.
▲,△ Ears warmed at low humidity.

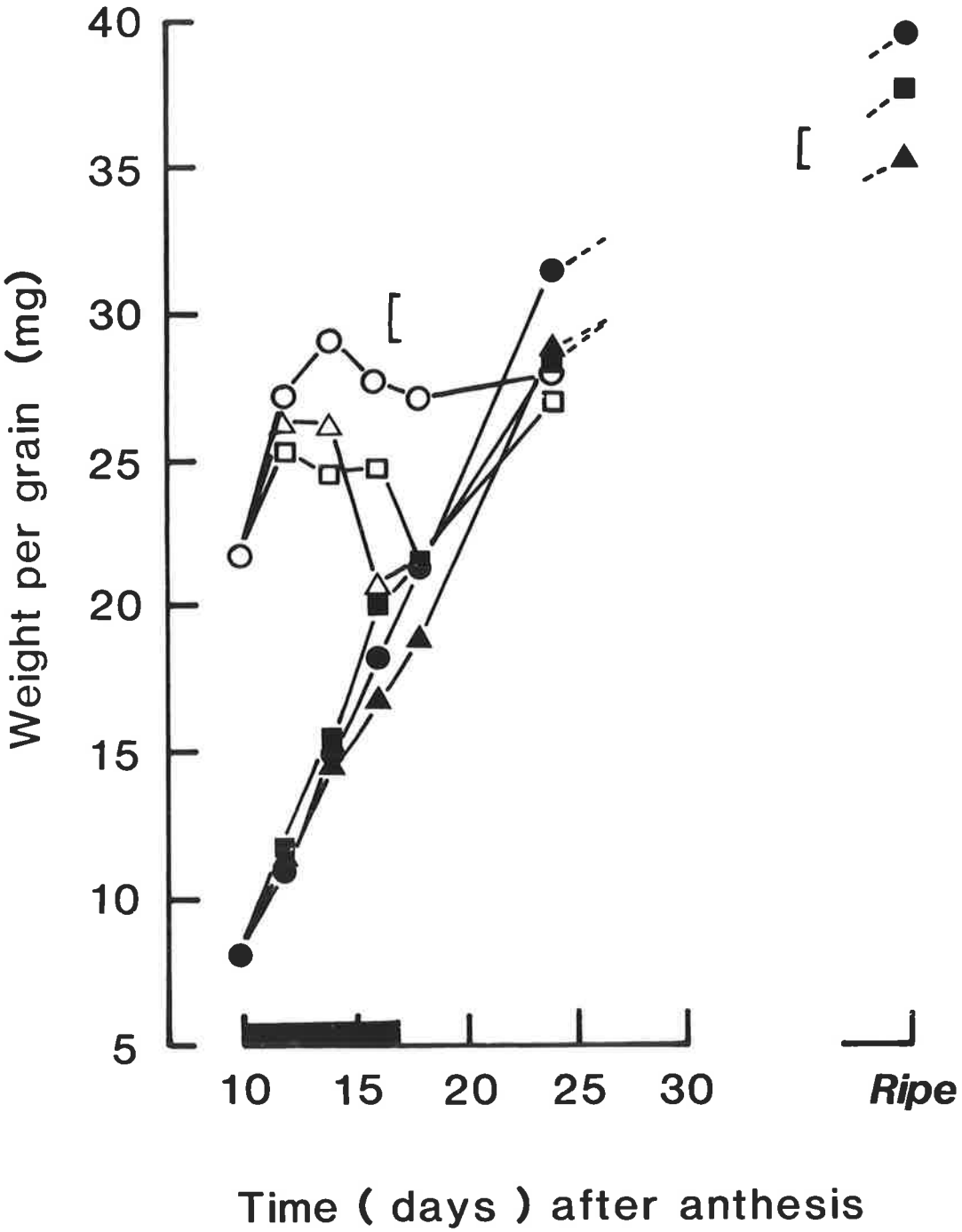

40
35
30
25
20
15
10
5
Weight per grain (mg)
10
15
20
25
30
Ripe
Time (days) after anthesis

Table 3.2.3

Effects of warming ears at low or high humidity on the water potential of the grain and the bracts. Treatment and sampling details are given in the text. (Expt. 5)

Days after anthesis	Ψ_w(MPa) of grains			Ψ_w(MPa) of bracts		
	Control	High humidity	Low humidity	Control	High humidity	Low humidity
12	-1.20	-1.26	-1.32	-0.80	-1.30	-1.02
14	-1.10	-0.89	-0.86	-1.07	-1.18	-1.37
16	-0.98	-0.89	-0.87	-1.16	-1.50	-0.86
18	-1.18	-1.09	-0.97	-1.07	-0.90	-1.24
22	-1.21	-1.07	-1.20	-1.19	-1.34	-1.11
24	-1.01	-1.05	-1.08	-1.40	-1.53	-1.47
Mean of days	-1.11	-1.04	-1.05	-1.11	-1.25	-1.18
Least significant differences						
Treatment		n.s.			0.06	
Interaction		n.s.			0.16	

the ears warmed at high humidity (38.0 mg) and least for the grains in the ears warmed at low humidity (35.1 mg).

Water content of the grains in warmed ears fell significantly after 4 days of ear warming at both levels of humidity (Fig. 3.2.3). Eight days after the imposition of ear warming the water content of the grains at high or low humidity were respectively 20.7 mg and 20.4 mg as compared to 27.1 mg in the cool ears. However, later still (day 24), there were no significant differences in water content of grains of warmed or unwarmed ears.

Water potential of the bracts and the grains

Water potential (Ψ_w) of the bracts was between -0.80 and -1.53 MPa (Table 3.2.3) and exhibited a downward trend with time. Ear warming did not affect Ψ_w of the bracts. Although there were significant interactions between treatments and time, there was no evidence that warming at low as compared to high humidity resulted in lower Ψ_w.

Water potential of the grains did not change significantly as a result of warming the ears. If anything, shortly after the imposition of the treatments the overall effect of warming was that Ψ_w was apparently slightly higher (more positive) in the warmed than in the unwarmed grains (Table 3.2.3).

Temperature at various positions within the floret

Transpiration from the ears warmed at low humidity was presumably faster than for the ears warmed at high humidity which could result in differences in temperature in the immediate vicinity of the grain. To check this possibility, temperature at various positions within the most central floret, for a number of ears (10 per treatment), on the ears warmed at high or low humidity was monitored (on day 3 after initiation of ear warming treatments) by inserting fine copper-constantan thermocouples (32 American wire gauge) and values are given in the tabulation on next page.

Although the temperature inside the floret was higher both in the control as well as for ears warmed at either humidity than the surrounding air, but there were no significant differences in temperature at various positions within a floret between the ear warming treatments.

Thermocouple position	Growth room	High humidity box	Low humidity box
In air	20.7	30.7	31.0
Between the Glume and the Lemma	22.1	32.8	31.8
Between the Lemma and the grain	22.1	32.0	32.4
Between the Palea and the grain	22.0	31.2	31.5

Experiments 6a and 6b

Warming ears at high humidity (for a brief period) resulted in a higher rate of dry matter accumulation in the grains (1.74 mg grain^{-1} day^{-1}) as compared to the grains (1.35 mg grain^{-1} day^{-1}) in ears warmed at low humidity (Fig. 3.2.4a and Table 3.2.4). Up to 30 days after anthesis the courses of dry matter accumulation in the grains in the unwarmed ears and in those warmed at high humidity were almost identical. However, final weight per grain was significantly reduced in both the ear warming treatments (45.5, 41.7 and 48.7 mg for high humidity, low humidity and the control treatments respectively).

Water content of the grains fell significantly (28.3, 26.7 and 32.2 mg for high humidity, low humidity and the control treatments respectively) in both ear warming treatments after 5 days, but there was no significant difference between the two humidity treatments (Fig. 3.2.4a). However, water content of the grains in the ears warmed at high humidity rose between days 25 and 30 (also see Fig. 3.2.3), while in the ears warmed at low humidity grain water content fell even further.

Although the average sucrose content was significantly different between the two experiments (0.39 mg per grain, experiment 6a; 0.32 mg per grain, experiment 6b; L.S.D.=0.03), there was no significant interaction of treatments with experiments. Sucrose content in the grains of the ears warmed at high or low humidity fell significantly after 5 days of warming (expressed on per grain basis; Fig. 3.2.4b, or

Fig. 3.2.4

Effects of warming ears at 33/25°C (day/night) at high humidity on grain dry matter (solid symbols) and water content (open symbols) (a), sucrose content (b) and other soluble sugars excluding sucrose (c) in the b grains from the four central spikelets. Bars indicate L.S.D. for treatments x times. (Mean of Expts. 6a and 6b)

●,○ Ears kept at 21/16°C throughout grain development.
■,□ Ears warmed at high humidity.
▲,△ Ears warmed at low humidity.

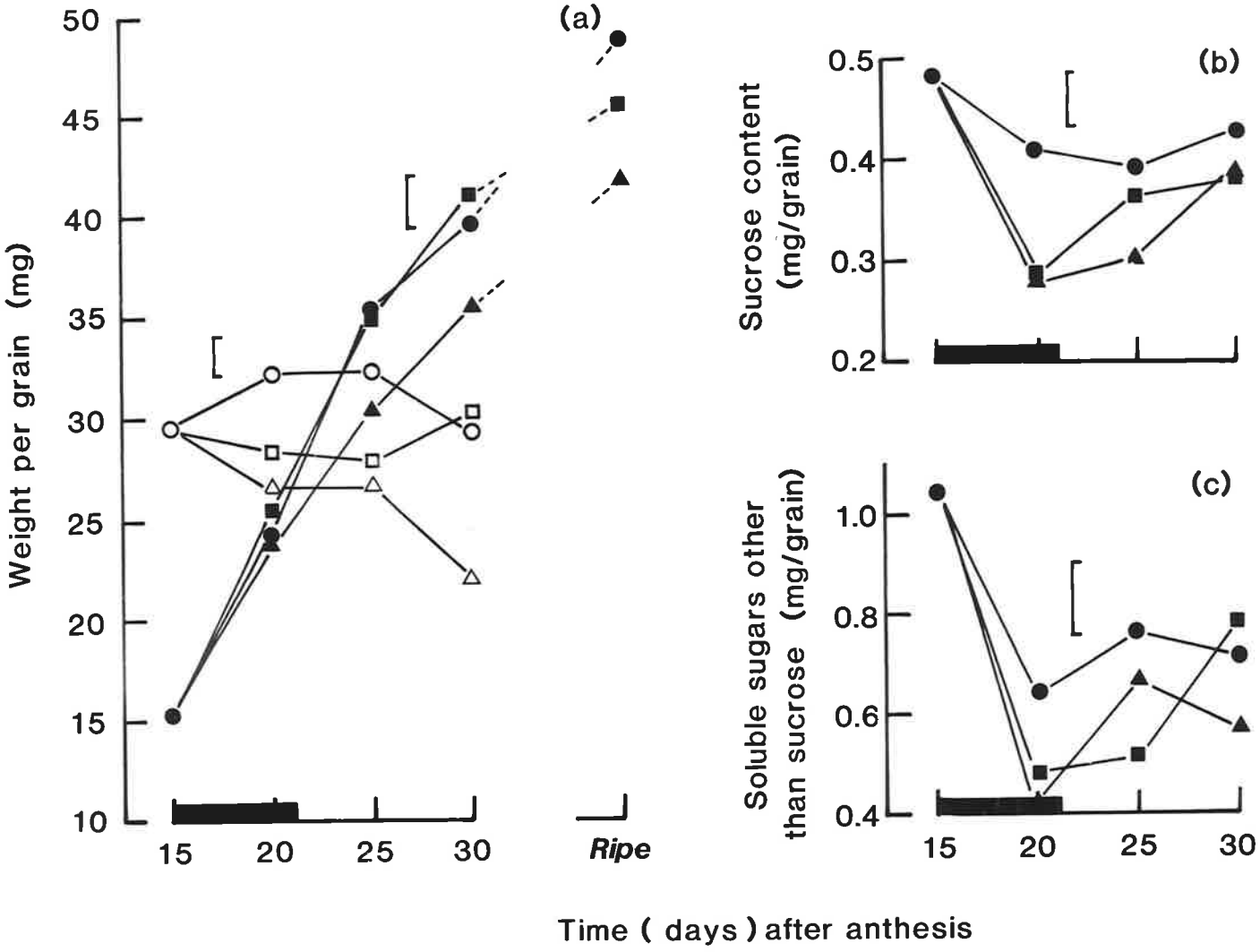

(a)
(b)
(c)
Weight per grain (mg)
Sucrose content (mg/grain)
Soluble sugars other than sucrose (mg/grain)
50
45
40
35
30
25
20
15
10
0.5
0.4
0.3
0.2
1.0
0.8
0.6
0.4
15
20
25
30
Ripe
Time (days) after anthesis

Table 3.2.4

Effects of warming ears at 33/25°C (day/night) at high humidity or at low humidity on fresh weight, dry weight and soluble sugars in the b grain. Grains were taken from the central four spikelets from control ears (C), or ears warmed at high humidity (TH), or at low humidity (T). Data are mean of expts. 6a and 6b.

Days after anthesis	Treatment	Weight per grain (mg) Fresh	Dry	Sucrose	Other soluble sugars (µg/mg grain dry weight)
*15	C	49.6	17.6	27.2	59.1
20	C	56.4	24.2	16.9	26.3
	TH	53.7	25.5	11.1	18.7
	T	50.7	24.0	11.5	18.0
25	C	67.6	35.3	11.1	21.6
	TH	63.0	35.0	10.4	14.7
	T	57.3	30.5	9.9	22.0
30	C	68.6	39.4	10.8	18.1
	TH	71.5	41.1	9.2	18.7
	T	57.6	35.6	10.9	15.9
Ripe	C	**N.E.	48.7	N.E.	N.E.
	TH	N.E.	45.5	N.E.	N.E.
	T	N.E.	41.7	N.E.	N.E.
L.S.D. Interaction		3.3	2.7	2.1	5.2

* Day 15 values not included in the analysis.
** Not estimated.

Table 3.2.5

Fresh weight, dry weight, sucrose and other soluble sugars in the c grain taken from the central four spikelets from control ears (C), or ears warmed at high humidity (TH), or at low humidity (T). Details as for Table 3.2.4.

Days after anthesis	Treatment	Weight per grain (mg) Fresh	Weight per grain (mg) Dry	Sucrose (µg/mg grain dry weight)	Other soluble sugars (µg/mg grain dry weight)
*15	C	40.1	13.4	34.7	75.8
20	C	44.8	19.2	20.8	38.6
	TH	41.7	20.0	17.3	20.1
	T	39.3	19.1	16.0	21.0
25	C	56.6	30.5	15.0	20.9
	TH	45.3	25.3	14.5	21.5
	T	47.4	25.7	13.4	27.1
30	C	59.0	33.8	12.8	20.7
	TH	57.7	33.5	13.1	17.9
	T	45.4	25.1	14.8	28.7
Ripe	C	**N.E.	40.6	N.E.	N.E.
	TH	N.E.	32.9	N.E.	N.E.
	T	N.E.	28.0	N.E.	N.E.
L.S.D. Interaction		3.9	2.8	2.4	5.5

* Day 15 values not included in the analysis.
** Not estimated.

on mg grain dry weight; Table 3.2.4) as compared to the grains developing at 21/16°C. However, 9 days after the warming treatments were finished, there were no differences between any of the means. Soluble sugars (excluding sucrose) decreased with the development of the grain (Table 3.2.4 and Fig. 3.2.4c) and warming either at high or low humidity resulted in a significant reduction of soluble sugars after 5 days of warming as compared to grains in the ears kept at 21/16°C.

Responses of c grains in terms of the rate of dry matter accumulation, amounts of sucrose and other soluble sugars (excluding sucrose) to ear warming at high or low humidity were similar to those reported for the b grain and are given in Table 3.2.5.

Warming ears at either humidity had no affect on fresh or dry weight of the rachis (Table 3.2.6), or the bracts (Table 3.2.7) at any sampling stage. There were no reductions apparent in sucrose concentration (on dry weight basis) in the rachis due to ear warming. On the contrary, on days 25 and 30 after anthesis, there was a significant increase in sucrose concentration in the rachis from ears which were warmed at high humidity. Total soluble sugars (excluding sucrose) concentration (on tissue water basis) in the rachis of unheated ears decreased between days 15 and 25 after anthesis, but there was no significant change in sucrose content over the experimental period (Fig. 3.2.5b). Warming at low but not at high humidity accelerated the decline in soluble sugars excluding sucrose (Fig. 3.2.5a). There were no significant differences between sucrose contents of the rachises of unwarmed ears and of ears warmed at low humidity at any stage of grain development (Fig. 3.2.5b).

Soluble sugars (other than sucrose) in the bracts did not display any temporal change and warming had no effect on the levels observed (Fig. 3.2.5c). Sucrose content, on the other hand, markedly incrased in both ear warming treatments (Fig. 3.2.5d, on dry weight basis; Table 3.2.7), but not in the unwarmed ears.

Water potential and Osmotic potential

Water and osmotic potentials of the grains in the ears warmed at low humidity were higher after the first 2 days of warming than in the unwarmed ears (Table 3.2.8), whereas warming at high humidity had no effect. However, 4, 6, and 15 days later water and osmotic potentials in the warmed ears were lower than in the control ears. Mean values

Table 3.2.6

Fresh weight, dry weight, sucrose and other soluble sugars for the piece of the rachis bearing the central four spikelets for control ears (C), or ears warmed at high humidity (TH), or ears warmed at low humidity. Details as for Table 3.2.4.

Days after anthesis	Treatment	Weight (mg) for 1 node and 1 internode of the rachis		Soluble sugars in the rachis	
		Fresh	Dry	Sucrose (µg/mg dry weight)	Other soluble sugars (µg/mg dry weight)
*15	C	12.6	4.1	29.0	51.0
20	C	12.3	4.1	29.7	37.6
	TH	11.7	4.1	27.3	36.2
	T	11.8	4.2	28.4	20.1
25	C	12.0	4.3	31.7	19.6
	TH	11.4	4.3	35.4	20.1
	T	12.1	4.3	28.5	17.5
30	C	11.3	4.1	27.8	22.0
	TH	12.2	4.6	35.6	18.8
	T	11.8	4.4	27.4	23.7
L.S.D. Interaction		n.s.	n.s.	n.s.	n.s.

* Day 15 values not included in the analysis.

Table 3.2.7

Fresh weight, dry weight, sucrose and other soluble sugars for the bracts enclosing the b grain from control ears (C), or ears warmed at high humidity (TH), or ears warmed at low humidity. Details as for Table 3.2.4.

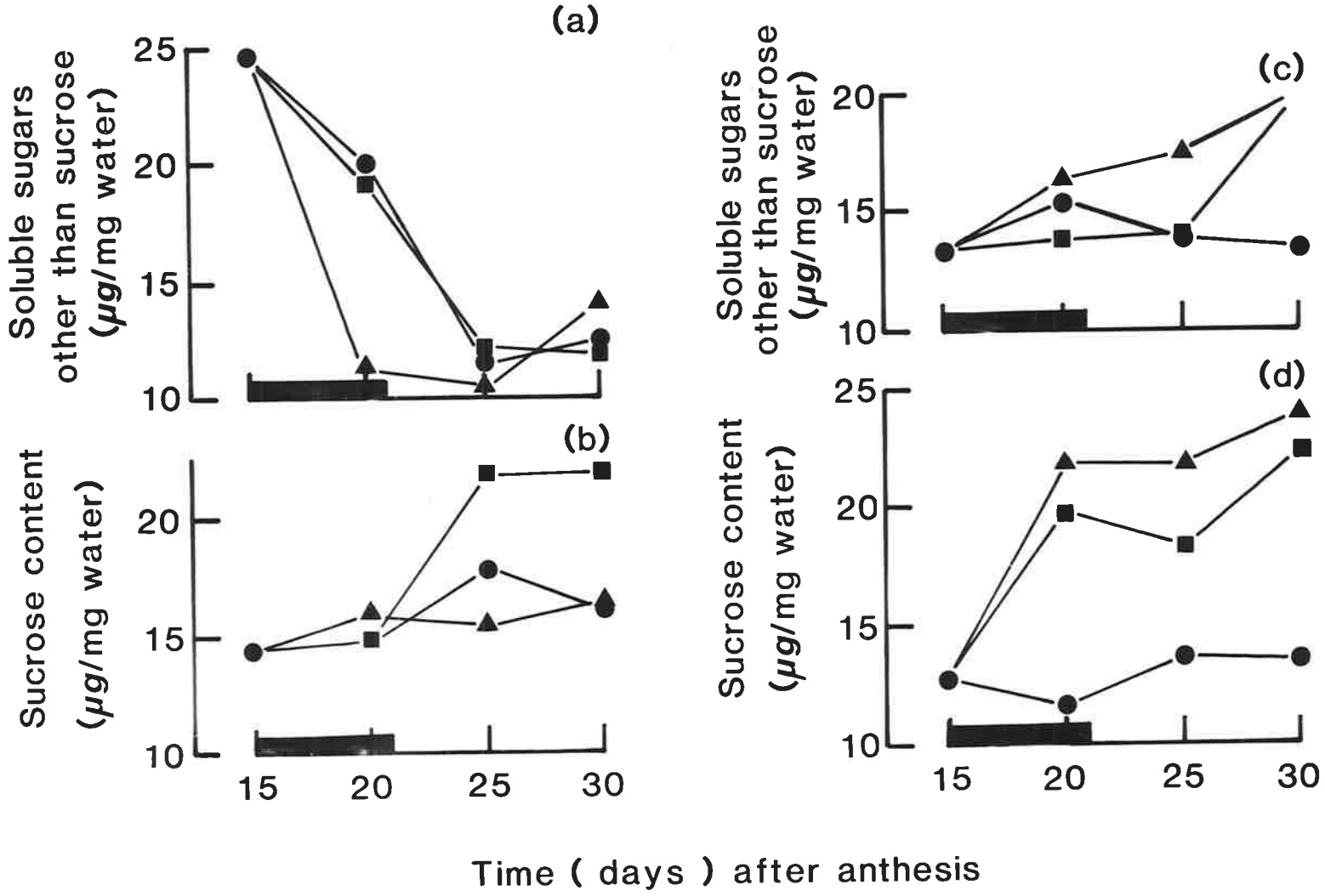
Days after anthesis	Treatment	Weight (mg) for the bracts enclosing one grain		Soluble sugars in the bracts	
		Fresh	Dry	Sucrose	Other soluble sugars
				(µg/mg dry weight)	
*15	C	16.3	6.8	18.0	18.6
20	C	16.5	6.9	16.9	22.1
	TH	16.2	7.1	26.0	17.9
	T	15.5	7.1	25.8	18.9
25	C	15.9	6.9	18.0	18.0
	TH	15.3	7.0	21.7	16.5
	T	15.6	7.3	25.0	19.9
30	C	16.1	7.1	17.1	17.0
	TH	16.5	7.6	26.4	24.4
	T	15.6	7.3	27.7	23.1
L.S.D. Interaction		n.s.	n.s.	n.s.	n.s.

* Day 15 values not included in the analysis.

Fig. 3.2.5

Effects of warming ears at 33/25°C (day/night) at high humidity (R.H. 85% during day and night) or at low humidity [R.H. 50% (day), 70%(night)] on apparent concentrations of soluble sugars (excluding sucrose) and sucrose in the rachis (a,b) and bracts (c,d). There were no significant interactions between treatments x times; overall treatment effects for sucrose in rachis (L.S.D.=2.3), soluble sugars (L.S.D.=1.9) and sucrose in bracts (L.S.D.=2.8) were significant.

● Ears kept at 21/16°C throughout grain development.
■ Ears warmed at high humidity.
▲ Ears warmed at low humidity.


(a)
Soluble sugars other than sucrose (μg/mg water)
25
20
15
10
(b)
Sucrose content (μg/mg water)
20
15
10
15
20
25
30
(c)
Soluble sugars other than sucrose (μg/mg water)
20
15
10
(d)
Sucrose content (μg/mg water)
25
20
15
10
15
20
25
30
Time (days) after anthesis

(average of sampling times) of Ψ_w and Ψ_π were most negative for the grains in the ears warmed at high humidity, followed by grains in the ears warmed at low humidity and least negative for grains in the ears grown at 21/16°C. So there does not seem to be any clear-cut relationship between the humidity of the air in which the ears were warmed and Ψ_w and Ψ_π of the grains or the bracts on the one hand, or between humidity and the rate of dry matter accumulation in the grain on the other.

Water and osmotic potentials of the bracts (Table 3.2.9) were lower than those observed in the grains and both the potentials were lower in the warmed ears than in the untreated ears. Moreover, warming at high humidity resulted in lower values than did warming at low humidity. The turgor potential (P) of the grains and the bracts (the differences between Ψ_w and Ψ_π) was relatively small throughout the grain filling period and there were no large differences between the average values for the grains and the bracts (0.31 and 0.22 MPa respectively) and no effects of warming the ears were discernible.

Discussion

Brief periods of ear warming during grain filling resulted in a reduction in total ear grain weight (Table 3.2.10) due mainly to an effect on individual grain weight and partly to a small reduction in grain number. Warming ears at high humidity appears to cause the grains to accumulate dry matter slightly faster than warming at low humidity, but differences were not significant. Both the duration and the rate of grain filling were affected by the imposition of episodes of elevated temperature to the ears (Figs. 3.2.1a and 3.2.4a). During the period of ear warming or immediately after the ear warming was discontinued, there were no visual differences between the control and the treated plants. About a week after the ear warming treatments were over, ears exposed to high temperature (at high or low humidity) appeared slightly yellower than the control ears, but at that stage there were no signs of enhanced flag leaf senescence. However, later senescence of both ears and flag leaves was enhanced in the plants in which ears were exposed to high temperature and ears in these plants matured at least 4-5 days earlier than control ears. Reduction in the duration of grain growth is well documented (Chowdhury and Wardlaw 1978) and will be dealt with in Section 4.

Table 3.2.8

Effects of warming ears at 33/25°C (day/night) at high humidity, or at low humidity on the water and the osmotic potentials of the grains. Details are given in the text. (Expt. 6)

Days after anthesis	Ψ_w(MPa) of grains			Ψ_π(MPa) of grains		
	Control	High humidity	Low humidity	Control	High humidity	Low humidity
17	-0.69	-0.61	-0.52	-1.03	-0.98	-0.87
19	-0.53	-0.65	-0.68	-0.88	-1.01	-0.98
21	-0.55	-0.70	-0.67	-0.94	-1.05	-0.96
30	-0.72	-0.99	-0.86	-0.87	-1.10	-1.09
Mean of days	-0.62	-0.74	-0.68	-0.93	-1.04	-0.98
		Least significant differences				
Treatment		0.05			0.06	
Interaction		0.10			0.11	

Table 3.2.9

Effects of warming ears at 33/25°C (day/night) at high humidity, or at low humidity on the water and the osmotic potentials of the bracts. Details are given in the text. (Expt. 6)

Days after anthesis	Ψ_w(MPa) of bracts			Ψ_π(MPa) of bracts		
	Control	High humidity	Low humidity	Control	High humidity	Low humidity
17	-0.81	-1.10	-0.81	-0.98	-1.16	-1.02
19	-0.73	-1.20	-1.60	-1.10	-1.35	-1.22
21	-0.74	-0.88	-0.85	-1.03	-1.15	-1.16
30	-0.93	-1.48	-1.26	-1.19	-1.59	-1.58
Mean of days	-0.80	-1.16	-1.08	-1.08	-1.31	-1.24
		Least significant differences				
Treatment		0.08			0.18	
Interaction		0.16			n.s.	

Table 3.2.10

Effects of a brief period of ear warming during grain filling on final ear grain weight. Details of experimental treatments are given in the text. Single grain weight is calculated from pooled data for the whole ear. Values in parentheses refer to per cent reductions over control.

Experiment no.	Parameter	Period (days after anthesis)	Treatment			L.S.D.
			21/16°C	Ears warmed at: High humidity	Low humidity	
4	Total ear grain wt.(g)	10–16	2.10	–	1.72 (–18.1)	0.27
	Single grain wt.(mg)		39.0	–	34.4 (–11.8)	3.9
5	Total ear grain wt.(g)	10–17	2.02	1.64 (–18.8)	1.52 (–24.8)	0.13
	Single grain wt.(mg)		33.2	29.4 (–11.4)	28.7 (–13.6)	3.6
6	Total ear grain wt.(g)	15–21	1.92	1.63 (–15.1)	1.39 (–27.6)	0.24
	Single grain wt.(mg)		39.1	34.1 (–12.8)	31.6 (–19.2)	3.8

However, reduction in the rate of dry matter accumulation as a result of warming the ears requires another kind of explanation. Two possible reasons, which could cause reduction in the rate of dry matter accumulation were hypothesized in the introduction to this section and are considered as follows.

(a) Water relations of the grain

There is little or no change in water content of the grain developing at uniform temperature during main grain filling phase (Figs. 3.2.1a, 3.2.3, 3.2.4a; Sofield et al. 1977b; Jenner 1982b) and the same seems to be true for water and osmotic potentials (Barlow et al. 1980). Although a period of ear warming during grain filling results in a significant reduction in the water content of the grain, this is not accompanied by substantial changes in water or osmotic potential of the grain.

Reduction in the grain water content must be due either to a more rapid loss of water from the grain, or to a slower influx of water into the grain, or due to both factors (examined in more detail in Section 3.4). Accelerated loss of water as a result of a possible downward shift in water potential in the bracts or other parts of the ear might be expected to lead in turn to a reduction in water and/or osmotic potentials of the grain, and yet such a response has not been detected. Indeed, the earliest significant response in water potential detectable (on day 17 in Table 3.2.8) is an *increase* (more positive) in water potential preceding a substantial decrease in water content three days later (Fig. 3.2.4a). Afterwards (day 19 and later: Table 3.2.8), water potential of grains from warmed ears is lower than that of the grain developing at lower temperature, but even this response is not observable in Table 3.2.3, although the grains from warmed ears in that experiment (Fig. 3.2.3) contained less water than the cooler ones.

As might have been expected, the water and osmotic potentials of the bracts (Table 3.2.9) have been shifted to more negative values by warming the ears. However, warming at high humidity has depressed water and osmotic potentials more than warming at low humidity.

The lack of an effect of warming on turgor potential of the grain indicates that water relations of the grain are dynamic and are subjected to homeostatic control. The apparent reversibility of the effect of heating on water content (Fig. 3.2.3) is further evidence of such a control.

Warming at high humidity and low humidity alike reduced water content, but heating at low humidity depressed the rate of accumulation of dry matter in the grains (Figs. 3.2.1a and 3.2.4a). This lack of parallelism, together with the disparate effects of heating at high humidity and low humidity on grain water relations and accumulation of dry matter, provide evidence that the effects of heating which result in smaller gains in dry matter are not mediated directly through an influence on water potential of the grain.

(b) Availability of soluble carbohydrates

Movement of ^{14}C-labelled photosynthate from flag leaf to ear increases with temperature between 21 and 30°C (Wardlaw et al. 1980). Also the rate of starch accumulation at 30°C is greater than at 15.5°C in detached ears of wheat cultured in solutions of sucrose (Jenner 1968). It might be expected that warming ears would result in an accelerated rate of grain growth. However, either there is no significant change in the rate of dry matter accumulation due to ear warming (high humidity treatment, Figs. 3.2.3 and 3.2.4a) or the rate is actually depressed (low humidity treatment, Figs. 3.2.1a and 3.2.4a). Both outcomes are consistent with the observation that amounts of soluble sugars declined significantly due to warming (Figs. 3.2.1b, 3.2.1c, 3.2.4b and 3.2.4c). It is conceivable that either the entry of photosynthate into the grain is depressed or carbohydrate levels in the grain are reduced due to high respiratory losses at high temperature (see references in introduction to this section).

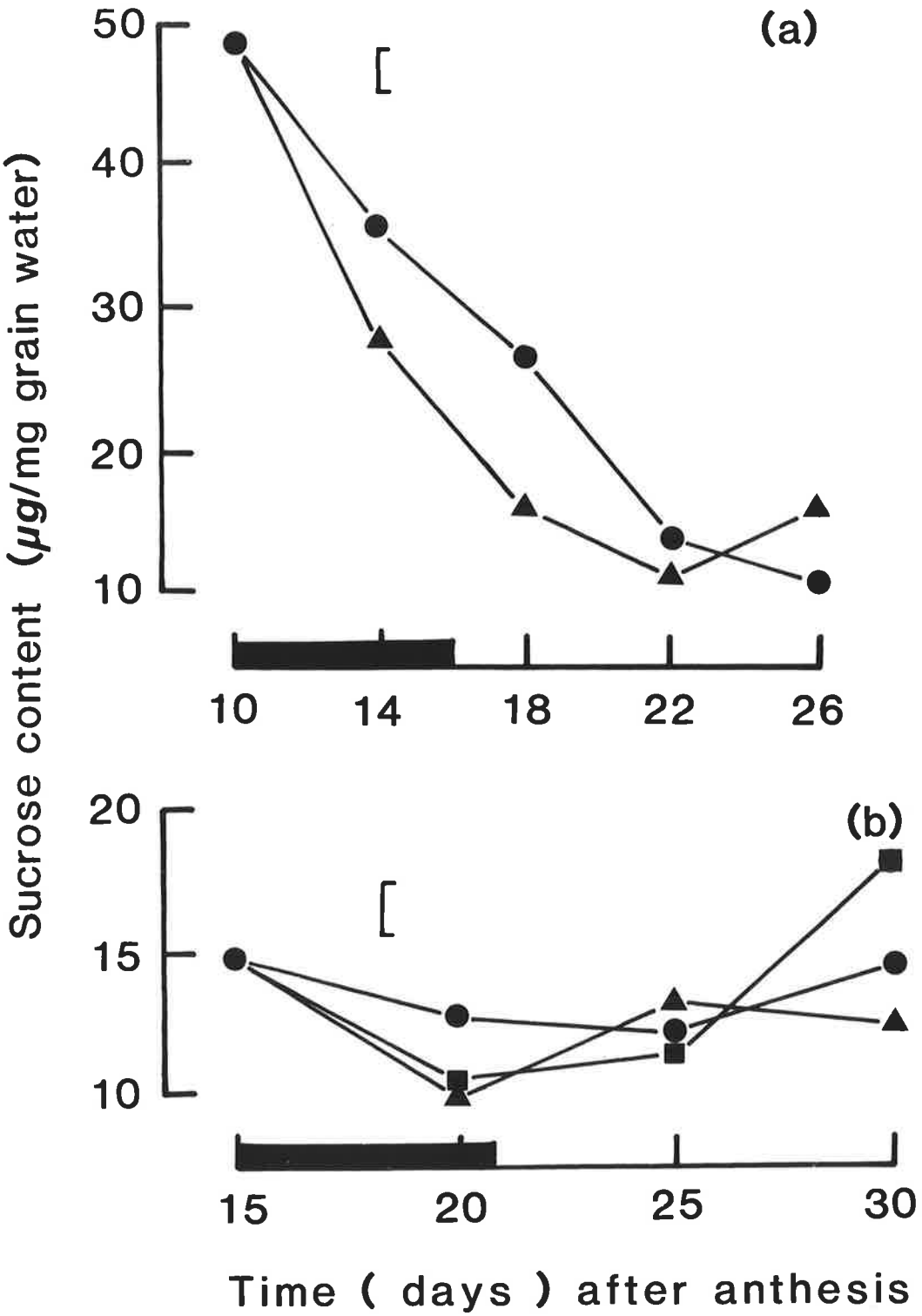
Amounts of sucrose and other soluble sugars in the rachis were either comparable to or higher in the treated ears as compared to the ears developing at lower temperatures at all sampling dates (Figs. 3.2.5a and 3.2.5b). Interestingly, the amounts of sucrose in the bracts at all sampling dates was significantly higher in warmed ears compared to ears developing at a lower temperature (Fig. 3.2.5d). From this information it seems unlikely that the supply of sugars available for distribution to the grain is depressed by elevated temperature.

The lower rate of dry matter accumulation in the grains in the ears warmed at low humidity might be attributed to a decline in apparent sucrose concentration during the period of warming (Fig. 3.2.6a). However, the differential response in dry matter accumulation due to ear warming at different humidities (Fig. 3.2.4a) is not explicable in terms of reduced sucrose concentration (Fig. 3.2.6b). Nevertheless it is

Fig. 3.2.6

Effects of warming ears at low or high humidity on apparent concentrations of sucrose in the grain. Details as for Fig. 3.2.1b and Fig. 3.2.4b for parts (a) and (b), respectively. Bars indicate L.S.D. for treatments x times.

● Ears kept at 21/16°C throughout grain development.
■ Ears warmed at high humidity.
▲ Ears warmed at low humidity.


(a)
50
40
30
20
10
10
14
18
22
26
(b)
20
15
10
15
20
25
30
Sucrose content (μg/mg grain water)
Time (days) after anthesis

likely that both humidity and temperature have independent effects on the rate of grain growth but it is difficult to make judgement on the interactive effects of humidity and temperature from the information presented in this section. Moreover, the responses to elevated temperature displayed in Fig. 3.2.6b may reflect accelerated development. If this is the case, this response could have no casual relation to the observed changes in rate of dry matter. Further, it is important to point out that as the vapour pressure deficits of the warm air in the high humidity treatment and in the control (0.75 kPa during the day and 0.50 kPa at night) were almost identical, high humidity may have some part in alleviating the effect of temperature on the rate of dry matter accumulation. As the development of the grain was advanced by warming at either humidity, it is reasonable to adduce that effects of temperature on development of the grain are independent of the humidity (or V.P.D.) of the warm air.

3.3 THE EFFECTS OF EAR WARMING ON THE STORAGE CAPACITY OF THE GRAIN

Introduction

Neither the reduction in the rate of grain growth nor the advancement in termination of grain growth, as a result of a brief period of ear warming, can be satisfactorily explained as attributable to : a change in the water and osmotic potentials of the bracts (palea, lemma and the sterile glume) and/or the grain, or alterations in the amounts of soluble carbohydrates present in the grain (Section 3.2).

A major problem with the interpretation of experiments designed to investigate the effects of temperature on grain filling is an inability to distinguish whether a reduction in final single grain weight is due to an effect of temperature on the availability of substrate within the grain for its growth, or on the processes involved in the synthesis of starch and protein. Many reports in the literature (Wardlaw et al. 1980 and reference cited therein) favour the notion that a reduction in single grain weight at high temperature is due to an effect of temperature on the processes within the grain rather than through the provision of substrate from other parts of the plant, but critical evidence in support of this hypothesis is lacking.

Liquid culture of detached ears permits some control over the provision of carbon and nitrogen for grain growth. It has been reported (Singh and Jenner 1983) that the grains grow and develop normally from anthesis until maturity when ears were cultured on solutions of sucrose and L-glutamine, and this technique has been used by many workers to study various aspects of grain growth and development, including : the effects of water stress on the water relations of the grain, and its effect on starch and protein accumulation in the grain (Barlow et al. 1983); the formation of protein bodies and endosperm morphology (Campbell et al. 1981); the mechanism(s) of transport of sucrose and amino acids into and along the vascular strand of the grain (Donovan et al. 1983a); and short-term (Jenner 1968) and long-term (Donovan et al. 1983b) effects of temperature on starch and protein accumulation in the grain.

Similarly, isolated grains cultured on ^{14}C-sucrose have been used by many workers (Jenner and Rathjen 1975, 1977; Radley 1978, 1981; Gifford and Bremner 1981a, 1981b) to assess the capacity of grain to absorb and to convert sucrose to starch.

The purpose of experiments, detailed in this section, by using the ear culture and the isolated grain culture technique, was to determine whether a reduction in single grain weight as a result of ear warming was due to an effect of temperature on the availability of substrate (sucrose within the grain) for grain growth, or on the synthetic processes involved in dry matter accumulation (storing ability) in the grain.

Grain filling in the experimentally warmed ears can be considered as biphasic : a part of grain filling during the period of ear warming and a part after the warming period is over. An increase in supply of substrate (by means of detached ear culture) during the warming period may give some information regarding the processes which are directly affected by temperature, while culturing the ears after they have been warmed may relate to delayed effect(s) of temperature on the processes related to dry matter accumulation.

Accordingly, in the first experiment (expt. 7) ears were warmed for a brief period and then cultured at a range of sucrose levels, both above (60 gl^{-1}) and below (20 gl^{-1}) the level of sucrose required to maintain the rate of grain growth normally observed on the plant (40 gl^{-1} of sucrose with 0.5 g N l^{-1} in the form L-glutamine, see Campbell *et al.* 1981; Singh and Jenner 1983). In the second experiment (expt. 8) ears were cultured on various levels of sucrose (as above) and warmed at the same time. In the third experiment (expt. 9) ears were warmed at high or low humidity and isolated endosperms from these ears were cultured on ^{14}C-sucrose to assess their capacity to absorb and metabolise sucrose.

Materials and Methods

Plants were raised in a glass house (for 2 weeks), and then transferred to a controlled environment growth room (Section 2.2.2), where they stayed until maturity.

Experiment 7

On day 10 after anthesis, plants were divided into two batches, and ears (only) in one batch were exposed to 30/25°C [V.P.D. kPa 1.7 (day) and 0.95 (night)] for 7 days (Section 2.3.2), while keeping the other batch at control conditions (21/16°C). One day after the ear warming was over (on day 18), ears both from control and warmed groups were cultured at 3 levels of sucrose (20, 40, or 60 gl^{-1}) with a constant amount of

L-glutamine (0.5 g N l^{-1}, details for solution preparation in Section 2.4) and the ears were allowed to stay at control conditions until maturity.

Samples of the b and the c grains from four central spikelets of the ears were taken on days 18 (start of ear culture), 23 and 28 and when they had ripened. The amounts of total soluble sugars and sucrose in the samples of the b grains were determined on days 18, 23 and 28 after anthesis.

At maturity, four ears for each sucrose concentration (in the culture medium) and from the intact plants were used to determine grain yield components. After recording dry weight of the b grains (taken from the four central spikelets), they were used for total nitrogen determination (Section 2.6.3).

Experiment 8

Ears were cultured on the same levels of sucrose as in experiment 7 on day 9 after anthesis. One day after the start of culture the ears were divided into two batches. One batch of the ears, and a set of ears attached to the plants, were warmed at 30/25°C for 7 days (details as for expt. 7), while keeping the other batch and some ears on the plants at control conditions.

The samples and the sampling procedure were the same as described in experiment 7, except that the samples were taken on days 10, 14, 16, 18 and 20 after anthesis and when the ears had ripened.

Experiment 9

Ears were warmed at 33/25°C at high humidity [V.P.D. kPa 0.75 (day) and 0.48 (night)] or at low humidity [V.P.D. kPa 2.51 (day) and 0.95 (night)] for 6 days, between days 10 and 16 after anthesis.

On days 14, 18, 25 and 30 after anthesis samples of the b grains (5 grains each from four separate ears), were taken from the five central spikelets of control ears or ears warmed at high or low humidity. The grains were dissected according to the procedure in Section 2.8.1, and were cultured on ^{14}C-sucrose solution (7.4 µCi g^{-1}, osmotically adjusted with PEG to -0.82 MPa) containing 30 gl^{-1} (89.5 mMolal) of sucrose by the procedure described in Section 2.8.2.3. The choice of the above grain culture procedure was based on the results of the experiments reported in Section 3.6.

Similar samples of the dissected b grains taken from the opposite side of the same ears were used for fresh weight, dry weight, and water content determinations.

After 8 h of incubation at 25°C, the dissected grains were rinsed (within 2 min) with three lots of deionised water (10 ml each) and then incubated in deionised water for the removal of ^{14}C present in the apparent free space (Section 2.9). The amounts of ethanol soluble ^{14}C in the cells (Section 2.10.1), the amounts of starch in the samples, and the amount of ^{14}C-incorporated into starch were determined by procedures described in Section 2.11.

Results

The responses of the b and the c grains in experiment 7 or 8, in all cases were qualitatively similar hence only the results for the b grain are discussed in detail here and the results for the c grain are presented only when some important point is to be made.

Data for the b grain for each experiment (expt. 7 or 8) were analysed as a whole and separately for each sucrose concentration in the medium, and for ears on the plants. Results are presented either separately for each sucrose concentration, or they are presented as a whole in a table and relevant interactions are plotted separately.

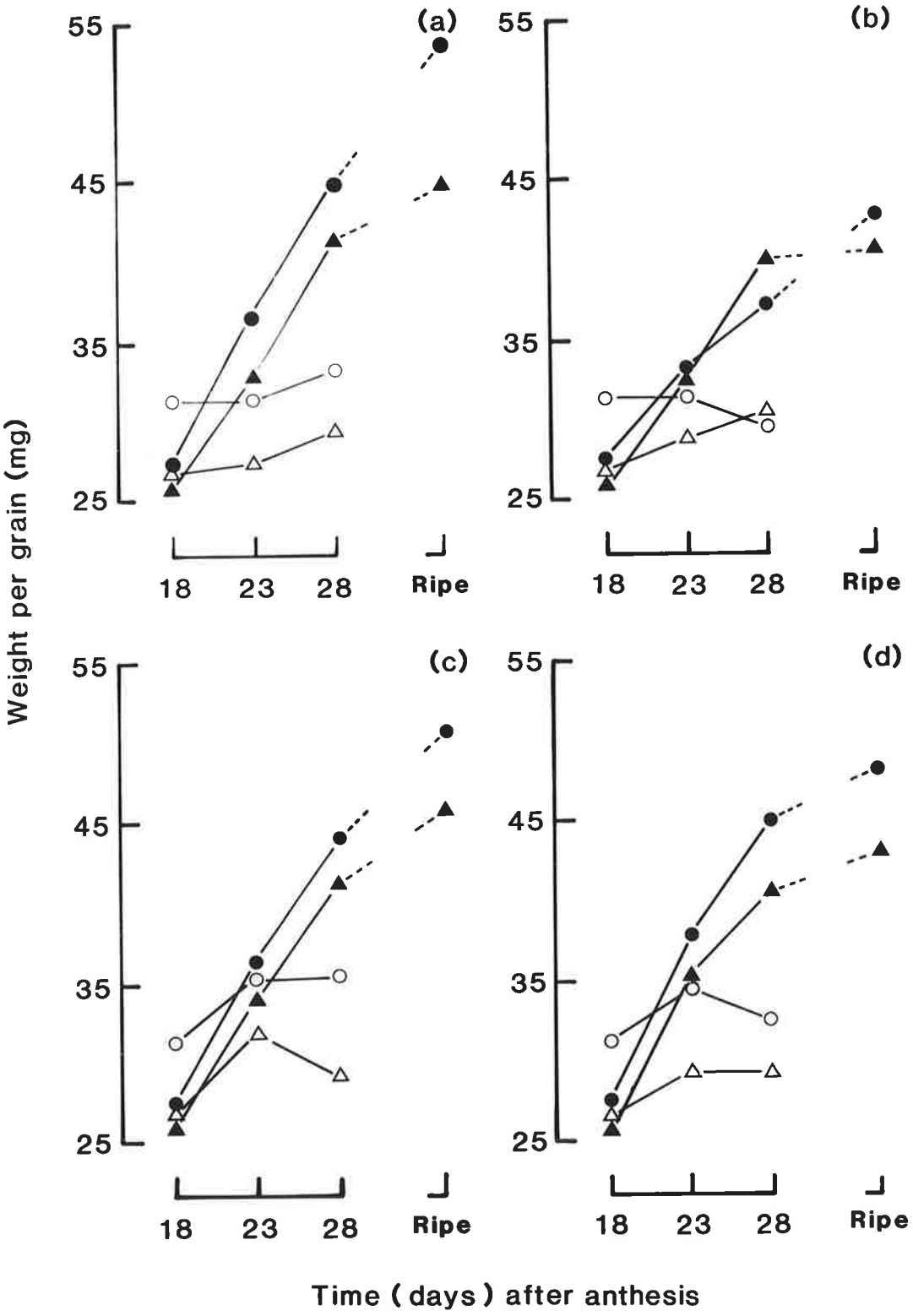
Experiment 7

Dry weights and water contents of the b grain from the control and the warmed ears left on the plant (part a), or for ears cultured with 20 (part b), 40 (part c), or 60 gl^{-1} (part d) of sucrose on day 18, and at subsequent stages of grain growth are given in Fig. 3.3.1. Warming of ears reduced the water content of the grains on day 18 (Fig. 3.3.1a) and at subsequent sampling times, as compared to grains from the ears kept at control conditions. Water content of the b grains both from the control a'd warmed ears cultured with 40 (Fig. 3.3.1c) or 60 gl^{-1} (Fig. 3.3.1d) were higher on days 23 and 28 than on day 18. Nevertheless, the amount of water in the grains from warmed ears was lower on both the sampling times at both these levels of sucrose.

Both in the control and warmed ears either left on the plant or cultured with sucrose, dry matter accumulated progressively between days 18 and 28 after anthesis. The rate of increase of dry weight, both in the control and the warmed ears increased with an increase in sucrose concentration in the medium from 20 to 40 gl^{-1} of sucrose, but further

Fig. 3.3.1

Dry weight (solid symbols) and water content (open symbols) of the b grain taken from the four central spikelets of the control (●, ○) and warmed ears (▲, △). Grains from the ears left on the plant (a), ears cultured with L-glutamine (0.5 g N l^{-1}) and 20 gl^{-1} (b), 40 gl^{-1} (c), or 60 gl^{-1} (d) of sucrose. Ears were warmed at 30/25°C between days 10 and 17 after anthesis and were cultured on solutions of sucrose from day 18 until they had ripened. (Expt. 17)


(a)
(b)
(c)
(d)
55
45
35
25
18
23
28
Ripe
Weight per grain (mg)
Time (days) after anthesis

increase to 60 gl^{-1} had no effect on the rate of grain filling in either control or warmed ears. The rates of grain growth (calculated by regression, between days 18 and 28 after anthesis) both in the control and the warmed ears cultured with 40 or 60 gl^{-1} of sucrose were comparable to their respective type of ears attached to the plant, as shown in the tabulation below.

Sucrose concn (gl^{-1})	Treatment	
	control	warmed
	Growth rate (mg $grain^{-1}$ day^{-1})	
20	0.99	1.45
40	1.65	1.55
60	1.76	1.49
on plant	1.77	1.56

Grains in the warmed ears cultured with 20 gl^{-1} of sucrose grew faster than the control ears cultured at the same level. But grain growth in the warmed ears cultured with 40 or 60 gl^{-1} of sucrose or left on the plant appears to be lower than the control ears cultured at the same levels. It should also be noted that while the rate of dry matter accumulation was influenced by the concentration of sucrose supplied in the control ears, in the warmed ears it was not.

The amounts of sucrose and other soluble sugars on a per grain basis are given in Table 3.3.1. The amount of sucrose on day 18 (start of ear culture) appeared to be lower in the grain from untreated ears than in those of warmed ears, while the reverse seemed to be true for other soluble sugars. On days 23 and 28, the amounts of sucrose (average of treatments, Table 3.3.1) in the grain increased with increase in sucrose from 20 to 40 gl^{-1} in the medium, but a further increase in sucrose in the medium to 60 gl^{-1} had little effect. On both the sampling times, the grains from the control ears on the plant had higher sucrose contents compared with the grains from the warmed ears on the plant.

The interactions (treatments x concentration of sucrose in the culture medium) for the amounts of sucrose (from Table 3.3.1) and the

Table 3.3.1

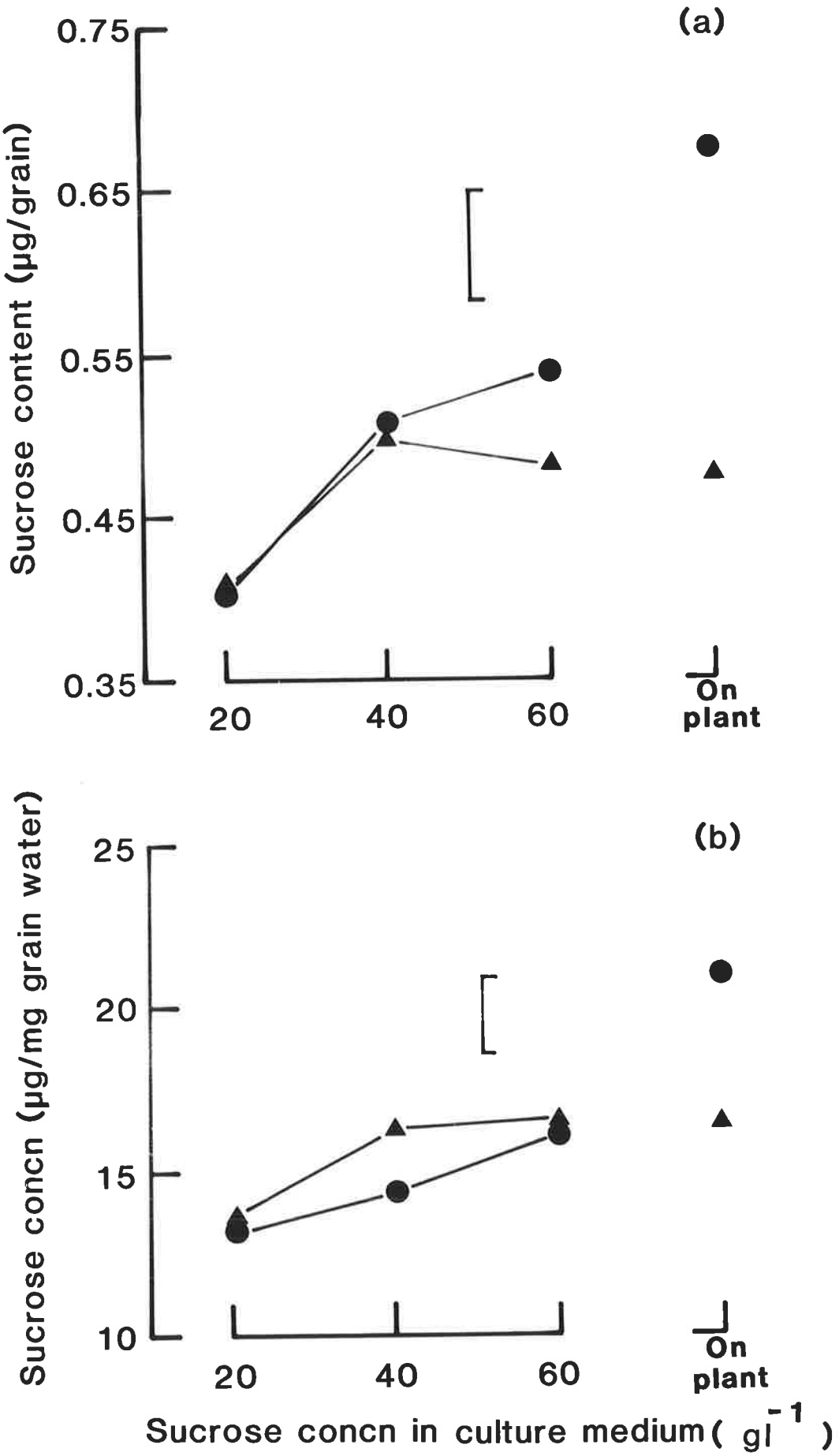
Amounts of sucrose and other soluble sugars in the b grain taken from the four central spikelets of control or warmed ears. Details are given in legend to Fig. 3.3.1. (Expt. 7)

Days after anthesis	Sucrose concn (gl^{-1})	Sucrose			Other soluble sugars		
		Control	Warmed	Mean	Control	Warmed	Mean
		µg per grain			µg per grain		
*18	on plant	446	470	458	735	590	663
23	20	384	373	379	552	352	452
	40	452	506	479	503	594	549
	60	562	435	499	556	481	520
	on plant	650	418	534	784	556	670
Mean		512	433	473	599	496	548
28	20	418	440	429	820	599	710
	40	557	489	523	660	532	596
	60	512	530	521	482	649	566
	on plant	701	532	617	674	604	639
Mean		547	498	523	659	596	628
Overall Mean		530	465		629	546	
		Least significant differences					
Treatment		33			68		
Days		36			94		

* Start of ear culture, values for this day were not included in the analyses.

Fig. 3.3.2

Amounts of sucrose per grain (a), and concentrations of sucrose (b) in the b grain taken from the four central spikelets of the control (●) and warmed ears (▲). Values are means over days 23 and 28 after anthesis. (Expt. 7)


(a)
0.75
0.65
0.55
0.45
0.35
Sucrose content (µg/grain)
20
40
60
On plant
(b)
25
20
15
10
Sucrose concn (µg/mg grain water)
20
40
60
On plant
Sucrose concn in culture medium (gl⁻¹)

concentrations of sucrose (on grain water basis) in the grain are plotted separately in Figs. 3.3.2a and 3.3.2b respectively. Both the amounts and the concentrations of sucrose in the grains (means of days 23 and 28) both from the control and warmed ears increased with increase in sucrose in the medium. Both amounts and concentrations in the grain from control or warmed ears at each concentration in the culture medium were the same. As described earlier, the grains from the control ears on the plant had higher absolute amounts as well as the concentrations of sucrose than the grains in the warmed ears on the plant.

On day 23, the amounts of other soluble sugars in the grain from control ears cultured at various levels of sucrose were the same (Table 3.3.1), whereas on day 28, an inverse relationship between the amounts of other soluble sugars in the grain and the sucrose levels in the medium was evident. But there was no apparent relationship observable between the amounts of soluble sugars in the grain and the sucrose levels in the culture medium for the warmed ears on either of the sampling times (Table 3.3.1). However, it is interesting to note that the average amount (mean of sampling times) of other soluble sugars in the grain from warmed ears was lower than in their counterparts in the control ears.

Final b grain dry weight was higher both in control and warmed ears cultured with 40 or 60 gl^{-1} of sucrose than in the ears cultured with 20 gl^{-1} sucrose (Table 3.3.2), and their weights were similar to the b grain dry weights in the respective type of ears left on the plant. Dry weight of the b grain in the warmed ears was reduced in comparison with the control ears cultured at the same level of sucrose in the external medium. The percentage reduction in the b grain weight in the warmed compared to control ears was greatest (16.7%) for ears left on the plant, followed by ears cultured with 40 (9.5%) or 60 gl^{-1} (10.0%) of sucrose and was least for ears cultured with 20 gl^{-1} (3.5%) of sucrose.

Despite the grains being smaller in the warmed ears as discussed above, the amount of nitrogen in the b grains from warmed ears was similar to the grains from the control ears cultured at the same level of sucrose. The amounts of nitrogen in the b grains both from control and warmed ears cultured with 40 gl^{-1} were similar to their respective type of ear attached to the plant. However, the amount of nitrogen in the b grain in both types of ears decreased with increase in sucrose concentration in the culture medium.

Table 3.3.2

Dry weight and nitrogen content (per grain) for mature b grain taken from the four central spikelets of the control and the warmed ears. Values in the parentheses are differences, as percentages, between the values for the control and warmed ears in that column. Details are given in the legend to Fig. 3.3.1. (Expt. 7)

Parameter	Treatment	Sucrose (gl^{-1}) in the culture medium			On plant	Mean
		20	40	60		
Dry weight (mg)	Control	42.4	50.6	48.0	53.4	48.6
	Warmed	40.9	45.8	43.2	44.7	43.7
		(-3.5)	(-9.5)	(-10.0)	(-16.7)	(-10.1)
	Mean	41.6	48.2	45.6	49.0	
Nitrogen (µg)	Control	1028	999	843	940	952
	Warmed	1062	980	859	859	947
	Mean	1045	989	851	914	
Per cent Nitrogen	Control	2.42	1.97	1.76	1.76	1.96
	Warmed	2.60	2.14	1.99	1.92	2.17
	Mean	2.51	2.06	1.88	1.84	

Parameter	Least significant differences		
	Treatment	concn	Interaction
Dry weight	2.5	3.5	n.s.
Nitrogen	n.s.	74	n.s.

Total ear grain weight, at maturity, was reduced by 21.4% (overall mean, Table 3.3.3) in the warmed ears compared to control ears, and this reduction was due in part to differences in the number of grains between the treatments. Average single grain weight in the control ears was higher in the ears cultured with 40 or 60 gl^{-1} of sucrose than in the ears cultured with 20 gl^{-1} of sucrose, but no such differences were discernible in the warmed ears cultured at those levels of sucrose. Average single grain weight both in control and warmed ears cultured with 40 or 60 gl^{-1} of sucrose were similar to their respective types of ears on the plant (Table 3.3.3). Interestingly, the effects of warming were less obvious on the ears cultured at 20 gl^{-1} than at higher concentrations or in the ears developing on the plant.

Experiment 8

Water content of the b grain in the control ears increased between days 10 and 14 after anthesis and stayed almost constant thereafter (Fig. 3.3.3a). Water content of the grains in the ears on the plant or in ears cultured with 40 (Fig. 3.3.3c) or 60 gl^{-1} (Fig. 3.3.3d) of sucrose was reduced by the fourth day of warming (day 14) and it continued to be lower at subsequent sampling times (days 16, 18 and 20). Warming the ears cultured on 20 gl^{-1} of sucrose, on the other hand, led to an increase in water content of the grains on day 14 over the control ears cultured at the same level (Fig. 3.3.3b). But on subsequent sampling times there were no differences in water contents of the grains from these two types of ears. As the response to warming of the water content of the grains from ears cultured with 20 gl^{-1} of sucrose is in contrast to the responses observed for the water content of the grains from the ears cultured with 40 or 60 gl^{-1} of sucrose or in the ears left on the plant, the differences in water content of the grains from the control and the warmed ears are presented separately in Table 3.3.5.

The rates of grain growth in the control as well as in the warmed ears cultured on solutions of sucrose increased with each increment in sucrose supplied in the external medium (Fig. 3.3.4), and at each level of sucrose the grains in the ears warmed to 30/25°C filled faster (the b and the c grains) than in the ears held at 21/16°C. However, the accelerating effect of temperature on the rate of grain filling was greatest in ears cultured with 20 gl^{-1} of sucrose (1.45 times and 1.44 times for the b and the c grains respectively) and least in the ears cultured with 60 gl^{-1} of sucrose (1.07 and 1.04 times for the b and the

Table 3.3.3.

Grain yield components for control and warmed ears. Details as for Table 3.3.2. (Expt. 7)

Comparison	Treatment	Sucrose (gl^{-1}) in the culture medium			On plant	Mean
		20	40	60		
Grains per ear	Control	46.8	48.5	44.5	40.5	45.1
	Warmed	41.5	39.8	40.3	41.0	40.6
	Mean	44.1	44.1	42.4	40.8	
Grain weight per ear (mg)	Control	1647	1964	1781	1681	1768
	Warmed	1388	1388	1401	1454	1408
		(-15.7)	(-29.3)	(-21.3)	(-13.5)	(-21.4)
	Mean	1518	1676	1591	1567	
Average single grain weight (mg)	Control	35.1	40.5	40.1	41.8	39.4
	Warmed	33.5	35.0	34.9	35.4	34.7
		(-4.6)	(-13.6)	(-13.0)	(-15.3)	(-11.9)
	Mean	34.3	37.8	37.5	38.6	

Parameter	Least significant differences		
	Treatment	concn	Interaction
Grains per ear	3.5	n.s.	n.s.
Grain weight per ear	111	n.s.	n.s.
Average single grain weight	2.0	2.8	n.s.

Fig. 3.3.3

Dry weight (solid symbols) and water content (open symbols) of the b grain taken from the four central spikelets of the control (●, ○) and warmed ears (▲, △). Grains from the ears left on the plant (a), ears cultured with L-glutamine (0.5 g N l^{-1}) and 20 gl^{-1} (b), 40 gl^{-1} (c), or 60 gl^{-1} (d) of sucrose. Ears were cultured on solutions of sucrose on day 9, warmed at 30/25°C between days 10 and 17, and allowed to stay in culture until they ripened. Solid horizontal bars refer to treatment period. (Expt. 8)

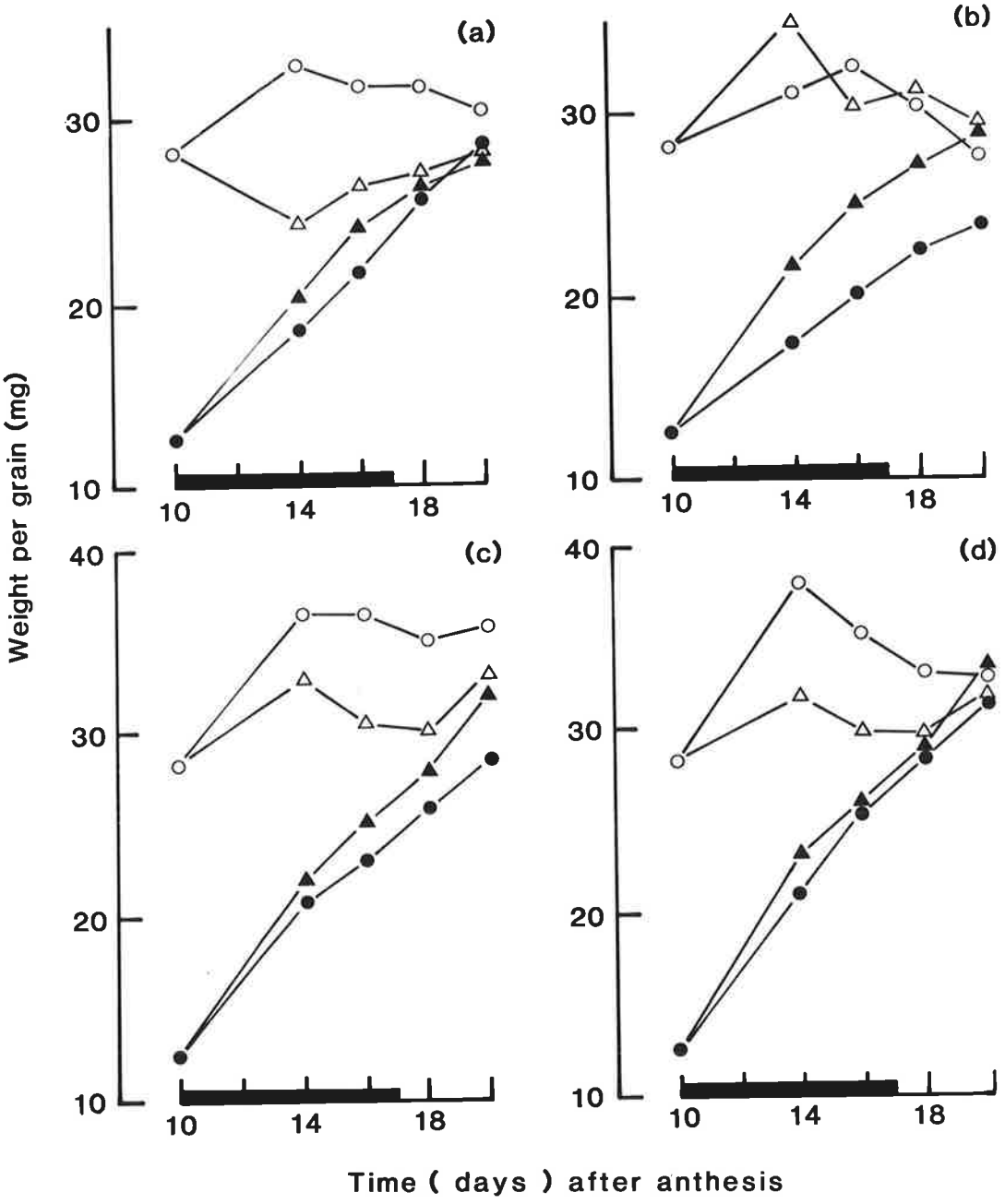

(a)
(b)
(c)
(d)
Weight per grain (mg)
Time (days) after anthesis
10
14
18
20
30
40

Table 3.3.4

Amounts of sucrose and other soluble sugars in the b grain taken from the four central spikelets of control or warmed ears. Details are given in legend to Fig. 3.3.3. (Expt. 8)

Days after anthesis	Sucrose concn (gl^{-1})	Sucrose			Other soluble sugars		
		Control	Warmed	Mean	Control	Warmed	Mean
		µg per grain			µg per grain		
*10	on plant	857	---	---	1131	---	---
14	20	376	323	350	703	468	586
	40	440	370	405	719	484	602
	60	420	323	372	798	467	633
	on plant	487	396	442	927	541	734
Mean		430	353	392	787	490	639
16	20	389	342	366	1099	778	939
	40	667	358	513	823	742	783
	60	629	371	500	667	585	626
	on plant	465	469	467	1101	669	885
Mean		538	390	464	923	693	808
18	20	326	355	341	621	675	648
	40	390	424	407	728	736	732
	60	437	461	449	937	546	742
	on plant	468	491	480	571	815	693
Mean		405	432	419	712	693	703
20	20	280	357	319	616	813	715
	40	435	424	430	764	696	730
	60	445	449	447	734	780	757
	on plant	464	534	499	834	803	819
Mean		406	441	424	737	773	755
Overall Mean		445	404		790	662	662

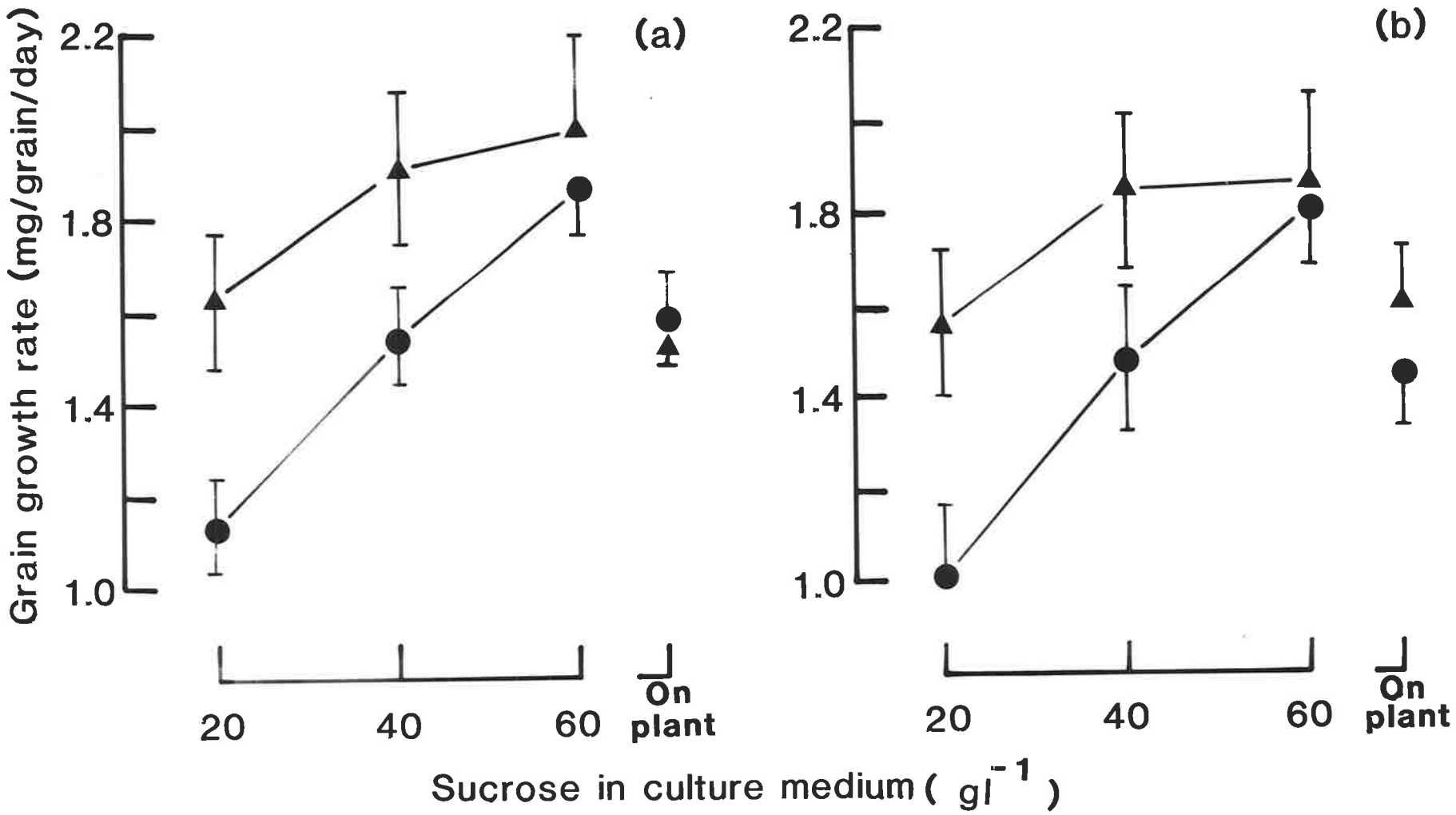
Least significant differences

Treatment	28	60
Days	32	62
Days x concn	n.s.	123

* Start of ear culture, values for this day were not included in the analysis.

Fig. 3.3.4

Effects of the concentration of sucrose in the culture medium on the rates of growth of the b (a) and the c (b) grains taken from the four central spikelets of the control ears (●) or ears held at 30/25°C (▲). The rates were calculated by Regression analysis on the original data between days 10 and 20 after anthesis. Details in the legend to Fig. 3.3.3 (Expt. 8)


Grain growth rate (mg/grain/day)
2.2
1.8
1.4
1.0
(a)
(b)
20
40
60
On plant
Sucrose in culture medium (gl^{-1})

c grains respectively). Warming of ears attached to the plant did not affect the rate of grain filling in either of the florets.

The values for the amounts of sucrose and other soluble sugars in the grains on a per grain basis are presented in Table 3.3.4. On days 14 and 16, the grains from the ears cultured on various levels of sucrose and warmed to 30/25°C had smaller amounts of sucrose than in the grains from the control ears cultured at the same level of sucrose. However, on days 18 and 20, one and three days after ear warming was over, the amounts of sucrose in the grains from the warmed ears were higher than in the grains from the control ears cultured at the same level of sucrose (Table 3.3.5). On day 14, the amount of sucrose in the grains from the warmed ears, attached to the plant, was lower than in the grains from untreated ears on the plant. On subsequent sampling occasions however, the amounts of sucrose in the grains from warmed ears were either similar to (day 16) or higher (days 18 and 20) than the amounts of sucrose in the grains from the control ears on the plant (Table 3.3.5).

Two main interactions (between treatments and days, and between treatments and sucrose levels in the culture medium) for the amounts and the concentrations (on a grain water basis) in the grain are plotted in Fig. 3.3.5. On average (mean overall sucrose levels) the amounts of sucrose in the grains from control ears, on days 14 and 16, were higher than in the grains from ears held at 30/25°C (Fig. 3.3.5a), while the differences on days 18 and 20 between the treatments were non-significant. The concentrations of sucrose (Fig. 3.3.5b) in the grain for both treatments displayed a similar trend as recorded above for the absolute amounts of sucrose, except that the concentrations of sucrose in the grains from warmed ears on days 18 and 20 were significantly higher than the concentrations of sucrose in the grains from the control ears.

Overall amounts of sucrose (means of sampling times, Fig. 3.3.5c) in the grains both in the control and warmed ears increased with increase in sucrose level from 20 to 40 gl^{-1} in the culture medium, but a further increment in the level of sucrose (to 60 gl^{-1}) did not result in a concomitant increase in the amount of sucrose in the grains in either of the treatments. The amounts of sucrose in the grains from control ears cultured with 40 to 60 gl^{-1} of sucrose were significantly higher than in the grains from the warmed ears cultured at same levels of sucrose. When the above amounts were expressed as concentrations of

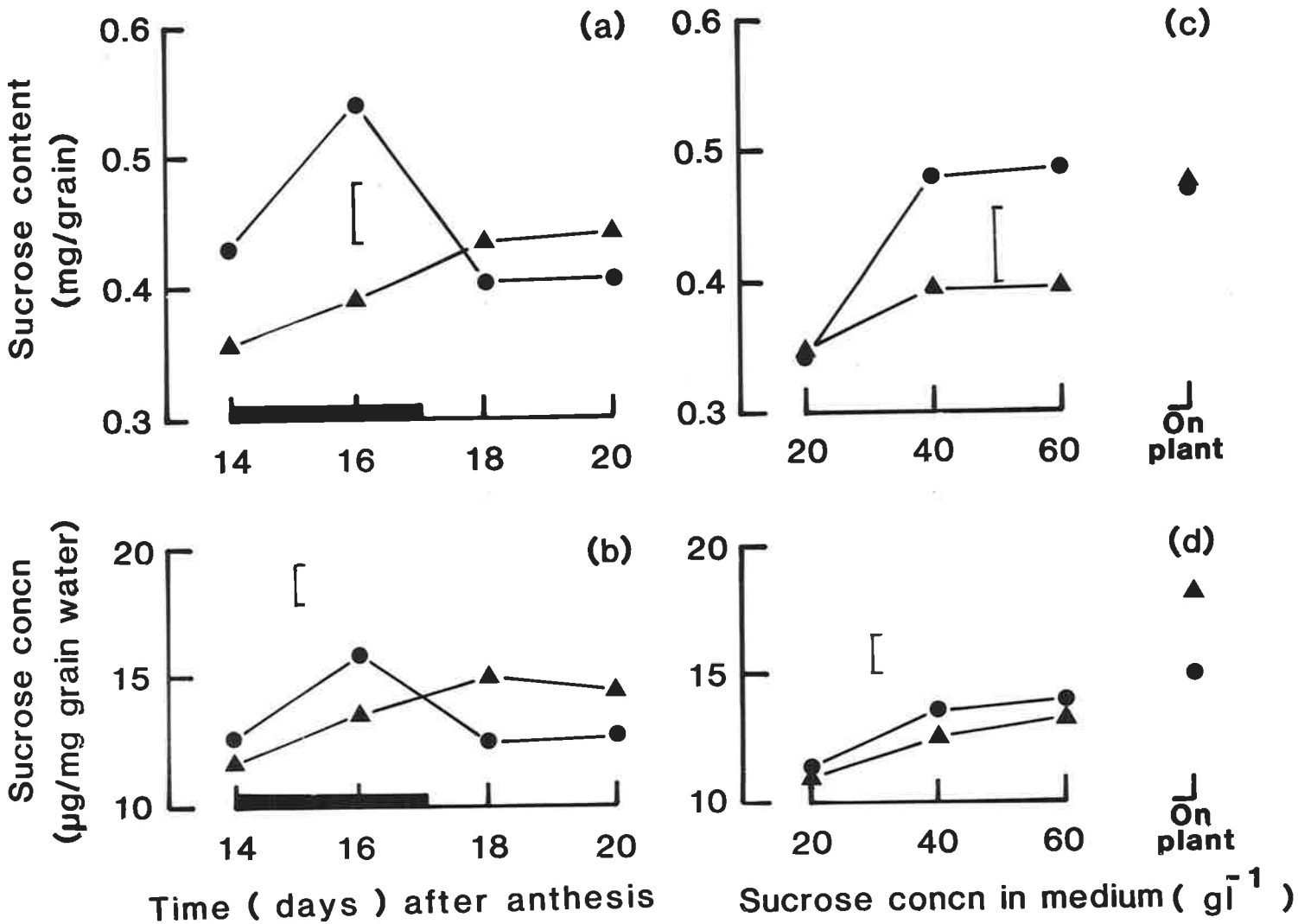
Table 3.3.5

Differences in water content, amounts of sucrose, and other soluble sugars of the b grain between control and warmed ears. Data are from Fig. 3.3.3 and Table 3.3.4. (Expt. 8)

Sucrose concn (gl^{-1})	Days after anthesis	Water (mg)	Difference in amount (µg per grain) of: Sucrose	Other soluble sugars
on plant	14	-8.1	-91	-386
	16	-5.5	4	-432
	18	-4.7	23	244
	20	-2.3	70	-31
Mean		-5.2	1.5	-151
20	14	3.9	-53	-235
	16	-2.0	-47	-321
	18	1.1	29	54
	20	1.7	77	197
Mean		1.2	1.5	-76
40	14	-3.4	-70	-235
	16	-6.0	-309	-81
	18	-4.8	34	8
	20	-2.6	-11	-68
Mean		-4.2	-89	-94
60	14	-6.3	-97	-331
	16	-5.2	-258	-82
	18	-3.6	24	-391
	20	-0.8	4	46
Mean		-4.0	-82	-190

Fig. 3.3.5

Amounts of sucrose (a,c) and concentrations of sucrose (b,d) in the b grain taken from the central four spikelets of the control (●) and warmed ears (▲). Values plotted in parts (a) and (b) are means of amounts of sucrose in the grain from ears left on the plant and in ears cultured with sucrose. Values plotted in parts (c) and (d) are means of the sampling times. Data for parts (a) and (c) are from Table 3.3.4. (Expt. 8)


(a)
(b)
(c)
(d)
Sucrose content (mg/grain)
Sucrose concn (µg/mg grain water)
0.6
0.5
0.4
0.3
20
15
10
14
16
18
20
40
60
On plant
Time (days) after anthesis
Sucrose concn in medium (gl⁻¹)

sucrose (on a grain water basis), there were no significant differences between the treatments at any level of sucrose in the culture medium (Fig. 3.3.5d). It is worth noting that whereas the average *amount* of sucrose in the grains from the control and warmed ears on the plant were similar (Fig. 3.3.5c), the *concentration* of sucrose in the grains from warmed ears was higher than in the grains from control ears (Fig. 3.3.5d).

The amounts of other soluble sugars of the grain in either control or warmed ears did not display any consistent relationship either with time or with change in sucrose level in the medium (Table 3.3.4). Nevertheless, the grains from the ears, warmed to 30/25°C, either cultured on solutions of sucrose or attached to the plant, on days 14 and 16, contained smaller amounts of other soluble sugars than the grains from the control ears (Table 3.3.5).

Warming the ears reduced the final b grain dry weight whether the ears were cultured on solutions of sucrose or left atached to the plant (Table 3.3.6). However, the per cent reduction resulting from heating in the b grain dry weight was smallest in the ears cultured with 20 gl^{-1} of sucrose (4.5%) followed by ears cultured with 40 or 60 gl^{-1} of sucrose (14.4%) and greatest in the ears left on the plant (20.2%). Dry weights of the b grains, both in the control and warmed ears, on the plant were higher than their respective type of ears cultured on sucrose. The b grains from the ears cultured with 40 or 60 gl^{-1} of sucrose were heavier than the b grains from ears cultured with 20 gl^{-1} of sucrose.

There was little effect of ear warming on the amount of nitrogen in the b grain (overall mean, 905 and 855 µg per grain for control and warmed ears respectively, Table 3.3.6). The amount of nitrogen in the b grain both in control and warmed ears decreased with an increase in sucrose concentration in the external medium. The amounts of nitrogen in the b grains from control as well as from the warmed ears cultured with 40 gl^{-1} of sucrose were similar to the amounts observed in their respective type of ears left on the plant. As the b grains from the control or warmed ears cultured with 40 or 60 gl^{-1} of sucrose were heavier and had smaller amounts of nitrogen than the ears cultured with 20 gl^{-1} of sucrose, the percentage of nitrogen increased with decrease in sucrose level (Table 3.3.6).

Total ear grain weights for the control ears cultured with 40 or 60 gl^{-1} of sucrose were similar to ears on the plant (Table 3.3.7) with the exception of ears cultured with 20 gl^{-1} of sucrose, in which case warmed

Table 3.3.6.

Dry weight and the amount of nitrogen (per grain) for mature b grain taken from the four central spikelets of the control and the warmed ears. Values in the parentheses are differences, as percentages, between the values for the control and warmed ears in that column. Details as for Table 3.3.4. (Expt. 8)

Parameter	Treatment	Sucrose (gl^{-1}) in the culture medium			on Plant	Mean
		20	40	60		
Dry weight (mg)	Control	33.2	39.7	41.0	48.0	40.5
	Warmed	31.7	34.0	35.1	38.3	34.8
		(-4.5)	(-14.4)	(-14.4)	(-20.2)	(-14.1)
	Mean	32.5	36.9	38.1	43.2	
Nitrogen (µg)	Control	945	882	854	938	905
	Warmed	947	840	732	900	855
	Mean	946	861	793	919	
Per cent Nitrogen	Control	2.85	2.22	2.08	1.95	2.23
	Warmed	2.99	2.47	2.09	2.35	2.46
	Mean	2.92	2.35	2.09	2.15	

Parameter	Least significant differences Treatment	concn	Interaction
Dry weight	2.2	3.3	n.s.
Nitrogen	50	70	n.s.

Table 3.3.7.

Grain yield components for control and warmed ears. Details as for Table 3.3.5. (Expt. 8)

Comparison	Treatment	Sucrose (gl^{-1}) in the culture medium			On plant	Mean
		20	40	60		
Grains per ear	Control	48.3	50.5	50.3	46.0	48.8
	Warmed	48.5	47.5	47.3	42.5	46.4
Mean		48.4	49.0	48.8	44.3	
Grain weight per ear (mg)	Control	1341	1700	1808	1897	1687
	Warmed	1286	1356	1288	1353	1321
		(-4.1)	(-20.2)	(-28.7)	(-28.7)	(-21.7)
Average single grain weight (mg)	Control	27.8	33.7	36.1	41.2	34.7
	Warmed	26.5	28.5	27.2	32.0	28.6
		(-4.7)	(-15.4)	(-24.7)	(-22.3)	(-17.6)

Parameter	Least significant differences Treatment	concn	Interaction
Grains per ear	1.8	2.6	n.s.
Grain weight per ear	n.s.	n.s.	204
Average single grain weight	n.s.	n.s.	4.0

ears had lower numbers of grains. Grain number was lowest for warmed ears left on the plant. A brief period of ear warming reduced the total ear grain weights and average single grain weights of the ears either cultured on solutions of sucrose or left on the plant. Per cent reductions in total ear grain weight and average single grain weight due to warming were greater in the ears cultured at 40 or 60 gl^{-1} of sucrose than in those cultured at 20 gl^{-1}. In other words, heating had less effect at low as compared to higher levels of sucrose supply to the grain.

Experiment 9

Dry matter accumulation in the endosperm was enhanced due to warming of ears to 33/25°C at both levels of humidity (day 14, Fig. 3.3.6a). However, 9 and 14 days after the warming treatments had finished the opposite effect was observed (days 25 and 30 after anthesis, Table 3.3.8). Thus, the average rates of dry weight increase (calculated by regression, between days 10 and 30 after anthesis) were lower for the endosperms from the ears warmed at high (1.19 mg endosperm^{-1} day^{-1}) or low humidity (1.26 mg endosperm^{-1} day^{-1}) than for the endosperms from the control ears (1.51 mg endosperm^{-1} day^{-1}). Starch accumulation in the endosperms from control ears and from ears warmed at high or low humidity was very similar until day 25, with the exception of day 18 where control endosperms had higher amounts of starch than the endosperms from treated ears (Table 3.3.8). On day 30, control endosperms had higher amounts of starch than the endosperms from the warmed ears (Table 3.3.8).

The amount of ^{14}C recovered from the apparent free space (in 1.5 h) from control endosperms decreased between days 10 and 14 and increased with further development of the grain (Table 3.3.8). On day 14, endosperms from ears warmed at high humidity had a lower amount ^{14}C in the apparent free space than the endosperms from control ears whereas the endosperms from the ears warmed at low humidity had higher amounts than the control endosperms. On days 18 and 25, radioactivity in the apparent free space of the endosperms from control ears and from ears warmed at high humidity was higher than for the endosperms from the ears warmed at low humidity, but during further development of the grain (day 30) there were no differences among the treatments.

The quantities of ethanol soluble ^{14}C extracted from the cells of the cultured endosperms, expressed as micrograms of sucrose equivalents

Fig. 3.3.6

Dry weight (a), the amount of ethanol soluble ^{14}C (in the cells) per mg endosperm water (b), and the amount of radioactive starch per mg dry weight (C) in the endosperm of the b grain taken from the five control spikelets of control ears (●), or from ears warmed at high (■), or low humidity (▲). Ears were warmed at 33/25°C between days 10 and 16 after anthesis. Solid horizontal bars refer to treatment period. Isolated endosperms were cultured with 30 mg ml^{-1} (89.5 mMolal, sp. activity 7.4 µCi g^{-1} sucrose) of sucrose at 25°C for 8 h. (Expt. 9.)

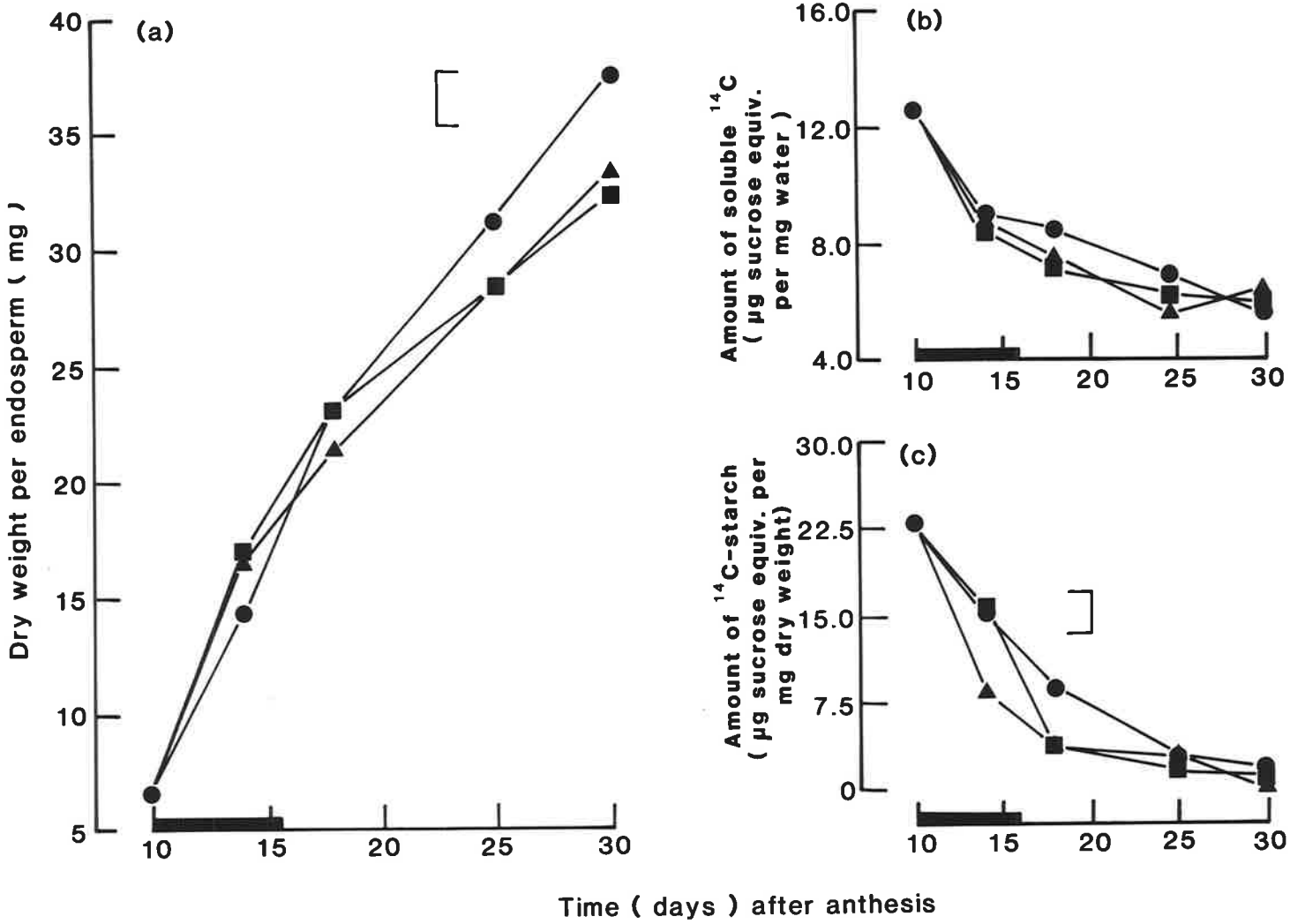

(a)
(b)
(c)
Dry weight per endosperm (mg)
Amount of soluble ^{14}C (µg sucrose equiv. per mg water)
Amount of ^{14}C-starch (µg sucrose equiv. per mg dry weight)
40
35
30
25
20
15
10
5
16.0
12.0
8.0
4.0
30.0
22.5
15.0
7.5
0
10
15
20
25
30
Time (days) after anthesis

Table 3.3.8

Fresh weight, dry weight, amount of starch, and the amount of ^{14}C present in different fractions of the endosperm. Data are for the b grain taken from the five control spikelets of the control (C), or ears warmed to 33/25°C at high (TH) or low humidity (T) between days 10 and 16 after anthesis. For details see legend to Fig. 3.3.6. (Expt.9)

Days after anthesis	Treatment	Fresh weight	Dry weight	Starch content	Amount of ^{14}C[g sucrose equivalents endosperm^{-1}] in: Apparent free space	Soluble fraction (in cells)	^{14}C-starch
		(mg per endosperm)					
10	C	20.7	6.6	3.8	43.0	177.8	152.1
14	C	35.7	14.3	7.2	35.2	187.1	211.0
	TH	35.7	17.0	8.6	31.3	157.4	263.4
	T	34.7	16.5	8.7	39.9	156.3	135.4
18	C	44.8	23.1	15.8	39.4	180.6	179.4
	TH	41.3	23.1	12.6	40.0	128.5	87.5
	T	39.1	21.4	12.8	33.8	132.7	82.3
25	C	50.6	31.1	18.2	46.9	132.6	84.4
	TH	46.2	28.4	19.1	43.1	108.5	54.1
	T	46.3	28.4	19.3	40.1	105.0	73.3
30	C	57.9	37.3	25.6	52.0	114.5	64.6
	TH	49.4	32.2	22.4	46.8	98.2	32.1
	T	51.3	33.3	21.4	48.0	111.8	18.2
L.S.D.		2.5	2.0	2.7	6.2	n.s.	60.3

(calculated by dividing the d.p.m. in the sample by the specific radioactivity of sucrose supplied) per endosperm are given in Table 3.3.8. The amount of ethanol soluble ^{14}C in the cells of the endosperms taken from the untreated ears did not change between days 10 and 18, and then fell significantly with the development of the grain. The quantities of soluble ^{14}C in the cells of the endosperms from ears warmed at high or low humidity did not differ from each other at any sampling occasion and were significantly lower than the quantity in the cells of endosperms from control ears at all sampling times. When the quantities of soluble ^{14}C are expressed on an endosperm water basis (Fig. 3.3.6b), the concentration of soluble ^{14}C in the endosperm cells decreased for the control as well as for the ears warmed at either humidity, and there were no differences among the treatments at any stage of grain development.

Shown in Table 3.3.8 are the amounts of radioactive starch produced in 8 h by the endosperms from the control ears or ears warmed at high or low humidity. The incorporation of ^{14}C into starch in the control endosperms increased between days 10 and 14 after anthesis, and declined thereafter with the development of the grain. With the sole exception of the value on day 14 for the endosperms from ears warmed at high humidity, where the amount of radioactive starch produced was more than in the endosperms from control ears or ears warmed at low humidity, the quantities of radioactive starch produced were the same in the endosperms from the ears warmed at either humidity, and were lower than the quantities produced in the endosperms from control ears. When the amounts of radioactive starch were expressed on a dry weight basis, the amount of radioactive starch produced in the endosperms from the control as well as in the endosperms from the ears warmed at high or low humidity decreased with development of the grain (Fig. 3.3.6c). On day 14, the amounts of radioactive starch produced (on a dry weight basis) by the endosperms from the control ears and from the ears warmed at high humidity were the same, and were higher than the amount produced by endosperms from ears warmed at the low humidity. On day 18, there were no differences in the amounts of radioactive starch produced by the endosperms from ears warmed at high or low humidity, and both values were significantly lower than the amount produced by control endosperms. But on days 25 and 30 after anthesis, the amounts of radioactive starch produced were the same for all three treatments.

At maturity, total ear grain weight and average single grain weight were lower for both humidity treatments, as recorded in the following tabulation.

Parameter	Treatment			L.S.D
	Control	High humidity	Low humidity	
Total ear grain weight (mg)	1462	1273(-12.9)	1198 (-18.1)	146
Average single grain weight (mg)	33.1	30.9 (-6.6)	28.3(-14.5)	2.4
Weight of the b grain (mg)	41.5	36.5(-12.0)	33.8(-18.6)	3.7

(values in parentheses are the differences, as percentages, between warmed and control ears)

Although the reductions in total ear grain weight and average single grain weight were greater in the ears which had been warmed at low humidity than in the ones that were warmed at high humidity, the differences between these two sets of ears were not significant.

Discussion

As all the nutrients provided in the medium enter the detached ears through the transpiration stream, results obtained with this technique are subject to the caution that they may not reflect the normal physiological state of the developing grain. Many reports to support the case that grains do grow and develop normally in detached ears are already referred to in the introduction to this section, and further evidence to support those reports is given as follows. The rate of grain filling in detached ears (from the control set of plants) cultured with 40 gl^{-1} of sucrose and 0.5 gl^{-1} of nitrogen (as L-glutamine), both in experiments 7 (compare Fig. 3.3.1c with Fig. 3.3.1a) and 8 (Fig. 3.3.4) are comparable to those observed for the grains on the plants. Also, in experiment 7, total ear grain weight and average single grain weight (Table 3.3.3), of the b grain and the amount of nitrogen in it at

maturity (Table 3.3.2) are similr for control ears cultured with 40 gl^{-1} of sucrose and for ears attached to the plant.

However, when the control ears are excised and cultured with 40 gl^{-1} of sucrose on day 9 (expt. 8), these ears have the same total ear grain weight at maturity as the ears on the plant, yet the average single grain weight (Table 3.3.7) and the weight of the b grain (Table 3.3.6) in cultured and attached ears are significantly different. These differences may partly be due to differences in grain numbers (50.5 for ears cultured with 40 gl^{-1} of sucrose and 46.0 for ears on the plant) between the two sets of ears and/or to a change in the distribution of grain weight among the florets within spikelets of cultured ears (see Table 2 in Singh and Jenner 1983). Nevertheless, if comparisons between grains developing in the control and warmed ears are restricted to within a level of sucrose in the culture medium, the above recorded small differences should have little bearing on the interpretation of the results.

The main objective of the experiments detailed in this section was to examine the hypothesis that a reduction in single grain weight due to a brief period of ear warming is mediated through the supply of substrate for grain growth rather than through more direct effects of temperature on the processes involved in the dry matter accumulation in the grain. Results presented in this section support the idea that a reduction in grain weight is due to effects of temperature on the processes related to dry matter accumulation in the grain, and evidence for it is summarized as follows.

In experiment 7, it was assumed that (a) warming of ears results in a reduced supply of substrate (sucrose) to the grain, lowered grain sucrose and hence lower accumulation of dry matter; (b) that feeding the detached ears with excessive sucrose (60 gl^{-1}) would make good the deficit in supply, will restore internal sucrose to normal levels and thus prevent reduction in weight per grain.

However, assumption (b) is not true, as feeding high levels of sucrose failed to raise the internal sucrose levels and the experiment failed to test this hypothesis properly. What this experiment does show, however, is that heating does not depress grain weight at low levels of sucrose supply (Fig. 3.3.1b). This observation is not consistent with the original notion (a) because it would be expected if (a) were true that heating would have more severe effects at low levels

of supply than at high levels. It is also clear from Figs. 3.3.2(a) and 3.3.2(b) that while the after-affects of heating include reduced amounts and concentrations of sucrose in the grain of attached ears heating does not have the same effect in the cultured ears even though dry matter deposition is reduced. Such a result tends to discount proposition (a) where heating is held to act through decreasing the availability of assimilate within the grain. An alternative view of these observations is that an episode of high temperature, somehow, limits the grain's capacity to accumulate dry matter subsequently. Moreover, the effects of this stress cannot be ameliorated by surplus supply of substrate in the external medium.

Further support for these inferences comes from experiment 9, where the endosperms from control ears or ears warmed at high or low humidity were cultured with ^{14}C-sucrose. The endosperms from the ears warmed at high or low humidity absorbed smaller quantities of ^{14}C in the cells (Table 3.3.8), presumably because there was a small volume of water in the endosperms (as the concentration of soluble ^{14}C is comparable to the control endosperms, Fig. 3.3.6b) and also produced less radioactive starch. The capacity of the endosperms from ears warmed at high humidity to produce more radioactive starch compared to endosperms from the ears warmed at low humidity, on day 14 (during the stress period), is interesting and is consistent with the finding that warming at high humidity is less damaging to the synthetic system of the grain than warming at low humidity (see expt. 6, Section 3.2).

However, the differing effects of high and low humidity are not maintained during subsequent stages of grain growth. The concentration of ethanol soluble radioactivity in the cells of endosperms from the warmed ears and those from control ears is the same on day 18, yet the former produced less radioactive starch than the latter (Table 3.3.8). Therefore, it seems reasonable to conclude that warming of ears has not acted by reducing the uptake of ^{14}C substrate, but has had a direct effect on the synthetic system converting sucrose to starch.

Experiment 8, was another test of the idea that heating acted by depriving the grain of assimilate, reducing levels of assimilate in the grain and depressing the rate of dry matter accumulation (or failing to increase it). Thus warming the ears cultured at higher levels of surcrose than normal should increase the rate of dry matter accumulation proportionately *more* than the ears cultured at low levels of supply. This expectation is not true. In fact the grain's (positive) response to

elevated temperature is greater at low levels of supply than at higher ones (Fig. 3.3.4).

One of the most important points from this experiment is that the relationship between the response to heating in terms of dry matter accumulation and internal level of sucrose has been broken. Heating stimulated the rate of dry matter accumulation in 20 gl^{-1} of sucrose, but not on the plant or at 60 gl^{-1} of sucrose, but heating reduces sucrose or other soluble sugars to the same extent in all cases (Table 3.3.5).

Thus it is not true to postulate that heating acts on dry matter accumulation through its effect on sucrose in the grain : the two effects appear to be independent of each other. Most interesting however, is the fact that warming the ears cultured at 20 gl^{-1} of sucrose did not reduce water content of the grain whereas water content of the grain was reduced in the other ear warming treatments. Therefore it is likely that under conditions of heat stress water content of the grain may be an important indicator of response to the stress. Warming of ears on the plant or ears cultured with 40 or 60 gl^{-1} of sucrose does reduce water content of the grain, and this may reflect a reduced cell volume and hence a smaller capacity of the grain to accumulate dry matter (cf. Radley 1976).

Warming of ears, in experiment 8, accelerated the rate of dry weight increase in ears cultured with 20 or 40 gl^{-1} of sucrose, but had no effect in the ears cultured with 60 gl^{-1} of sucrose or in ears left on the plant (Fig. 3.3.4). Final dry weight was reduced in all cases however. This reduction in single grain weight is possibly due to an earlier termination of grain growth in the warmed ears.

Despite the substantial reduction in dry weight of the b grain in warmed ears (Table 3.3.2 and 3.3.6) there is little or no effect on the amount of nitrogen in it. As most of the nitrogen in the grain at maturity is in the form of protein (Jennings and Morton 1963a; Donovan and Lee 1978), it seems that a brief period of ear warming during grain filling has a differential effect on the processes related to starch and protein accumulation. As determinations of nitrogen, in these experiments, were carried out only on mature grains, no inferences regarding the effect of temperature on the pattern of protein accumulation can be drawn.

In summary, both the rate and the duration of grain growth are sensitive to temperature. A reduction in single grain weight, at

maturity, is most likely due to direct effects of temperature on the process of dry matter accumulation and on the rate of development rather through a lack of availability of substrate for grain growth. Apparently, a brief period of ear warming seems to have a differential effect on starch and protein accumulation.

3.4 EFFECTS OF EAR WARMING ON THE TRANSFER OF WATER AND DRY MATTER THROUGH THE STALK OF THE GRAIN

Introduction

Grain growth in wheat consists of a series of developmental stages (Rogers and Quatrano 1983). On the basis of changes in water content of the grain various developmental stages can be grouped into three distinct phases of grain growth. During the first phase of grain growth both water and dry matter build up in the grain; in the second phase the grain accumulates one-half to two-thirds of its eventual mass without any change in water content, and in the third phase the grain loses water rapidly with little or no change in its dry matter (Fig. 1.5, Section 1.6.1). Despite a large number of reports on various aspects of grain growth and development very little is known about the mechanism(s) of the transfer of dry matter and water between the grain and the plant.

The continuity of the tracheary elements where the grain is attached to the rachilla is interrupted by a core of thick-walled cells which is presumed to restrict flow of material in the xylem from the rachilla to the pericarp (Zee and O'Brien, 1970a); flow of water into the grain through the apoplast may, therefore, be restricted. As already pointed out, a major proportion of grain's eventual mass accumulates without any change in its water content, so either the bulk of the dry matter is transported into the grain without any accompanying flow of water, or if material (assimilates) flows in as a solution the water entering the grain must be lost either through the pericarp or by some other means. In fact, Jenner (1982b) has reported that proportional unloading of the assimilate and water into the apoplast of the grain is unlikely and has suggested that there is some form of recirculation of water in the grain or, alternatively, that a mechanism exists whereby solute is transferred to the endosperm from the parent without any accompanying transfer of water.

Warming the whole plant (expt. 3), or the ears alone at the early stage (10 days after anthesis; when water content of the grain is rising, expts. 4 and 5) or at a later stage (15 days after anthesis, expt. 6) results in a reduction of water content of the grain, which may be due to (a) an increase in the evaporative loss of water from the surface of the grain, or to (b) a lower influx of water into the grain, or to (c) a combination of both (a) and (b).

The method for determining the net fluxes of water and dry matter into the grain at a range of water potential gradients (between the plant and the surroundings of the grain) has been described by Jenner (1982b). Accordingly, this method was used to evaluate the effects of ear warming on the transport of water and dry matter through the stalk of the grain. Also, the rates of water loss by isolated grains were determined to examine the effects of ear warming on loss by evaporation from the surface of the grain.

Materials and Methods

Experiment 10

Plants were raised in the glasshouse for 2 weeks and then transferred to the growth room (Section 2.2.2).

On day 15 after anthesis, the plants were divided into three batches. In one batch ears alone were warmed to 33/25°C (while keeping the rest of the plant at 21/16°C), between days 15 and 20 after anthesis, at high humidity [V.P.D. 0.75 kPa (day) and 0.48 kPa (night)]. Ears in the second batch were warmed to the same day and the night temperatures over the same period at low humidity [V.P.D. 2.5 kPa (day) and 0.95 kPa (night)], while the plants in the third batch were kept at control conditions (21/16°C).

On day 20, the boxes containing plants having untreated ears, or plants on which ears were warmed at high or low humidity were brought to a laboratory maintained at a constant temperature (21±1°C), where further manipulations of the ears were carried out.

From each ear used for encapsulation, grains from the a florets of spikelets 6, 8, and 10 were isolated from their enclosing bracts and weighed immediately (fresh weight) and then after drying (dry weight), and these weights were used to estimate corresponding weights of the b grains. The b florets from these spikelets were manipulated (individually) to prepare either the attached, or the detached, or the empty assemblies (for details see Section 2.5.7). Similarly, from the other side of the same ear, the a grain from spikelet 7, or 9, or 11 was used to estimate the initial weight of the b grain which was left invested in its enclosing bracts for comparison with the grains in the attached assemblies. The capsules in which the grains were enclosed were injected with either 0.1 ml of deionised water or with 0.1 ml of a solution of polyethylene glycol 4000 at water potentials of -1.0 or

-2.5 MPa. Treatments were allocated systematically in Latin Square designs (3 x 3 for spikelets 6, 8, and 10 for encapsulation, and spikelets 7, 9 and 11 for intact florets) to each spikelet position in turn.

On day 24 after anthesis, all the three types of assemblies on an ear were dismantled, and the individual components of the assembly were weighed separately. The net fluxes of water (F_w) and dry matter (F_{dm}) into the grain in four days (between days 20 and 24 after anthesis) were calculated by the procedures described in Section 2.5.7.8.

Results

Experiment 10

At the beginning of the encapsulation (on day 20 after anthesis), grains in the ears warmed at high or low humidity had smaller amounts of water than those in the untreated ears (Table 3.4.1). Dry weight per grain was highest in the ears warmed at high humidity (28.6 mg), followed by control ears (27.1 mg) and was lowest in the ears warmed at low humidity (26.3 mg). Between days 20 and 24 after anthesis grains in the florets left intact on the control, high humidity warmed and low humidity warmed ears respectively grew at daily rates of 1.53, 1.15, and 1.28 mg. Over the same period, grains (in the intact florets) in the control ears lost 1.3 mg of water while grains in the ears which had been warmed at high humidity or at low humidity gained 0.9 and 0.3 mg of water respectively.

Total net flux (F), and net fluxes of dry matter (F_{dm}) and water (F_w - calculated by difference) into the b grains encapsulated with water or polyethylene glycol at water potentials of -1.0 or -2.5 MPa are given in Table 3.4.2.

In the control ears as well as in the ears warmed at either humidity, F was negative at 0 MPa (i.e. there was net flux of material from the grain into the rachis), and F was least negative for the grains in the ears previously warmed at low humidity. At water potentials of -1.0 and -2.5 MPa, F was positive for all the three types of ears, and the values for the warmed ears were higher than those for the control ears (Fig. 3.4.1a).

The encapsulated grains accumulated dry matter (F_{dm}) at slightly slower rates than the grains in the intact florets in the same ears (Table 3.4.2). However, there was no simple relationship evident between

Table 3.4.1

Changes in fresh weight, dry weight and water content of the b grain of the intact florets between days 20 and 24 after anthesis. Values are means over spikelets 7, 9 and 11, and initial values were calculated from the corresponding a grain values. The grains were taken from the control ears (C), or ears warmed to 33/25°C (between days 15 and 20 after anthesis) at high (TH) or low humidity (T). (Expt. 10).

Treatment	Fresh weight	Dry weight	Water content
	Amount per grain (mg)		
(a) Values on day 20 after anthesis			
C	59.0	27.1	31.9
TH	57.8	28.6	29.2
T	54.3	26.3	28.0
L.S.D.	2.6	1.5	1.7
(b) Values on day 24 after anthesis (after 4 days of encapsulation)			
C	63.8	33.2	30.6
TH	63.3	33.3	30.1
T	59.9	31.5	28.4
L.S.D.	3.1	1.6	1.7
(c) Change in 4 days			
C	4.8	6.1	-1.3
TH	5.5	4.6	0.9
T	5.5	5.1	0.4
L.S.D.	n.s.	1.2	1.4

Fig. 3.4.1

Total net flux of matter (a), and the net flux of water (b), through the stalk of the grain in 4 days (between days 20 and 24 after anthesis) at various water potentials in the capsule. The values are for the control ears (●), or ears which were warmed to 33/25°C at high (■) or low humidity (▲) between days 15 and 20 after anthesis. The data are derived by averaging the estimates for both methods in Tables 3.4.2 and 3.4.3. Details in the text. (Expt. 10)

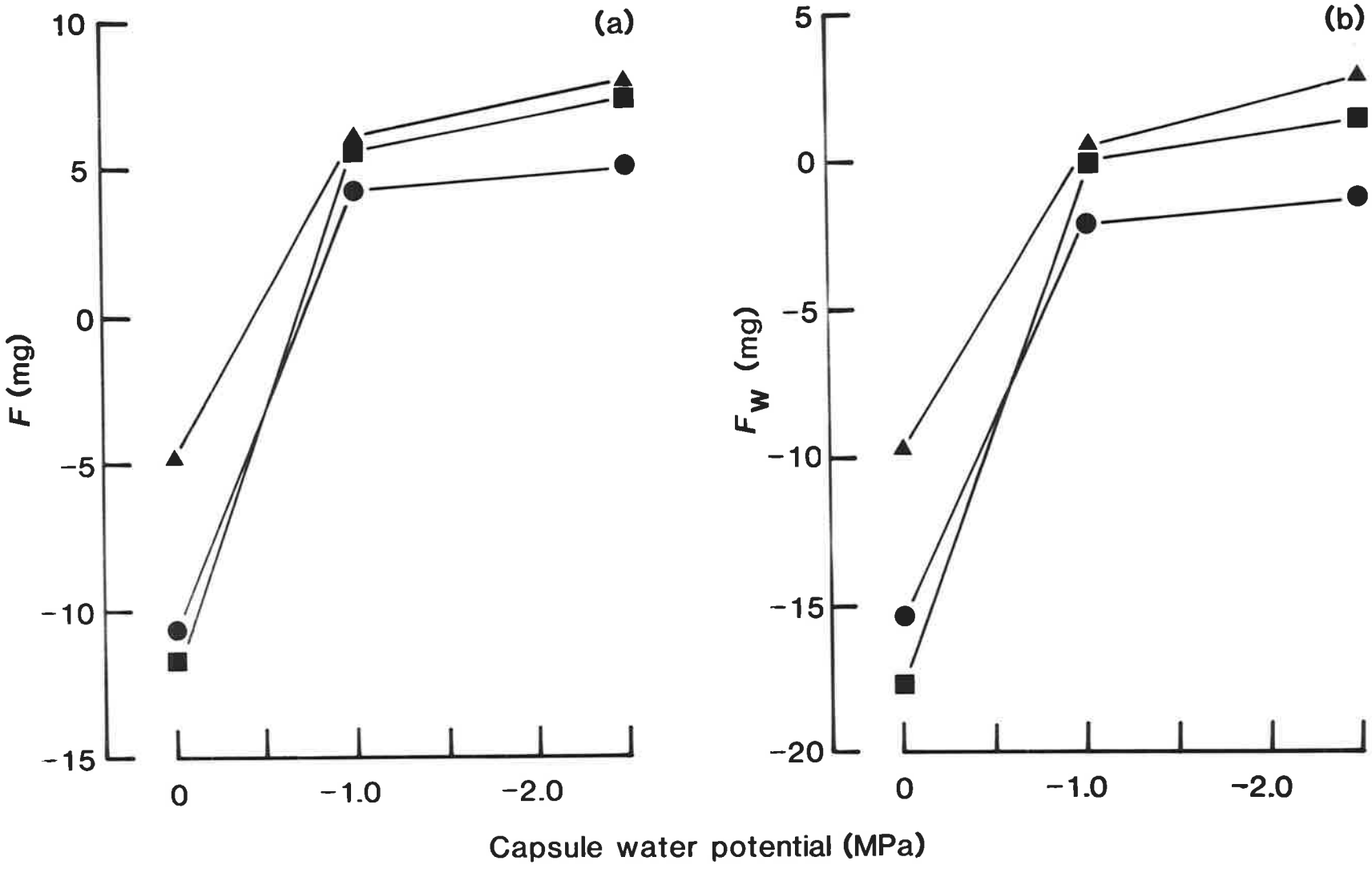

(a)
(b)
10
5
0
−5
−10
−15
F (mg)
5
0
−5
−10
−15
−20
F_W (mg)
0
−1.0
−2.0
Capsule water potential (MPa)

Table 3.4.2

Total net flux (F) and net fluxes of dry matter (F_{dm}) and water (F_w) into b grains encapsulated for 4 days with de-ionised water or polythylene glycol. The encapsulation of the grains was done on control ears (C) or ears which were warmed to 33/25°C (between days 15 and 20 after anthesis) at high (TH) or low humidity (T). Details in the text. F_w is calculated by difference. (Expt. 10).

Parameter	Treatment	Intact floret	Value of parameter (mg) for encapsulated grains at water potential (MPa):			
			0	-1.0	-2.05	Mean
F	C	-	-10.5	4.1	4.8	-0.5
	TH	-	-11.7	5.5	7.1	0.3
	T	-	-5.0	5.8	7.8	2.8
	Mean	-	-9.1^{A}	5.1^{B}	6.5^{B}	
F_{dm}	C	6.1^{A}	4.0	4.7	4.9	4.5
	TH	4.6^{B}	4.6	4.7	4.3	4.5
	T	5.1^{AB}	4.2	4.0	3.9	4.0
	Mean	5.3	4.3	4.5	4.4	
F_w	C	-	-14.5	-0.7	-0.1	-5.1^{A}
	TH	-	-16.3	0.8	2.8	-4.2^{AB}
	T	-	-9.2	1.7	3.9	-1.2^{B}
	Mean	-	-13.3^{A}	0.6^{B}	2.2^{B}	

Values within a row or a column with the same letter are not significantly different.

the net fluxes of dry matter into the encapsulated grains and water potential in the capsule, nor were any significant differences among the treatments.

F_w was calculated both by difference (Table 3.4.2) and by addition (Table 3.4.3) methods. The values of F_w estimated by addition were consistently lower than the values obtained by difference at all levels of water potential in the capsule and for all types of ears. These differences were unexpected and no explanation for this discrepancy can be given. But it seems important to point out that separate sets of assumptions are made while calculating F_w by the two methods. Although the values of F_w calculated by both methods (difference or addition) are not numerically identical, the values show the same general trends, therefore, the average values for both methods have been calculated for comparisons between the control and the warmed ears (Fig. 3.4.1b). Furthermore, as the errors involved in determining F_w were not the same at all the water potentials in the capsule, values for F_w at 0 MPa, and values at -1.0 and -2.5 MPa were analysed separately. F_w for the grains encapsulated with water showed that there had been net fluxes of 15.2, 17.9 and 9.9 mg of water from the grains into the rachis (in 4 days) in control ears, in ears which had been warmed at high humidity, and in ears which had been warmed at low humidity respectively. However, these values were not significantly different (least significant difference 9.2 from each other. At -1.0 MPa in the control and warmed ears (at either humidity) there was a little flux in either direction. At -2.5 MPa the net fluxes of water in the encapsulated grains in the ears warmed at high humidity (1.2 mg) and in the ears warmed at low humidity (2.6 mg) were small and were in the direction from the rachis into the grains. On average (mean of -1.0 and -2.5 MPa), F_w was significantly higher for warmed ears (0.52 and 1.49 mg for ears warmed at high and low humidity respectively) than for the control ears (-1.99 mg).

The change in the water content of the grains in the attached assemblies (ΔAG_w) was greater at -2.5 MPa than at 0 MPa, but at all water potentials the changes were not large (Table 3.4.3).

At 0 and -1.0 MPa, there was a net loss of water from the capsules of the attached (ΔAC, Table 3.4.3) and the detached (ΔDC, Table 3.4.4) assemblies; at -2.5 MPa water content of the capsules in the attached as well as detached assemblies increased, and there were no significant differences among different types of ears. In general values for ΔD were negative indicating that the detached assemblies lost some weight, and

Table 3.4.3

Changes in various parameters required for the determination of net flux of water (F_w) into grains encapsulated for 4 days with water or polyethylene glycol 4000. Details in table 3.4.2. (Expt. 10)

Parameter	Treatment	Value of parameter (mg) for encapsulated grains at water potential (MPa): 0	-1.0	-2.05	Mean
ΔAG_w	C	-0.8	-2.3	-3.9	-2.3^A
	TH	1.9	-1.4	1.9	-0.5^B
	T	1.5	-0.5	-0.9	0.0^B
	Mean	0.9^A	-1.4^B	-2.2^B	
ΔAC	C	-18.2	-2.5	0.1	-6.9
	TH	-24.3	-3.6	0.2	-9.2
	T	-16.0	-2.2	1.5	-5.6
	Mean	-19.5^A	-2.8^B	0.6^C	
ΔD	C	-0.8	2.2	-0.7	0.2
	TH	-1.7	-1.9	-0.2	-1.1
	T	-0.6	0.0	-0.6	0.0
	Mean	-1.0	0.1	0.0	
BA	C	1.0	1.2	0.9	1.0^A
	TH	0.8	0.6	0.7	0.7^A
	T	2.3	1.4	2.0	1.9^B
	Mean	1.4	1.1	1.2	
WL	C	1.1	0.8	0.7	0.9
	TH	1.0	0.9	0.7	0.9
	T	1.0	0.7	0.7	0.8
	Mean	1.0^A	0.8^B	0.7^B	
F_w by addition*	C	-15.9	-3.7	-3.5	-7.7^A
	TH	-19.6	-1.2	-0.3	-7.0^A
	T	-10.6	-0.9	1.3	-3.4^B
	Mean	-15.3^A	-1.9^B	-0.8^B	

* Values are calculated by the equation (7) in Section 2.5.7.8. values within a row or a column marked with the same letter are not significantly different.

Table 3.4.4

Changes in the amount of water in the capsule (ΔDC), and in the water content of the grain (ΔDG_w) in the detached assembly over 4 days of encapsulation. Details in table 3.4.1. (Expt. 10).

Parameter	Treatment	Value of parameter (mg) for encapsulated grains at water potential (MPa):			
		0	-1.0	-2.0	Mean
ΔDC	C	-7.3	-0.5	1.4	-2.1
	TH	-7.7	-0.9	2.2	-2.2
	T	-5.7	-0.9	2.3	-1.5
	Mean	-6.9^{A}	-0.8^{B}	1.9^{C}	
ΔDG_w	C	0.5	-3.5	-6.7	-3.2
	TH	1.1	-4.3	-6.4	-3.2
	T	0.7	-3.3	-6.0	-2.9
	Mean	0.8^{A}	-3.7^{B}	-6.4^{C}	

Values within a row marked with the same letter are not significantly different.

these losses among treatments or among water potentials in the capsules were not significant. BA was positive (at all levels of water potential) indicating that the Blu-Tack seal gained some weight probably from the rachis as well as from the atmosphere. (The relative humdiity of the air in the growth room was higher than that in the laboratory where the ears were encapsulated). Some water evaporated (0.7 to 1.0 mg) from the components of the assemblies (WL) as they were being weighed, and these losses were consistently higher in the assemblies containing water than those containing polyethylene glycol solution (Table 3.4.3).

Grains in the detached assemblies in all types of ears, gained some water (ΔDG_w) when encapsulated with water, and lost some water at lower levels of water potential (-1.0 and -2.5 MPa, Table 3.4.4) in the capsule.

Loss of water from the isolated grains

Ten to fifteen b grains were removed, on day 20, from control and warmed ears at random and weighed individually on a microbalance (Mettler ME 30) sited in a constant temperature laboratory (21±1°C) with an average relative humidity of 60%. The weight of the grain was recorded immediately after its removal from the ear, and then at intervals of 1 min for 5 min. The rate of water loss from the grain was calculated by regression. Due to differences in the numbers of grains used for different types of ears, initial weights of the grains were compared by using the t-Test for samples of unequal sizes, the values for fresh weights and the rates of water loss are given in the following tabulation.

Parameter	Treatment		
	Control	High humidity	Low humidity
Fresh weight per grain (mg)	58.9^{A}	57.8^{B}	55.5^{C}
Rate of water loss ($\mu l\ h^{-1}$)	2.20	2.02	2.04

Fresh weights of the b grain recorded here for the various treatments were similar to ones recorded in Table 3.4.1, and there were no significant differences in the rates of water loss among the treatments.

Discussion

The mechanisms involved in the transfer of dry matter (assimilates) and water between the grain and the plant are poorly understood. The main problem in studying these mechanisms in wheat is that these processes cannot be studied while the grain is invested in its bracts, and some degree of manipulation is essential. Jenner's (1982b) encapsulation technique, however, permits evaluation of the net fluxes of dry matter and water through the stalk of the grain, and hence was used to study the effects of temperature on the properties of the stalk and the grain itself related to the transfer of dry matter and water.

Grains encapsulated in the impermeable capsules appeared to accumulate slightly less dry matter compared to grains left in the intact florets (F_{dm}, Table 3.4.2). However, as the errors involved in estimating F_{dm} (in intact florets as well as in the encapsulated grains) are relatively large (least significant difference of 1.2 mg) the mean values (Table 3.4.2) for dry matter increments in the grains of the intact florets and the encapsulated grains were not significantly different. So it seems reasonable to assume that the encapsulated grains functioned normally.

Warming of ears at high or low humidity has minor effects on the water potentials of the grains or the bracts, and the values for grains lay between -0.5 and -0.7 MPa (days 17, 19, and 21 after anthesis, Table 3.2.8) and values for the bracts over the same days range from -0.7 to -1.2 MPa (Table 3.2.9). Presumably, the water potential of the air in the space surrounding the grain (which cannot be determined by available techniques), is not far removed from the above values. F_w values for the grains in the warmed ears at either humidity were similar to each other but were higher than that of the control ears at capsule water potentials of -1.0 and -2.5 MPa (Fig. 3.4.1b). Therefore, the notion that warming of ears reduces the movement of water through the apoplast of the stalk (and of the grain) is not true.

Some inferences regarding the effect of heating on water conducting properties of the pericarp can be drawn by comparing the rates of water loss from isolated grains, from the control ears and ears warmed at high

or low humidity, in relatively dry atmosphere (V.P.D. 1.0 kPa), and also from the changes in the water content of the grains sealed in the detached assemblies at water potentials of -1.0 and -2.5 MPa (ΔDG_w), Table 3.4.4). In both cases these losses from grains of control and warmed ears were the same, so there would appear to be little or no effect of ear warming on the resistances to water flow from the grain to its surroundings through the pericarp.

A reduction in water content of the grain in heat stressed ears cannot simply be explained by effects on the water conducting properties of the pericarp. So, what is the cause of this reduction in water content. Two possible explanations are discussed here.

The encapsulated grain on average (overall mean of treatments from Table 3.4.2) increases in weight by 4.4 mg in 4 days. Assuming this dry weight enters the grain (as sucrose) by way of phloem (Section 1.8) as a bulk-flow of solution and both solute and solvent are unloaded into the apoplast of the grain, and assuming further that the concentration of sucrose in the phloem (sieve tubes) is as high as 1M (cf. Jenner 1982b), then the net influx of sucrose would require an influx of 12.9 µl of solution, which would include 10.1 µl of water. This volume of water is 3.9 times the highest value of F_w observed for the grains in the ears warmed at low humidity (Fig. 3.4.1b). Moreover, in the control ears at a water potential of -2.5 MPa there is net influx of 4.9 mg of dry weight without any accompanying net influx of water. These observations are consistent with Jenner's (1982b) view that too little water flows through the apoplastic phase of the grain to account for the transport of assimilates by a mechanism involving mass flow and proportional unloading of the solute and the solvent in the grain. However, the possibility that some sort of recirculation of water between the grain and the plant takes place in a pathway isolated from the apoplast of the grain (also speculated by Cochrane 1983) is not at present ruled out by these findings. According to this view, heat stress could increase the export of water from the grain by way of this pathway.

Water movement in the plant is presumably determined by gradients in water potential and by the resistances to its flow. However, the grain has the capacity to maintain high (more positive) water potential under conditions of water stress (Barlow *et al.* 1980; Brooks *et al.* 1982), and this capacity is possibly due to the special morphological and anatomical features of the grain and the surrounding parts. Water potential of the grain in the ears warmed at high or low humidity is

higher than the control ears (on day 17, Table 3.2.8, also see Table 3.2.3) and furthermore, the differences in water potential between the bracts and the grains is increased as a result of ear warming (Tables 3.2.8 and 3.2.9 for the grains and the bracts respectively - Section 3.2). Thus, it seems possible that due to the increased water potential gradient in the heat stressed ears more water could flow through the pericarp to the space surrounding the grain (with little or no change in the resistances to its flow), or it could flow back from the grain to the rachis and be lost through the bracts (also see Oparka and Gates 1981).

3.5 Effects of high temperature on starch and protein accumulation in the grain

Introduction

Starch and protein are the major constituents of mature wheat grain (Section 1.5). The protein content of the grain is one of the principal quality parameters (Bell and Simmonds 1963; Orth and Bushuk 1972, 1973), and is determined by the interaction of the genotype with the environment (Miezan et al. 1977; Section 1.4.2.2). The relative proportion of protein to starch in the grain, between temperatures of 15 and 30°C, increases with increase in temperature during the grain filling period (Sosulski et al. 1963; Campbell and Read 1968; Kolderup 1975; Sofield et al. 1977b; Spiertz 1977), and even a brief period of ear warming during the early stages of grain filling (between days 10 and 17 after anthesis) seems to be effective in eliciting the above response (Section 3.3). Increase in per cent protein in the grain has also been reported under conditions of water stress (Hutcheon and Paul 1966; Salter and Goode 1967; Brooks et al. 1982), low light intensity (Spiertz 1977), or a brief period of shading during the early parts of grain filling (Jenner 1979, 1980b).

The protein content, as percentage of dry matter, is essentially determined by the relative rates and the durations of both protein and starch deposition, so the response reported above could conceivably be achieved in a number of ways. Increase in protein per cent in the grain, at maturity, with increase in temperature, between 15 and 21°C, during grain filling seems mainly to be due to an increase in the absolute amount of protein in the grain with little or no change in the amount of starch, whereas with further increase in temperature to 30°C amounts of both protein and starch are reduced, but the amount of starch is reduced more than that of protein (Table 2 - Sofield et al. 1977b).

It is generally accepted (Meredith 1981 and references cited therein) that starch in the wheat grain is deposited in two distinct types of granule: large, lenticular (commonly known as A-type) which are initiated early during the development of the endosperm (between days 4 and 14 after anthesis), continue to grow throughout grain growth and are normally larger than 10 µm in diameter at maturity, and smaller, spherical granules (B-type) which are initiated later than the A-type (Section 1.4.2.1.2) and do not exceed 10 µm in diameter. Brooks et al. (1982) have shown that grains from wheat and barley plants grown at low

water potential had smaller A-type and fewer B-type granules than the grains from well watered plants (also see Brocklehurst et al. 1978). Similarly, some workers (Hoshikawa 1962; Moss 1963) have reported that high temperature during grain filling seems to have similar effects to water stress on granule number and size, but to date no critical measurements of starch granule size and/or number in the grains from heat stressed plants have been made.

Heat tolerant and susceptible genotypes have been identified, based on fruit set and yield, under high temperature field conditions, in tomato (Stevens and Rudich 1978; Ahmadi Al and Stevens 1979), bean (*Phaseolus vulgaris* L.) (Halterlein et al. 1980), soybean (Martineau et al. 1979) and potato (Mendoza and Estrada 1979). Differences in response among wheat cultivars, in terms of grain growth and mature grain weight (or grain yield), to high temperature have been reported (Bagga and Rawson 1977; Winzeler and Nosberger 1980), but little is known about the biochemical or physiological factors related to the superior performance of some cultivars over others.

Grain filling results from a series of partial processes which determine the eventual amount of starch and protein to be stored in the grain. In most of the work related to the effects of temperature, on grain growth and on grain weight at maturity, the plants have been kept at a high temperature from anthesis (or soon afterwards) until maturity, and little is known about the effects of a brief period of high temperature (a more likely situation in South Australian fields) on starch and protein accumulation in the grain. Accordingly, four wheat cultivars RC-81-429 (The Roseworthy Agricultural College, Crossing block 1981 - Plot #429) - The Oregon Collection Oo 58 Burgas II 2002; WW15 - ANZA Spring Wheat from CIMMYT ; Kite - Spring Wheat from Australia ; and Sonora 64 - Spring Wheat from CIMMYT, were compared to examine: (a) any differences in the responses of the cultivars (in terms of grain yield) to a brief period of heat stress ; (b) the effect of heat stress on patterns of starch and protein accumulation ; (c) the effects of a brief period of heat stress on starch granule size and number.

Materials and Methods

Experiment 11

Plants of all the cultivars were raised, in 1.2 l plastic bags arranged in boxes (Section 2.2.1), for 45 days in the glasshouse and

then shifted to the growth room (Section 2.2.2). As no vernalisation of the seeds was carried out, anthesis in cv. Sonora was one week earlier than the other cultivars. Soon after anthesis, plants of each cultivar were divided into two almost equal sets. On day 10, one set of plants of each cultivar was shifted to the growth cabinet (Section 2.3.2) set at 33/25°C while keeping the other set of plants in the growth room. After the heat stress treatment was over (day 20 after anthesis) the plants were put back in the growth room where they stayed until maturity.

The b grains from one side of the ear, from 4 separate ears (except at maturity when five ears were used) from the four central spikelets on days 10, 15, 20, 25, 30, and 35 after anthesis and when the plants had ripened were used for fresh and dry weight determinations (Freeze-drying-Section 2.5.1), and the dried grains were used for nitrogen estimations (Section 2.6.3). The b grains from the other side of the same ears (except for day 35 when no starch granule measurements were made) were used for starch granule number and size determinations (Section 2.5.6). On days 10, 15, and 20 after anthesis isolated endosperms were used for starch granule measurements whereas the intact grains were used on the other sampling times.

At maturity, five plants each of control and heat stressed plants for each cultivar were sampled to determine grain yield components. Also to compare the effects of heat stress on grain weight in different floret positions, the grains a and c from the four central spikelets were weighed separately.

Experiment 12

The same four wheat cultivars were used in this experiment. The plants were raised, in 15 cm pots (3 plants per pot) containing about 1.2 l of soil, for two weeks in the glasshouse and then transferred to the growth room. After anthesis, the plants in each cultivar were divided into two uniform sets and one set of plants for each cultivar was warmed at 27/22°C between days 10 and 30 after anthesis while keeping the other set of plants at ambient conditions.

Sampling procedure for this experiment was exactly the same as described for experiment 11, except that sampling was done on days 10, 20, 30, and 35 after anthesis and when ears had ripened. No measurements for starch granule size or number were made in this experiment.

Results

Experiment 11

Changes in dry weight and water content of the grain

The changes in dry weight and water content on a per grain basis at various stages of grain development, plotted separately for each cv., are given in Fig. 3.5.1. Except for cv. Sonora where there was no change in water content of the grain in control plants over the main period of grain filling (between days 10 and 35), water content of the grain in other cultivars increased between days 10 and 20 after anthesis and then remained almost steady at subsequent stages of sampling. Heat stress reduced the water content of the grain in all cultivars, and it was also evident that maturation of the grains in warmed plants in cultivars RC-81-429 and Sonora started at least 5 days earlier than in cv. Kite or WW15. Further, to separate the direct effects of high temperature (during the period of its imposition) from the delayed effects (when the plants were allowed to grow in a common environment), the rates of dry matter and protein accumulation between days 10 and 20 after anthesis (Table 3.5.1), and the changes in dry weight (Fig. 3.5.2) and protein content (Fig. 3.5.4) in the grain at subsequent stages (from day 20 onwards) of grain growth, have also been presented.

Warming the plants to 33/25°C accelerated the rate of dry matter accumulation in all cultivars, and this increase in the rate was smaller in cv. RC-81-429 as compared to other cultivars (Table 3.5.1). Over the same period grain growth rate in the control plants was in the order of RC-81-429 > WW 15 > Kite > Sonora, but these rates were poorly correlated to the grain weight at maturity (Table 3.5.3).

The after effects of warming on dry matter accumulation are shown in Fig. 3.5.2. With the exception of cv. RC-81-429 where the rate of grain growth in heat stressed plants between days 20 and 25 seems to be higher than control plants, the rate of grain growth in heat stressed plants was lower than in the control plants in the other cultivars (Figs. 3.5.2b-d). In heat stressed plants some differences in the courses of grain growth towards maturity were also discernible among the cultivars. For instance, in cultivar RC-81-429 (Fig. 3.5.1a) maximum grain weight was reached by day 25 and there was little or no change thereafter (indication of earlier maturity), whereas in cvv. Kite (Fig. 3.5.1b), Sonora (Fig. 3.5.1c), and WW 15 (Fig. 3.5.1d) grain

Fig. 3.5.1

Changes in dry weight (closed symbols) and water content (open symbols) of the b grains taken from the four central spikelets of the control (●, ○) and heat stressed (▲,△) plants of cv. RC-81-429 (a), cv. Kite (b), cv. Sonora (c), and cv. WW 15 (d). Plants were warmed, between days 10 and 20 after anthesis (indicated by solid horizontal bars), to 33/25°C and then transferred to the control environment (21/16°C) where they stayed until maturity. Vertical bars represent the interaction L.S.D. between times x treatments. (Expt. 11)

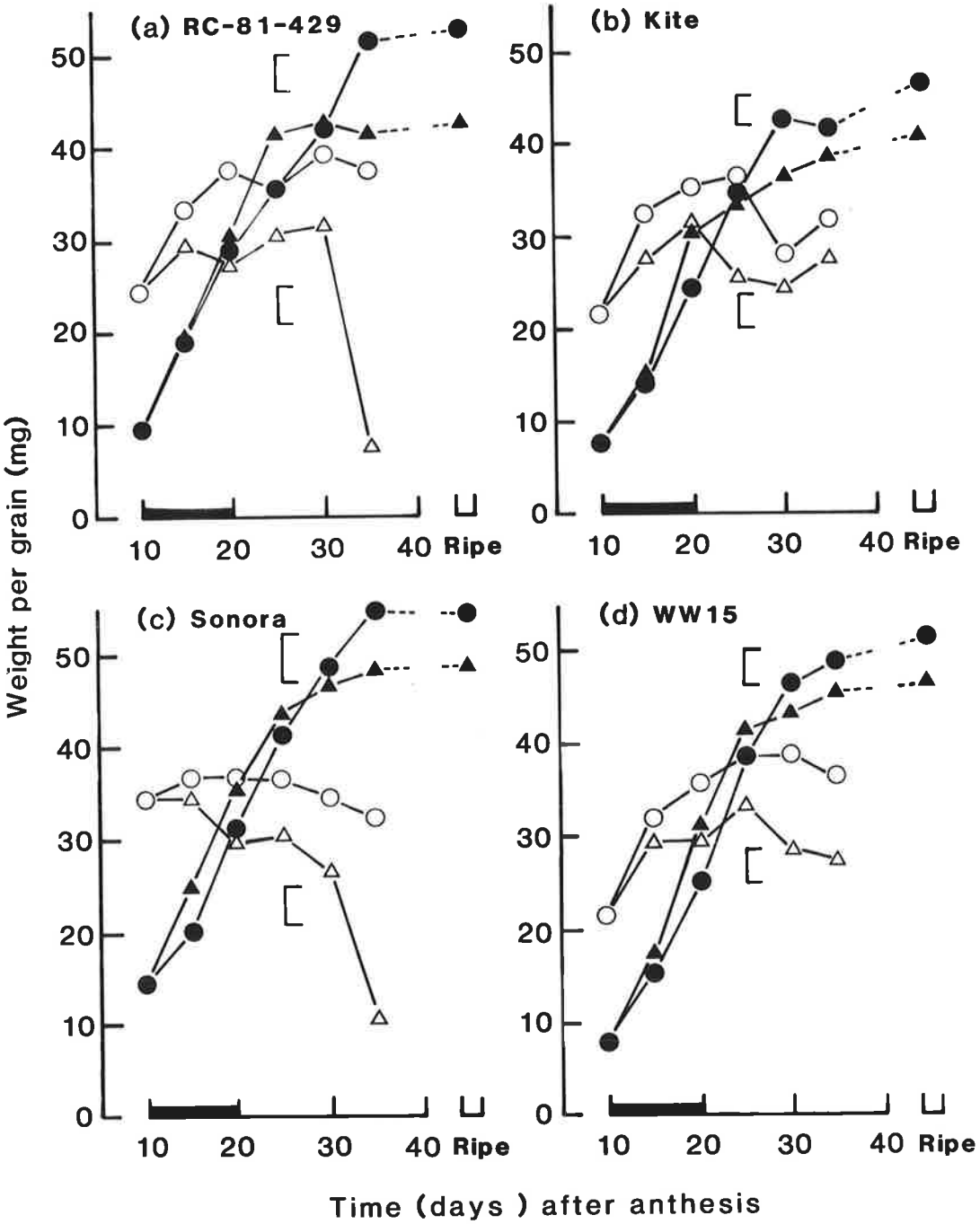

(a) RC-81-429
(b) Kite
(c) Sonora
(d) WW15
Weight per grain (mg)
0
10
20
30
40
50
10
20
30
40 Ripe
Time (days) after anthesis

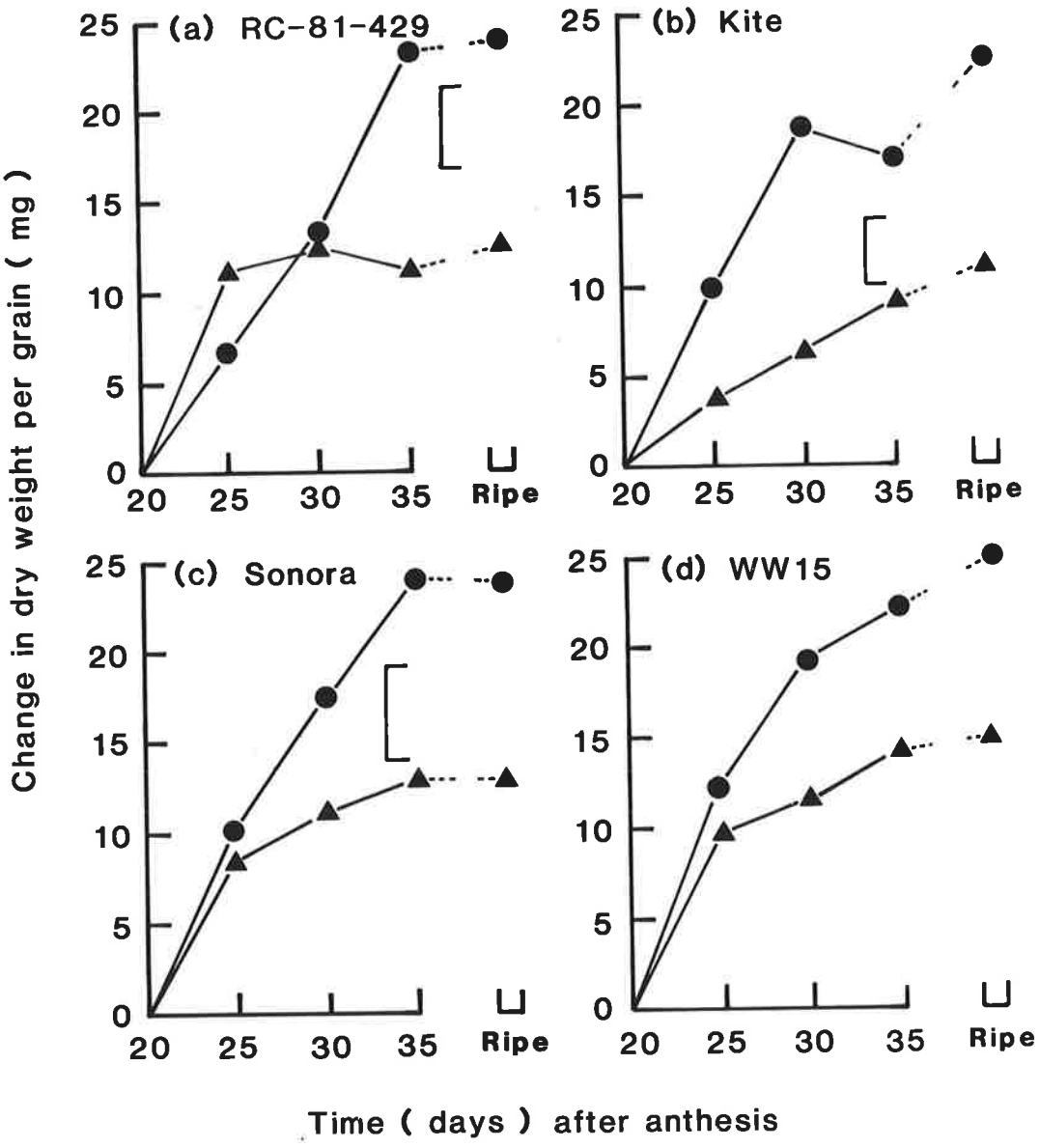
Table 3.5.1

Effects of whole plant warming on the rates of dry matter and protein accumulation (±S.E.), between days 10 and 20 after anthesis, in the grains of the four cultivars of wheat. Details of warming treatment are given in the legend to Fig. 3.5.1. Values in the parentheses are per cent of control values. (Expt. 11)

Cultivar	Treatment	Rate of increase of: Dry weight (mg grain^{-1}	Protein content day^{-1})
RC-81-429	Control	1.94±0.09	0.17±0.01
	Warmed	2.07±0.07	0.27±0.02
		(107)	(159)
Kite	Control	1.72±0.15	0.15±0.02
	Warmed	2.28±0.17	0.25±0.02
		(133)	(167)
Sonora	Control	1.65±0.18	0.20±0.05
	Warmed	2.06±0.19	0.29±0.05
		(125)	(145)
WW15	Control	1.89±0.20	0.21±0.03
	Warmed	2.37±0.20	0.30±0.03
		(125)	(143)

Fig. 3.5.2

Changes in dry weight of the b grain in the control (●) and warmed plants (▲) after the warmed plants were returned to 21/16°C. Other details as in legend in Fig. 3.5.1. (Expt. 11)


(a) RC-81-429
(b) Kite
(c) Sonora
(d) WW15
25
20
15
10
5
0
20
25
30
35
Ripe
Change in dry weight per grain (mg)
Time (days) after anthesis

growth continued, albeit at a slower rate, even until day 35 after anthesis.

At maturity, total ear grain weights in the control plants for cv. RC-81-429, Sonora, and WW 15 were almost equal and were higher than that of cv. Kite (Table 3.5.2). Lower total ear grain weight in cv. Kite was attributable both to a smaller number and smaller size of the grains. There was a small reduction in the number of grains per ear in warmed plants, but most of the reduction in ear grain weight resulting from heat stress was due to lower single grain weight. All the cultivars were affected and there were equal reductions at all floret positions (Tables 3.5.2 and 3.5.3). Both total ear grain weight and individual grain weight reductions were greater in cv. RC-81-429 than in the other cultivars.

Protein accumulation

In cvv. RC-81-429 (Fig. 3.5.3a), and Sonora (Fig. 3.5.3c) the amount of protein (N x 5.7) per grain in the control plants increased linearly until day 35 without much change thereafter, whereas in cvv. Kite (Fig. 3.5.3b), and WW 15 (Fig. 3.5.3d) the increase in protein content appears to have continued beyond day 35 after anthesis. Warming of plants accelerated the rate of protein accumulation in all the cultivars (between days 10 and 20, Table 3.5.1) and warming affected protein accumulation more than total dry matter. At the subsequent stage of grain development, between days 20 and 25, except for cv. RC-81-429 (Fig. 3.5.4a) where the increase in the protein content in the grains of heat stressed plants was greater compared to the grains from the control plants, the increase in the protein content in the grain of heat stressed plants was either comparable to (cv. WW 15, Fig. 3.5.4d) or slightly lower (cvv. Kite and Sonora, Fig. 3.5.4b and 3.5.4c respectively) than it was in the grains of the control plants. More importantly however, except for cv. RC-81-429 (Fig. 3.5.3a) where warming resulted in an earlier termination of protein accumulation (about 30 days after anthesis) there seems to be little effect of heat stress on the duration of protein accumulation in the other cultivars.

Unlike dry weight per grain at maturity, which was reduced in all the cultivars in the warmed plants, protein content per grain was hardly affected (Fig. 3.5.3 and Table 3.5.3) and hence on average there was about 2.5 per cent increase in percentage protein. The amount of protein

Table 3.5.2

Effects of a brief period of heat stress (33/25°C ; between days 10 and 20 after anthesis) on components of grain yield at maturity in four cultivars of wheat. Values in the parentheses are percentage reductions of the control values. (Expt. 11)

Cultivar	Treatment	No. per ear: Spikelets	No. per ear: Grains	Total ear grain weight (mg)	Single grain weight: Floret a (mg)	Single grain weight: Floret c (mg)
RC-81-429	Control	16.6	49.4	2065	50.7	44.4
	Warmed	16.6	46.4	1563 (-24.3)	39.7 (-22.1)	34.6 (-22.1)
Mean		16.6	47.8	1814	45.2	39.5
Kite	Control	16.4	41.8	1684	46.2	36.3
	Warmed	17.2	38.6	1307 (-22.4)	40.2 (-13.0)	33.1 (- 8.8)
Mean		16.8	40.2	1496	43.2	34.7
Sonora	Control	16.2	45.6	1954	50.9	46.0
	Warmed	16.2	43.0	1643 (-16.0)	44.4 (-12.8)	40.2 (-12.6)
Mean		16.2	44.3	1798	47.6	43.1
WW15	Control	14.2	44.2	1961	50.4	44.6
	Warmed	15.2	42.6	1644 (-16.2)	44.6 (-11.4)	37.8 (-15.2)
Mean		14.7	43.4	1802	47.5	41.2
Mean of cultivars	Control	15.9	45.3	1916	49.5	42.8
	Warmed	16.3	42.7	1539 (-19.7)	42.2 (-14.7)	36.5 (-14.7)
Least significant differences						
Treatment		n.s.	2.5	149	2.4	2.9
Cultivar		0.9	3.6	105	1.7	2.0

Table 3.5.3

Effects of raising temperature to 33/25°C between days 10 and 20 after anthesis on dry weight and protein content (N x 5.7) of the b grain at maturity in four cultivars wheat. Grains were taken from the central four spikelets of the ear. Values in parentheses are per cent reductions of control values. (Expt. 11)

Cultivar	Treatment	Dry weight (mg per grain)		Protein content (mg per grain)	Protein per cent
RC-81-429	Control	50.3		6.8	13.6
	Warmed	42.1	(-16.3)	6.7	15.9
Mean		46.2		6.7	14.8
Kite	Control	47.1		7.6	16.1
	Warmed	40.2	(-14.6)	7.8	19.4
Mean		43.7		7.7	17.8
Sonora	Control	54.4		8.8	16.4
	Warmed	47.5	(-12.7)	8.7	18.2
Mean		50.9		8.8	17.3
WW15	Control	51.6		6.9	13.3
	Warmed	46.4	(-10.1)	7.4	15.9
Mean		49.0		7.1	14.6
Mean of cultivars					
	Control	50.9		7.5	14.9
	Warmed	44.0	(-13.6)	7.7	17.4
		Least significant differences			
Treatment		1.8		N.S.	1.3
Cultivar		2.5		0.9	1.9

Fig. 3.5.3

Changes in protein content (N x 5.7) in the b grains taken from the four central spikelets of the ears from the control (●) and heat stressed (▲) plants of cv. RC-81-429 (a), cv. Kite (b), cv. Sonora (c), and cv. WW 15 (d). Details in legend to Fig. 3.5.1. (Expt. 11)

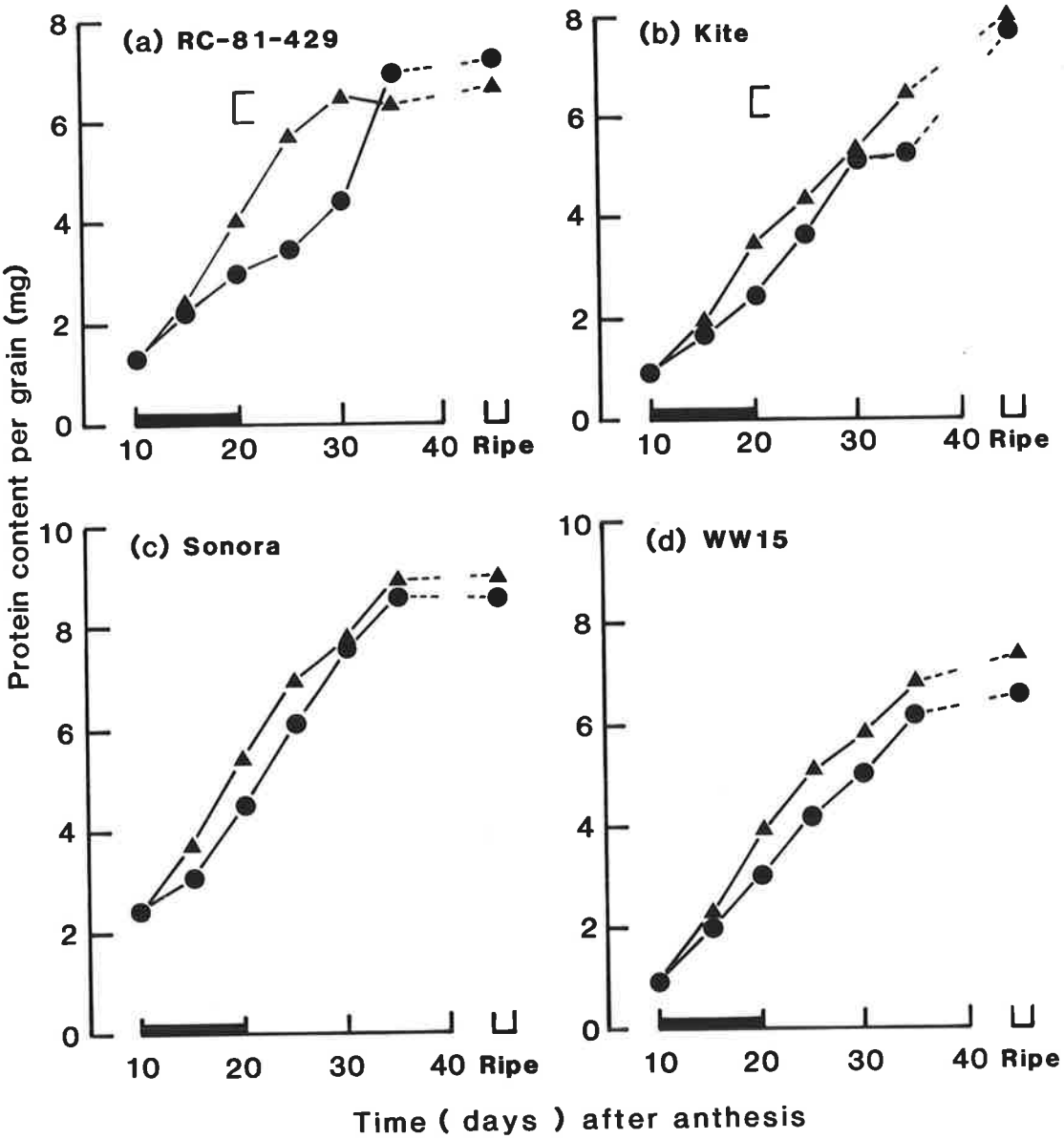
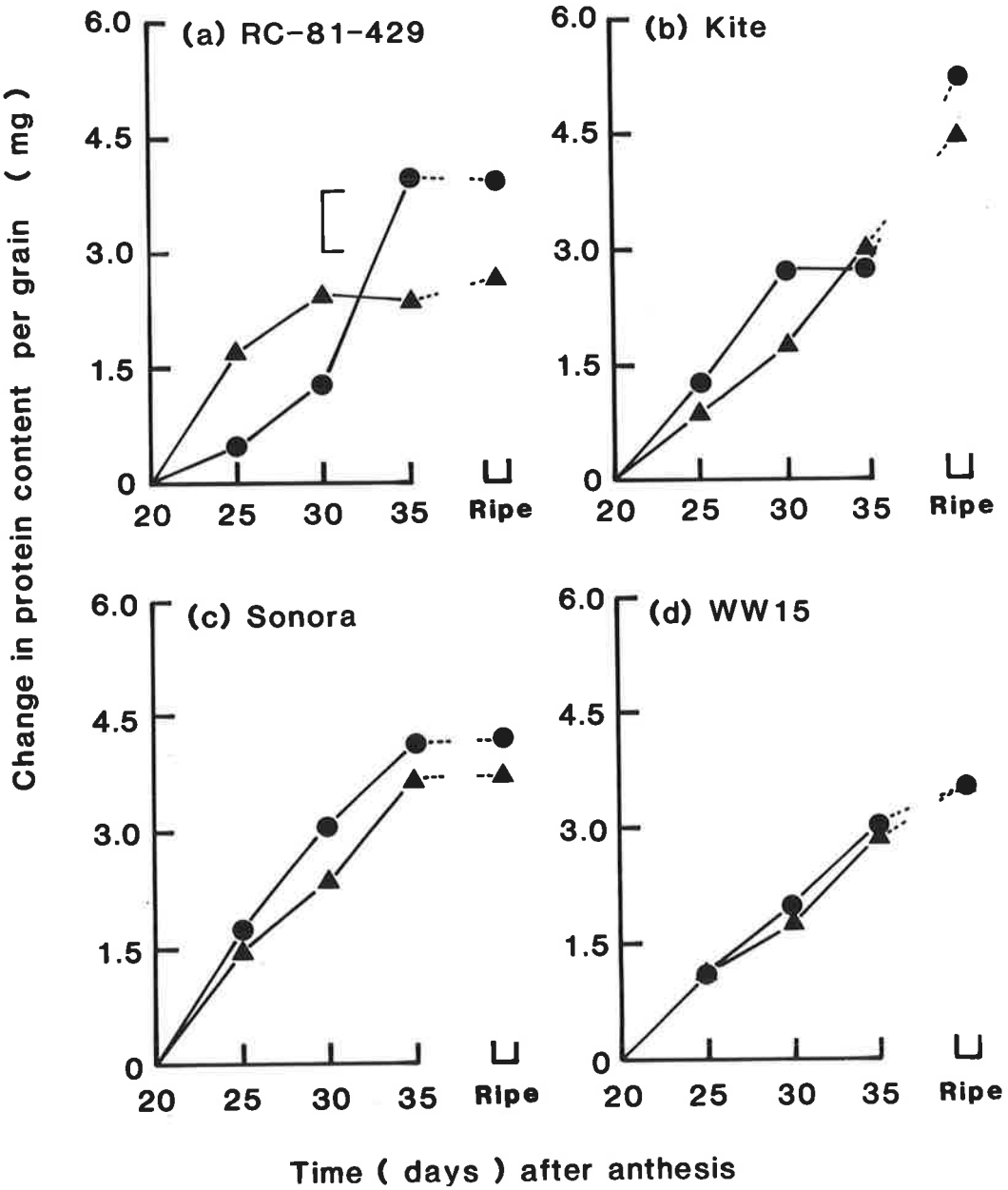

(a) RC-81-429
(b) Kite
(c) Sonora
(d) WW15
Protein content per grain (mg)
Time (days) after anthesis
Ripe
0
2
4
6
8
10
20
30
40

Fig. 3.5.4

Changes in protein content (N x 5.7) of the b grain in the control (●) and the heat stressed plants (▲) after the warmed plants were returned to 21/16°C. Other details as in the legend to Fig. 3.5.1. (Expt. 11)


(a) RC-81-429
(b) Kite
(c) Sonora
(d) WW15
6.0
4.5
3.0
1.5
0
20
25
30
35
Ripe
Change in protein content per grain (mg)
Time (days) after anthesis

per grain also differed among cultivars and was in order of Sonora > Kite > WW 15 > RC-81-429.

Changes in starch granule size and number

The classification of starch granules into large (> 10 µm) A-type and smaller (< 10 µm) B-type granules was purely based on the largest diameters of the granules and may not necessarily conform with the conventional way of designating A- and B-type granules as proposed by May and Buttrose (1959). Therefore, it is necessary to point out that the granules classed as the B-type during the early stages of grain development (before day 15 after anthesis), may in fact all be A-type granules, but because of their smaller diameters have been counted as B-type granules. Furthermore, at the later stages of grain development, some granules belonging to earlier generation (A-type) may eventually be included in B-type because they may have not had the time to develop completely. Therefore, the numbers of B-type granules in this study may be the mixture of true B-type granules and immature A-type granules.

The numbers of both A- and B-type starch granules at various stages of grain development in the control and the heat stressed plants are given, for each cultivar separately, in Tables 3.5.4a-d, and for easy comparison among the cultivars total numbers of starch granules are plotted in Figs. 3.5.5a-d. At the beginning of the warming treatment, day 10 after anthesis, as expected from dry weight data (Figs. 3.5.1a-d) cv. Sonora had higher numbers of both A- and B-type granules relative to other cultivars. Despite the initial differences in A-type granules among the cultivars the progression in A-type granules at subsequent stages of grain growth was similar for all the cultivars. B-type granules were initiated earlier (at least by 4-5 days, Tables 3.5.4a-d) and their rate of appearance was faster (from slopes of the curves in Figs. 3.5.5a-d) in cvv. Sonora and WW 15 as compared to the other two cultivars. Also, at maturity cultivars differed widely in total number of starch granules (Figs. 3.5.5a-d and Table 3.5.5) mainly because of the differences in B-type granules as there were no significant differences in A-type granules.

Warming had no effect on the rate of appearance or the number of A-type granules at any stage of grain development in any of the cultivars. However, warming seemed to accelerate temporarily the rate of appearance of B-type granules but the effects were not large enough to be significant. Due to an earlier termination of grain growth, the number

Table 3.5.4a

Effects of a brief period of heat stress on starch granule production in cv. RC-81-429 of wheat. Details are given in the legend to Fig. 3.5.1.

Days after anthesis (DAA)	Treatment	No. (in millions) of granules A-type	B-type	Total	Ratio of B-type to A-type
*10	Control	1.54	1.62	3.16	1.05
15	Control	4.79	6.40	11.19	1.35
	Warmed	4.82	6.50	11.32	1.35
Mean		4.81	6.45	11.26	1.35
20	Control	7.92	~~6.40~~ 17.20	~~14.32~~ 25.12	2.20
	Warmed	8.19	~~6.50~~ 20.10	~~14.69~~ 28.29	2.45
Mean		8.10	~~6.45~~ 18.70	~~14.55~~ 26.80	2.33
25	Control	8.17	~~17.20~~ 49.60	~~25.37~~ 57.77	6.06
	Warmed	8.53	~~20.10~~ 58.60	~~28.63~~ 67.13	6.86
Mean		8.35	~~18.70~~ 54.10	~~27.05~~ 62.45	6.46
30	Control	8.35	~~49.60~~ 88.34	~~57.95~~ 96.69	10.58
	Warmed	9.04	~~58.60~~ 91.30	~~67.64~~ 100.34	10.10
Mean		8.70	~~54.10~~ 89.96	~~62.80~~ 98.66	10.34
RIPE	Control	9.82	129.80	139.62	13.25
	Warmed	10.27	90.90	101.17	8.87
Mean		10.05	110.40	120.40	11.06
L.S.D. Interaction		n.s.	10.31	10.82	1.04

* Day 10 values not included in the analysis.

Table 3.5.4b

Effects of a brief period of heat stress on starch granule production in cv. Kite. Details are given in the legend to Fig. 3.5.1.

Days after anthesis (DAA)	Treatment	No. (in millions) of granules A-type	B-type	Total	Ratio of B-type to A-type
*10	Control	0.51	0.81	1.32	1.59
15	Control	4.27	5.10	9.30	1.23
	Warmed	4.49	7.00	11.50	1.57
Mean		4.38	6.05	10.40	1.40
20	Control	4.83	9.70	14.50	2.00
	Warmed	5.84	12.40	18.20	2.12
Mean		5.33	11.05	16.40	2.06
25	Control	7.99	37.80	45.80	4.82
	Warmed	7.39	47.60	55.00	6.51
Mean		7.69	42.70	50.40	5.66
30	Control	7.86	67.10	75.00	8.54
	Warmed	7.97	56.50	64.50	7.13
Mean		7.92	61.80	69.70	7.83
RIPE	Control	9.72	77.80	87.50	8.16
	Warmed	10.03	58.70	68.70	6.08
Mean		9.88	68.20	78.10	7.12
L.S.D. Interaction		n.s.	9.50	9.50	1.56

* Day 10 values not included in the analysis.

Table 3.5.4c

Effects of a brief period of heat stress on starch granule production in cv. Sonora. Details are given in the legend to Fig. 3.5.1.

Days after anthesis (DAA)	Treatment	No. (in millions) of granules A-type	B-type	Total	Ratio of B-type to A-type
*10	Control	3.09	2.49	5.58	0.89
15	Control	5.82	11.00	16.82	1.88
	Warmed	5.94	14.90	20.84	2.51
Mean		5.88	12.95	18.83	2.20
20	Control	6.37	16.50	22.87	2.61
	Warmed	6.28	22.30	28.58	3.54
Mean		6.32	19.40	25.72	3.07
25	Control	7.67	50.60	58.27	6.61
	Warmed	7.56	53.10	60.66	6.97
Mean		7.62	51.85	59.47	6.79
30	Control	8.68	61.90	70.58	7.15
	Warmed	8.34	57.20	65.54	6.87
Mean		8.51	59.55	68.06	7.01
RIPE	Control	10.53	60.20	70.73	5.70
	Warmed	9.99	50.80	60.79	5.10
Mean		10.26	55.50	65.76	5.40
L.S.D. DAA		0.47	7.43	7.70	0.76

* Day 10 values not included in the analysis.

Table 3.5.4d

Effects of a brief period of heat stress on starch granule production in cv. WW 15. Details are given in the legend to Fig. 3.5.1.

Days after anthesis (DAA)	Treatment	No. (in millions) of granules A-type	B-type	Total	Ratio of B-type to A-type
*10	Control	0.70	1.43	2.13	1.49
15	Control	5.14	4.19	9.33	0.84
	Warmed	5.94	10.77	16.71	1.84
Mean		5.54	7.48	13.02	1.33
20	Control	6.96	21.45	28.41	3.08
	Warmed	6.97	34.64	41.61	4.98
Mean		6.97	28.04	35.01	4.03
25	Control	7.76	63.26	71.02	8.16
	Warmed	7.74	51.57	59.31	6.63
Mean		7.75	57.41	65.16	7.40
30	Control	8.73	95.48	104.21	10.95
	Warmed	8.79	97.55	106.34	11.09
Mean		8.76	96.51	105.27	11.02
RIPE	Control	11.02	99.48	110.49	9.05
	Warmed	11.19	84.14	95.33	7.53
Mean		11.10	91.81	102.91	8.29
L.S.D. Interaction		n.s.	9.09	9.37	0.96

* Day 10 values not included in the analysis.

Fig. 3.5.5

Changes in total number of starch granules in the b grains taken from the four central spikelets of the ears from the control (●) and heat stressed (▲) plants of cv. RC-81-429 (a), cv. Kite (b), cv. Sonora (c), and cv. WW 15 (d). Details in the text. (Expt. 11)

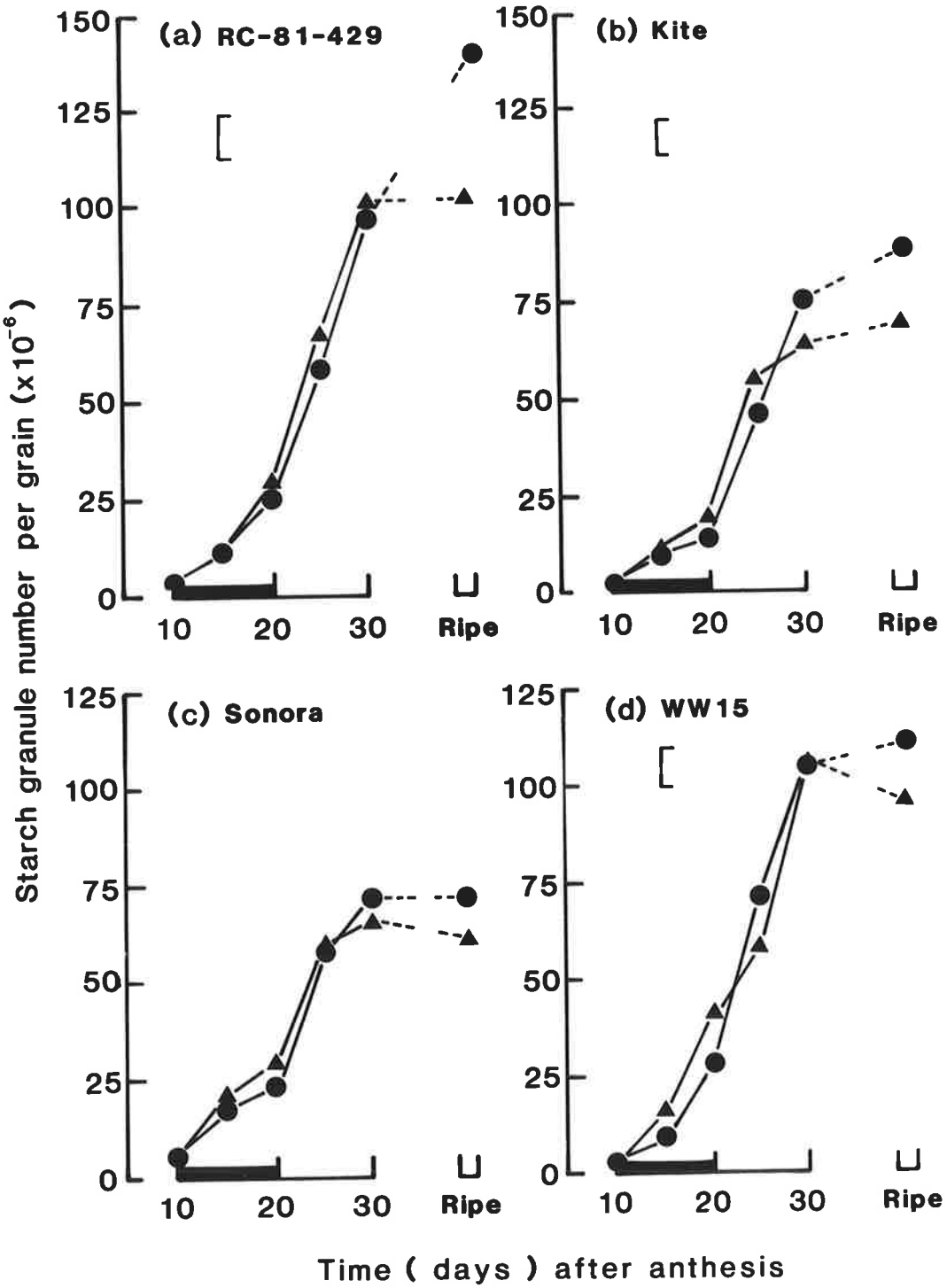

(a) RC-81-429
(b) Kite
(c) Sonora
(d) WW15
Starch granule number per grain (x10⁻⁶)
150
125
100
75
50
25
0
10
20
30
Ripe
Time (days) after anthesis

Table 3.5.5

Effects of a brief period of heat stress (33/25°C; between days 10 and 20 after anthesis) on final starch granule number in the b grain in four cultivars of wheat. (Expt. 11).

Cultivar	Treatment	No. (in millions) of granules per grain			Ratio of B-type to A-type
		A-type (>10 µm)	B-type (<10 µm)	Total	
RC-81-429	Control	9.82	129.8	137.9	13.3
	Warmed	10.27	90.9	101.2	8.9
Mean		10.04	110.4	120.4	11.2
Kite	Control	9.72	77.8	87.5	8.2
	Warmed	10.03	58.7	68.7	6.1
Mean		9.88	68.2	78.1	7.1
Sonora	Control	10.53	60.2	70.8	5.7
	Warmed	9.99	50.8	60.7	5.1
Mean		10.26	55.5	65.8	5.4
WW15	Control	11.01	99.5	110.5	9.1
	Warmed	11.19	84.1	95.3	7.5
Mean		11.10	91.8	102.9	8.3
Mean of cultivars					
	Control	10.27	91.8	102.1	9.0
	Warmed	10.37	71.1	81.5	6.9
Least significant differences					
Treatment		n.s.	7.7	8.1	0.8
Cultivar		n.s.	10.9	11.4	1.1

of B-type granules in the grains in the warmed plants was significantly reduced in all the cultivars (Table 3.5.5). The cultivars differed widely in the ratios of B-type to A-type granules both during the development of the grain and at maturity. The ratios of the B- to A-type granules increased both in control and heat stressed plants in all the cultivars until day 30 after anthesis, and thereafter seem to drop towards maturity.

Experiment 12

The intention of this experiment was to investigate the effects of a longer period of heat stress during the linear phase of grain filling at a relatively lower day/night (27/22°C) temperature differential, but keeping the total number of degree-days of warming (above ambient temperature, 21/16°C) about the same as in experiment 11. To achieve this goal plants were warmed at 27/22°C, between days 10 and 30 after anthesis, giving total heat stress of 120 degree-days as compared to 107.5 degree-days in experiment 11.

On average, the ears in this experiment were smaller, and had fewer and lighter grains in comparison with experiment 11 (compare Table 3.5.7 with Table 3.5.2), so, the total ear grain weight was about 80% of total ear grain weight in experiment 11. Smaller weight of the grains in this experiment seems to be due both to smaller rates and durations of dry matter accumulation in the grains (compare Fig. 3.5.6 with Fig. 3.5.1). In cv. RC-81-429 (Fig. 3.5.6a) and Kite (Fig. 3.5.6b) warming accelerated the rate of dry weight increase between days 10 and 20 after anthesis, but had the opposite effect during the following 10 days of grain growth. Therefore, the average rate of grain filling was depressed by warming in both the cultivars (Table 3.5.6) whereas in cvv. Sonora (Fig. 3.5.6c) and WW 15 (Fig. 3.5.6d) warming had no effect on the rates of dry matter accumulation during the period of heat stress. With the exception of cv. Kite (Fig. 3.5.6b) where there was no grain dry weight increase after day 30, grains in the control plants in the other cultivars continued to grow until day 35 after anthesis, but there was virtually no grain growth in the heat stressed plants in any of the cultivars after the warming period was over. Moreover, it is interesting to note that in cv. Kite (both in the control and heat stressed plants, Fig. 3.5.6b), and Sonora (in the heat stressed plants) there was a significant drop in grain dry weight after a maximum value had been obtained at about day 30.

Fig. 3.5.6

Changes in dry weight (closed symbols) and water content (open symbols) of the b grains taken from the four central spikelets of control (●, ○) and heat stressed (▲, △) plants of cv. RC-81-429 (a), cv. Kite (b), cv. Sonora (c), and cv. WW 15 (d). Plants were warmed, between days 10 and 30 after anthesis (indicated by solid horizontal bars), to 27/22°C and then transferred to control environment (21/16°C) where they started until maturity. Vertical bars represent interaction L.S.D. between times x treatments. (Expt. 12)

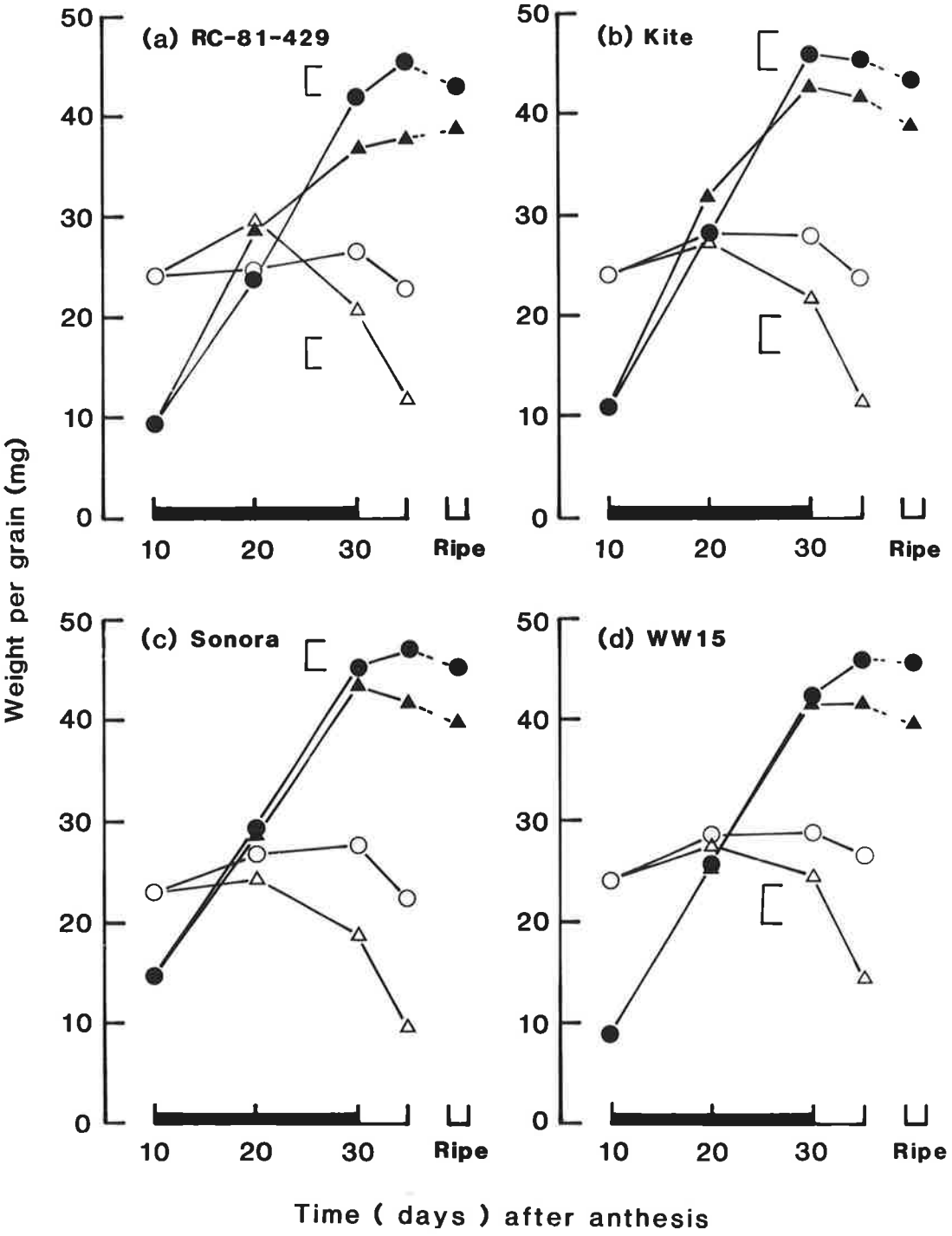

(a) RC-81-429
(b) Kite
(c) Sonora
(d) WW15
Weight per grain (mg)
0
10
20
30
40
50
10
20
30
Ripe
Time (days) after anthesis

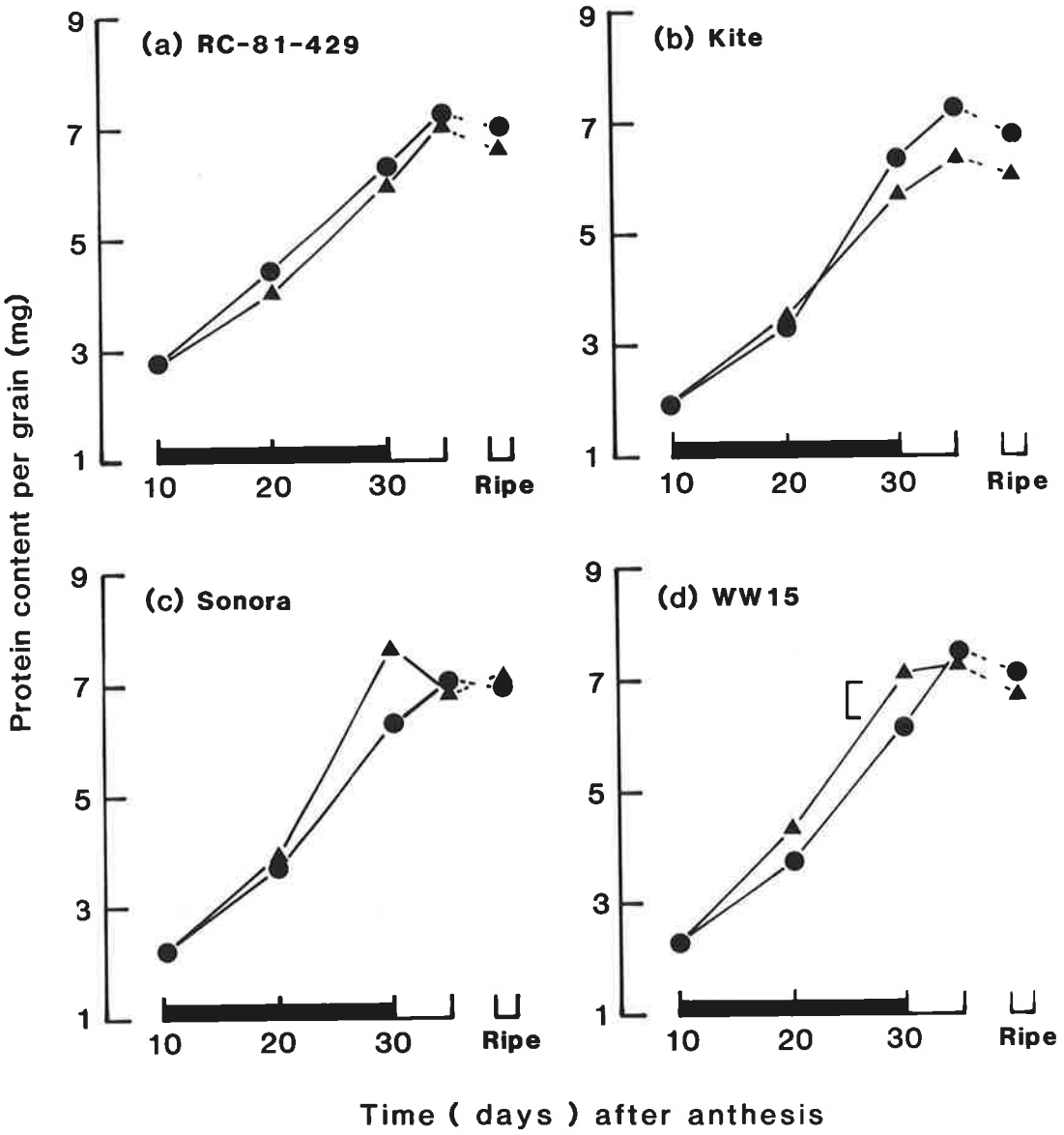
Table 3.5.6

Effects of whole plant warming on the rates of dry matter and protein accumulation (±S.E.), between days 10 and 30 after anthesis, in the grains of the four cultivars of wheat. Details of warming treatment are given in the legend to Fig. 3.5.6. Values in the parentheses are per cent of control values. (Expt. 12)

Cultivar	Treatment	Rate of increase of: Dry weight (mg grain^{-1}	Protein content day^{-1})
RC-81-429	Control	1.62±0.07	0.18±0.02
	Warmed	1.37±0.11	0.16±0.01
		(85)	(89)
Kite	Control	1.74±0.06	0.22±0.02
	Warmed	1.56±0.12	0.19±0.01
		(90)	(86)
Sonora	Control	1.50±0.08	0.20±0.02
	Warmed	1.41±0.07	0.28±0.02
		(94)	(140)
WW15	Control	1.66±0.08	0.19±0.02
	Warmed	1.65±0.07	0.24±0.02
		(99)	(126)

Fig. 3.5.7

Changes in protein content (N x 5.7) in the b grains taken from the four central spikelets of the ears from the control (●) and heat stressed (▲) plants of cv. RC-81-429 (a), cv. Kite (b), cv. Sonora (c), and cv. WW 15 (d). Details in legend to Fig. 3.5.6. (Expt. 12)


(a) RC-81-429
(b) Kite
(c) Sonora
(d) WW15
Protein content per grain (mg)
9
7
5
3
1
10
20
30
Ripe
Time (days) after anthesis

As recorded in experiment 11, at maturity total ear grain weight in this experiment was also reduced in the warmed plants in all the cultivars (Table 3.5.7), and these reductions were due to a reduction in dry weight at all the floret positions. The per cent. reduction in the weight of the b grain (compare Table 3.5.7 with Table 3.5.8) was consistently less than in floret c in all the cultivars.

The amounts of protein per grain in the control or the heat stressed plants in all the cultivars were the same, and were comparable to the values observed in experiment 11 (compare Tables 3.5.3 and 3.5.8). Warming had no significant effect on the concentration of protein in the grain in experiment 12.

It is apparent that the cultivars did not exhibit the same response, in terms of the rate and the duration of protein accumulation, to warming in the two experiments. For instance, warming the plants in experiment 11 accelerated the rate of protein accumulation in all the cultivars (Table 3.5.1) but such responses were observed only in cvv. Sonora and WW 15 (Table 3.5.6) in experiment 12. Furthermore, warming had no effect on the duration of protein accumulation in cvv. Sonora (Fig. 3.2.3c) and WW 15 (Fig. 3.5.3d) in experiment 11, but the duration in these cultivars was curtailed at least by 5 days in experiment 12.

Discussion

Warming the plants during the major period of grain filling decreased the final grain yield per ear by reducing individual grain weight. From the number of degree-days of warming imposed in the two experiments (120 degree-days in experiment 12 compared to 107.5 in experiment 11), it was expected that the reductions in total grain weight per ear and in individual grain weight would if anything be greater in experiment 12 compared to those in experiment 11. On the contrary, reductions both in total grain weight per ear and in individual grain weight in experiment 11 were greater than those observed in experiment 12 (compare Tables 3.5.2 and 3.5.7) and may be due to a higher temperature differential used in the former than in the latter experiment. Furthermore, in experiment 11 the reductions in total ear grain weight and individual grain weight in the warmed plants in cv. RC-81-429 were larger than in the other cultivars, but no differences were noticeable among the cultivars in experiment 12. So, it may indicate that cv. RC-81-429 may suffer relatively more than the others

TABLE 3.5.7

Effects of raising temperature to 27/22°C, between days 10 and 30 after anthesis, on components of grain yield at maturity in the four cultivars of wheat. Values in the parentheses are per cent reductions of the control values. (Expt. 12)

Cultivar	Treatment	No. per ear: Spikelets	No. per ear: Grains	Total ear grain weight (mg)	Single grain weight: Floret a (mg)	Single grain weight: Floret c (mg)
RC-81-429	Control	14.6	39.2	1463	40.5	36.0
	Warmed	14.6	38.2	1288 (-12.0)	36.1 (-10.9)	30.1 (-16.4)
Mean		14.6	38.7	1376	38.3	33.1
Kite	Control	15.0	40.0	1473	44.8	37.6
	Warmed	14.8	38.4	1264 (-14.2)	38.8 (-13.4)	30.3 (-19.4)
Mean		14.9	39.2	1368	41.8	34.0
Sonora	Control	14.8	40.0	1499	44.5	35.1
	Warmed	15.0	39.2	1256 (-16.2)	34.8 (-21.8)	27.3 (-22.2)
Mean		14.9	39.6	1377	39.6	31.2
WW15	Control	15.0	40.6	1524	45.0	35.8
	Warmed	14.4	40.6	1323 (-13.2)	39.3 (-12.7)	29.9 (-16.5)
Mean		14.7	40.6	1423	42.1	32.8
Mean of cultivars						
	Control	14.9	40.0	1490	43.7	36.1
	Warmed	14.7	39.1	1288 (-13.6)	37.3 (-14.6)	29.4 (-18.6)
				Least significant differences		
Treatment		n.s.	n.s.	74	2.0	2.0
Cultivar		n.s.	n.s.	n.s.	2.8	n.s.

Table 3.5.8

Effects of raising temperature to 27/22°C between days 10 and 30 after anthesis on final dry weight and protein content (N x 5.7) of the b grain (taken from the central four spikelets) in four cultivars of wheat values in parentheses are per cent reductions of control values. (Expt. 12)

Cultivar	Treatment	Dry weight (mg per grain)	Protein content	Per cent protein
RC-81-429	Control	42.0	7.1	16.8
	Warmed	40.2(-4.3)	6.7	16.8
Mean		41.1	6.9	16.8
Kite	Control	43.1	6.6	15.3
	Warmed	38.3(-11.1)	6.2	16.1
Mean		40.7	6.4	15.7
Sonora	Control	44.9	7.2	16.1
	Warmed	39.2(-12.7)	7.0	17.8
Mean		42.1	7.1	16.9
WW15	Control	45.3	6.9	15.2
	Warmed	39.4(-13.0)	6.7	16.9
Mean		42.3	6.8	16.1
Mean of cultivars	Control	43.8	6.9	15.9
	Warmed	39.3(-10.3)	6.6	16.9
		Least significant differences		
Treatment		1.6	n.s.	n.s.
Cultivar		n.s.	n.s.	n.s.

at high temperatures. Cultivars also differed in their response to elevated temperature, in terms of starch and protein accumulation both within and between the experiments. Most importantly, in experiment 11, the rate of dry matter accumulation was enhanced at high temperature in all the cultivars (although to a different extent, Table 3.5.1), but this effect was observed only in cvv. RC-81-429 (Fig. 3.5.6a) and Kite (Fig. 3.5.6b) and then only between days 10 and 20 after anthesis. Similarly the enhancing effect of warming on protein deposition was observable in all the cultivars in experiment 11 (Fig. 3.5.3 and Table 3.5.1) but only in cvv. Sonora (Fig. 3.5.7c) and WW15 (Fig. 3.5.7d) in experiment 12. However, in these cultivars there was no effect of warming on the rate of dry matter accumulation (Figs. 3.5.6c and 3.5.6d). As the acceleration in protein deposition was greater than that of starch in almost all cases (except in cvv. RC-81-429 and Kite in experiment 12) it is reasonable to conclude that protein synthesis is enhanced relatively more than starch synthesis as a result of elevation in temperature during grain growth (Campbell and Read 1968; Spiertz 1977).

It is frequently reported that percentage of protein increases at high temperature during grain growth (Sosulski et al. 1963; Kolderup 1975; Campbell and Davidson 1979; Campbell et al. 1981), but as protein content per grain is seldom given, it is difficult to judge whether the effects of temperature on protein percentage is accompanied by an effect on protein quantity *per se* or due solely to the smaller starch content in the heat stressed grains. From Tables 3.5.3 and 3.5.8 it is clear that the increase in per cent protein, in all cultivars in both the experiments, as a result of high temperature is almost entirely due to a reduction in starch content of the grain rather than the change in protein quantity. A smaller effect or no effect, of heat stress on protein content, in any of the cultivars, is a conclusion primarily supported by the fact that warming had little or no effect on the duration of protein accumulation (Figs. 3.5.3 and 3.5.7), contrary to its effect on the duration of dry matter (mainly starch) accumulation (Figs. 3.5.1 and 3.5.6). However, in instances (Figs. 3.5.3a, 3.5.7c and 3.5.7d) where heat stress seems to have curtailed the duration of protein accumulation, enhanced rate of protein synthesis at elevated temperature (Tables 3.5.1 and 3.5.6) seems to have compensated for the shorter duration. Barlow and co-workers (1983), and also Campbell et al. (1981), have shown that starch and protein storage in the grain are

independent of each other. There are also indications in many reports that protein and starch accumulation seem to be asynchronous (i.e. protein appears to accumulate for a longer period) both under normal conditions (Thatcher 1915 cited by Jenner 1982a; Sofield 1974, 1977b; Dalling et al. 1976; Waters et al. 1980) and under conditions of water stress (Brooks et al. 1982). Acceleration in the rate of protein deposition as a result of elevation in temperature may be explained either by enhanced remobilization of nitrogen from the rest of the plant (enhanced senescence) and/or to more direct effects on the rate of conversion of amino acids to protein. How protein continues to accumulate for longer periods than does starch is open to speculation however. Whatever the physiological reasons for the above responses, it is evident that starch and protein accumulation in the grain have different susceptibilities to brief periods of heat stress during grain filling.

Broadly, the changes in the A- and B-type granules during the development of the grain are almost identical for all the cultivars and are in agreement with the reports in the literature (Section 1.4.2.1.2) that the A-type granules are initiated during the early stages of grain development and continue to grow throughout grain growth (Tables 3.5.4a-d) whereas the B- granules are initiated later in the development and increase in number very rapidly. The cultivars differed significantly in numbers of B-type granules without any significant differences in numbers of A-type granules, hence the ratios of the numbers of B- to A-type granules differed significantly among the cultivars at maturity (Table 3.5.5). These ratios, however, were poorly related to apparent amount of starch or dry weight per grain and is in agreement with the observations made by Gleadow and co-workers (1982).

The contribution of B-type granules towards total weight of starch in the grain has been evaluated by using a number of techniques (sedimentation, microsieving, Coulter counter analysis, Quantimet image analysis, and stereological analysis) by a number of workers (Evers 1973; Briarty and Hughes 1976; Brocklehurst and Evers 1977; Evers and Lindley 1977; Meredith 1981) and has been shown to be between 30 to 50% for a number of cultivars. Assuming that the contribution of B-type granules to the weight of starch in all the cultivars was the same and was 30%, the reductions in the weight of starch as a result of heat stress attributable to the reductions in numbers of B-type granules can be estimated and are given in the following tabulation.

Cultivar	Reduction in weight of starch per grain (mg)	Per cent reduction in B-granule number	Reduction attributable to reduction in no. of B-granules (mg)
RC-81-429	8.1	30.0	3.9
Kite	7.1	24.6	2.9
Sonora	6.8	15.6	2.1
WW15	5.7	15.5	2.1

It is clear from the above tabulation that reduction in the number of B-type granules accounts only from 30.9 to 48.1% of the observed reductions. Therefore, the balance of the reduction is either due to a lesser weight contribution by existing B-granules or to a reduction in the weight contributed by A-type granules (also see Hoshikawa 1962).

It is well known that amylose content of the grain increases with maturity (Matheson 1971; Kulp 1973; Duffus and Murdoch 1979). So, in the grains where the duration of starch accumulation is curtailed as a result of heat - or water-stress are likely to have a higher percentage of amylopectin. As the percentage amylopectin in the flour is linearly related to the paste viscosity (Moss and Miskelly 1984), an important factor governing the eating quality of udon, or Japanese white noodles (Moss 1979) it is tempting to speculate that wheat crops grown under moderately limited water supply and/or moderately high temperatures, although they may yield poorly, are likely to have flour of better bread making quality and have higher potential for the manufacture of Japanese white noodles.

3.6 Effect of temperature on some metabolic processes of grains cultured *in vitro*

Introduction

Grain growth and development in wheat is regulated by complex interactions : between the maternal plant and the environment ; between the developing grain and the maternal plant, and among the different parts of the grain itself. With the developing grain attached to the plant, it is difficult to study the effects of environmental factors on the metabolic processes of the grain in isolation from the rest of the plant. As changes in temperature would inevitably affect all physiological and biochemical processes of the whole plant, the effects of temperature on grain growth and development observed in previous sections may either be confounded with, or even masked by indirect effects of temperature on the other parts of the plant. So, to study the effects of temperature on the metabolic processes of the grain itself, in isolation from the other parts of the plant, a different technique than warming the whole plant or warming the ears is needed.

In vitro tissue culture techniques, for maize kernels, have been used to study the effects of temperature on carbohydrate metabolism (Jones et al. 1981, 1984), and the effects of sucrose supply on the black layer formation (Afuakwa et al. 1984). Similarly, liquid culture of wheat grains from a few hours duration (Jener 1974a, 1974b ; Jenner and Rathjen 1975, 1977 ; Radley 1978, 1981 ; also see Section 3.3) to a number of days (Gifford and Bremner 1981a, 1981b) has been used to investigate the processes related to sucrose transport and its conversion into starch. However, in all reports related to wheat, grains were cultured either at 20 or 25°C and no attempts have been made to examine the effects of temperature on sucrose uptake or its conversion into starch. Hence the main objective of the experiments in this section was to study the effects of temperature on the processes of sucrose uptake and its conversion into starch, and also to determine the effect of temperature on the respiratory activity of the *in vitro* cultured grains.

Materials and Methods

Batches of plants, in succession (at weekly intervals), were raised in the glass house for two weeks and then transferred to the controlled environment growth room (Section 2.2.2). The ears were marked

individually with the date when the first anthers exserted on an ear and all subsequent operations were performed with reference to this date.

Grains between days 15 and 21 after anthesis were used in all the experiments reported in this section, and the exact age of the grains is given in the legend to a figure or a table. Other procedures for various analyses are outlined in Section 2 or in the text to each experiment.

Results

Sucrose during its transport from the phloem of the vascular strand in the furrow of the grain to the site of starch accumulation (endosperm cells) passes through the endosperm cavity and intercellular space (Section 1.8). It has been reported by the earlier workers (Jenner 1968; Gifford and Bremner 1981a) that intact grains isolated from the plant when placed on solutions of sucrose do not accumulate starch (because of the limited permeability of the pericarp), and some degree of manipulation of the grain to expose its apoplast (endosperm cavity and/or intercellular space) to the culture medium is necessary for the grain to absorb and metabolise sucrose. Therefore, the first few experiments were designed to characterize the apoplast of the grain.

Determination of endosperm cavity sap composition

Collection of the sap

The centrifugation method of Ho and Gifford (1984) was used, with some modifications, for collection of endosperm cavity sap. The collection assembly and a part of the procedure employed for sap extraction was described in Section 2.7.1.

In preliminary work it was found that centrifugation at 5000 rpm was insufficient to collect the sap consistently and spinning at 9000 rpm resulted in structural damage to the cut surface of the grains, hence in further experimentation centrifugation at 7000 rpm (4100 x g, r_{min}) was used for collection of endosperm cavity sap. At this speed, although there were no visible signs of structural damage to the cut surface of the grain, some starch granules (10-50) were detected in some samples and these starch granules presumably were from the cut cells. Further, it was also experienced that the grains younger than 15 days were structurally too weak to withstand this centrifugal force, and from the grains older than 20 days an inadequate amount of sap for sugar

analysis was collected. So, 15-18 days old grains were best suited for this purpose.

A major proportion of the sap was extracted in the first 5 min of centrifugation, but significant though small amounts of the sap were collected from the same set of grains at subsequent spins (Table 3.6.1). The concentration of total sugars (determined by the anthrone method after filtration of the diluted sap through Whatman GF/A filter) in the sap declined almost linearly with each successive spin. However, when the grains were spun firstly for 25 min and then the same set of grains for two successive spins of 5 min each, the concentration of total sugars was significantly reduced in the latter samples (Table 3.6.1). From the above results it seems reasonable to assume that the sap collected in the first 25 min of spinning was from one compartment of the grain (i.e. endosperm cavity) but does not necessarily exclude the possibility that there was contamination of the sap from the other parts of the grain (e.g. intercellular free space). Therefore, a shorter spinning time (15 min) in which the sap is most likely to come only from the endosperm cavity was used to spin the sap for compositional analysis.

Composition of endosperm cavity sap

Using the procedure outlined earlier, it was possible to collect 1.01 µl (specific gravity at 21°C was 1.01) of sap (mean of 6 samples each of 12 grains) from 15-16 days old b grains (from the central 10 spikelets of the ear). Immediately after the collection of the sap, each sample was diluted, by adding 40 µl of 80% (v/v) ethanol and stored at -16°C until analysed by the anthrone method and other more specific methods outlined in Section 2.6.1.2. The partial composition of the sap is given in the tabulation (values presented as mean ± S.E.)

Of the three samples measured sucrose was present in greatest amount, followed by fructose and a relatively small amount of glucose. However, the sum of the amounts of sucrose, glucose and fructose accounted for only 76.5% of the amount of all sugars in the sap as determined by anthrone method. On the molarity basis, however, the sap had the greatest concentration of fructose, followed by sucrose and least of glucose.

Table 3.6.1

Changes in the amount of total sugars (anthrone positive material) in the endosperm cavity sap with the time of spinning.

Time intervals (min)	Amount of cavity sap extracted per grain (mg)	Cumulative amount of the cavity sap per grain (mg)	Total sugars (µg per mg sap)
Set I			
0 - 5	0.50	0.50	60.0
5 - 10	0.22	0.72	53.0
10 - 15	0.15	0.87	51.1
15 - 20	0.16	1.03	49.6
20 - 25	0.12	1.15	47.7
Set 2			
0 - 25	1.04	1.04	60.8
25 - 30	0.18	1.22	32.4
30 - 35	0.13	1.35	23.0

Concn expressed as	Total sugars (Anthrone + ve)	Sucrose	*Glucose	**Fructose
mg mg^{-1} sap	64.7±1.9	26.3±1.9	4.5±0.5	18.7±0.9
mg μl^{-1} sap	64.1±1.9	26.0±1.9	4.5±0.5	18.5±0.9
m Molar	-	76.0	25.0	102.7
Per cent of total sugars	-	40.6	7.0	28.7

* may contain glucose-6-phosphate.
** may contain fructose-6-phosphate.

Osmotic potential of the sap

Osmolality of the undiluted sap was determined by using a Wescor vapour pressure osmometer (Model 5100C) using KCl solutions as standards (range 200 to 1000 mosmolal) at 25°C. As the volume of the sap per sample was small (8-12 µl), two samples were combined for one reading (which required 8 µl of solution). The osmotic concentration of the sap was between 399 to 481 mosmolal with a mean value of 446.3 ±24.5. Osmotic potential of the sap was calculated from freezing point depression values for the same osmolal solution of sucrose using the equation given by Michel (1972), or by using the relationship of concentration to osmotic potential for sucrose or NaCl solutions given by Slavik (1974). The conversions needed to obtain mosmol concentrations from mMolar (for sucrose solution) or mMolal (for NaCl solution) were calculated by using the tables from the CRC Handbook of Physics and Chemistry (60th Edition, 1979-1980). As expected, there was no difference in the osmotic potential calculated by either method, and was between -1.0 and -1.2 MPa with a mean value of -1.1 ±0.06 MPa.

Apparent free space volume

The volume of the intercellular free space (Briggs et al. 1961) was determined from the uptake of ^{14}C-PEG 4000 by the dissected grains (details in Section 2.7.3). The pattern of ^{14}C-PEG uptake by the grain is shown in Fig. 3.6.1. Uptake was fast during the first 4 h of incubation and then continued at a slower rate until 24 h from the beginning of incubation. This pattern of ^{14}C-PEG uptake was not

Fig. 3.6.1

Uptake of ^{14}C-PEG 4000 over 24 h by 20 days old wheat grains. Grains were incubated individually with 150 µl of osmotically adjusted sucrose solution [-0.82 MPa, containing 20 gl^{-1} (59.4 mMolal) sucrose]. The amount of label taken into a grain at each time was converted into the equivalent volume of bathing solution using the known radioactivity (538 dpm μl^{-1}). Each point is mean of 5 replicate grains.

Fig. 3.6.2

Time-course of ^{14}C-PEG 4000 exodiffusion into osmotically adjusted sucrose solution [-0.82 MPa, containing 20 gl^{-1} (59.4 mMolal) sucrose] from the preloaded wheat grains (as in Fig. 3.6.1) for 1 (●), 6 (■), or 24 h (▲). Details in the text. Each point is the mean of five replicate grains.

Fig.3.6.1

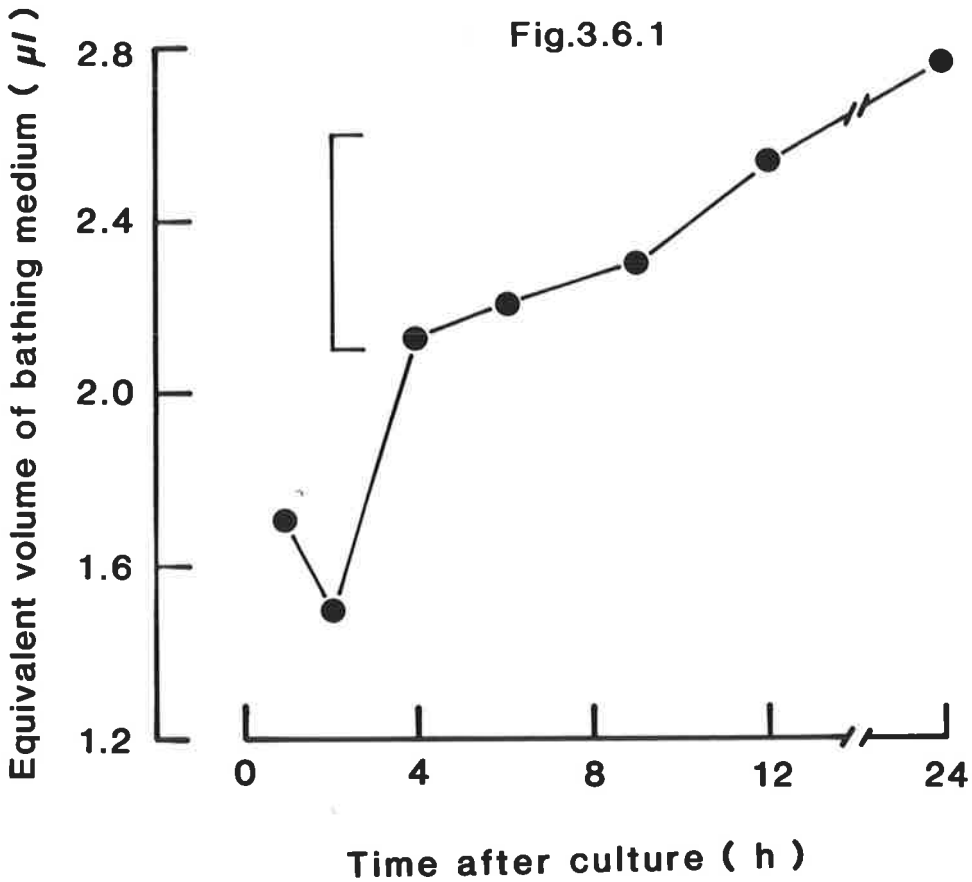
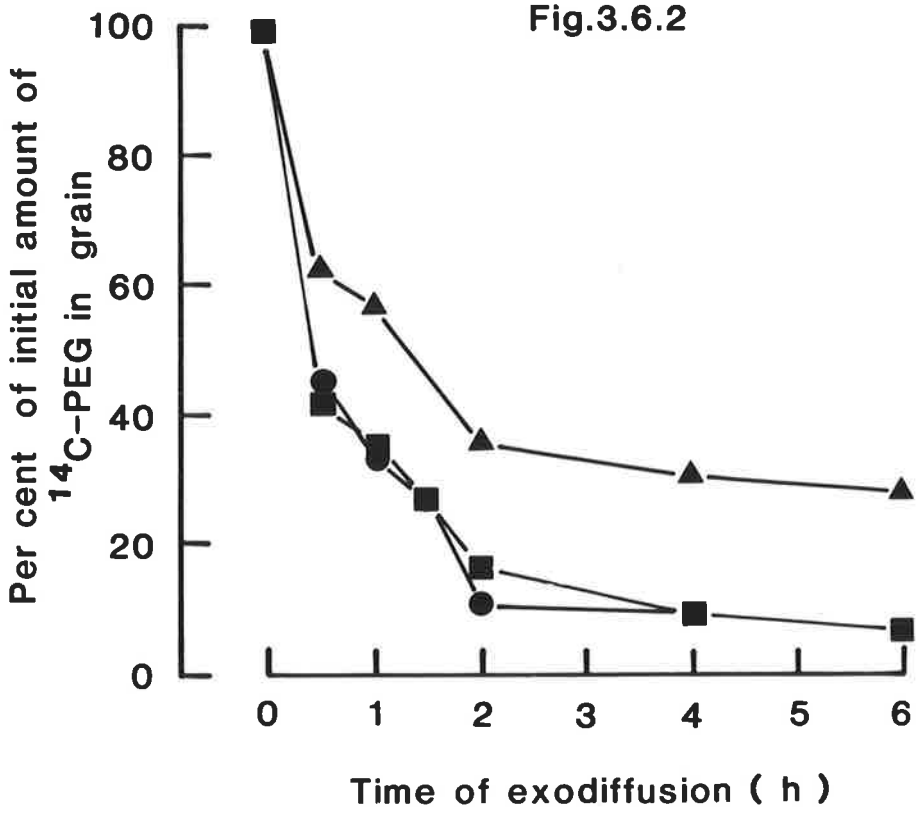


Fig.3.6.2



expected, as PEG used for this purpose had a high molecular weight and should not have permeated the cells and should not have been metabolised nearly as rapidly as sucrose (Lawlor 1970). The pattern may reflect that either the intercellular free space of the grain did not equilibrate with the medium even in 24 h of incubation, or ^{14}C-PEG was being absorbed into the cells and/or adsorbed on the cell walls.

To test the above possibilities, the dissected grains were pre-loaded with ^{14}C-PEG either for 1, 6 or 24 h, washed for 10-20 s in a large volume of water, and allowed to exodiffuse in 2 ml of 20 gl^{-1} (59.4 mMolal) sucrose (osmotically adjusted to -0.82 MPa with non-radioactive PEG) solution at about 4°C. The grains were transferred to the fresh solution every 30 min for the first 2 h and then every hour until 6 h. The amount of ^{14}C-PEG left in the grains at various times after the beginning of the exodiffusion was determined by the procedure given in Section 2.10.2. The grains which were preloaded for 1 or 6 h, lost about 90% of their label in the first 2 h of washing whereas the grains which were preloaded for 24 h lost the label slowly during the first 2 h of washing and retained 28.3% of the initial label even after 6 h of exodiffusion (Fig. 3.6.2). From the above results it is evident that the volume of the intercellular free space, if estimated from the grains which were allowed to take up ^{14}C-PEG for 24 h, would give an over estimate of apparent free space volume by 28.3%. So to determine the apparent free space volume of the grain more accurately, either it can be calculated from the difference in the volume of grain occupied by ^{14}C-PEG at the beginning and at the end of 2 h exodiffusion from grains preloaded for 6 or 24 h, or from the intercept of the linear regression equation over the period of 4 to 24 h incubation (Fig. 3.6.1). Between 4 and 24 h, the ^{14}C-PEG uptake by the grain can be described by the linear regression equation $Y = 2.03 + 0.032x$ ($r = 0.962$) which gives a value for the apparent free space volume of 2.0 µl which is the same as calculated by the difference method (2.0 and 1.9 µl for 6 and 24 h preloaded grains respectively).

Amounts and concentrations of total soluble sugars and sucrose in various parts of the grain

The amounts of total soluble sugars and sucrose in various parts of 20 day-old grains are presented in Table 3.6.2. The endosperm had higher amounts of both total soluble sugars and sucrose than in the crease tissue, or in the apparent free space. However, when these amounts were

Table 3.6.2

Amounts and concentrations of total soluble sugars and sucrose in some parts of 20 day-old wheat grain. Five b grains from the central part of the ear from one side of the ear were used for fresh weight and dry weight determinations, and five corresponding b grains from the other side of the ear were used for sugar analysis. Values in parentheses are concentration of sugars (gl^{-1} of tissue water).

Grain part	Fresh weight (mg)	Dry weight (mg)	Water content (µl)	Total soluble sugars (µg/grain part)	Sucrose
Outer pericarp	4.2 ±0.5	1.4 ±0.2	2.8 ±0.4	197 ±18 (70.4)	57 ±5 (20.4)
Crease tissue	1.69±0.15	0.49±0.04	1.20±0.12	103 ±6 (85.8)	81 ±9 (67.5)
*Endosperm cavity sap	-	-	-	64.7±1.9 (64.1)	26.3±1.9 (26.0)
Apparent free space	-	-	-	**124 ±6 (62.0)	**91 ±3 (45.5)
Endosperm with chlorophyllous layer (in cells)	49.9±3.3	23.2±1.2	26.7±2.2	633 ±19 (23.7)	268 ±15 (10.0)

* Value for 15-16 day old grains, estimated in the previous experiment

** Values calculated by dividing the total amounts in apparent free space by the estimated volume of apparent free space.

expressed on a tissue water content basis, the crease tissue had higher concentrations of both total soluble sugars and sucrose than those in the endosperm. The proportion of total soluble sugars as sucrose differed among the tissues, the crease tissue and the apparent free space having a higher proportion of sucrose (78.6 and 73.4% respectively) as compared to the endosperm (42.3%).

Liquid culture of isolated grains

In the first three experiments, the main aim was to study the effect of various manipulations on the uptake of ^{14}C-sucrose and its incorporation into starch, and also to study the effect of the presence or absence of the embryo on the above processes. For convenience, 10% sucrose solution which has a osmotic potential of -0.82 MPa (close to osmotic potential of endosperm cavity sap) was used.

In the first experiment, grains without the outer pericarp and the crease tissue (referred to as depericarped grains from now on), with or without the embryo, or sliced into two halves in the long-axis or in the short-axis of the grain were cultured with 50 µl of ^{14}C-sucrose for 10 h (Method I - Section 2.8.2.1). At the end of incubation the grains or halved grains were washed in a large volume of deionised water for 10-20 s, and the embryos were picked out with the tip of the needle and discarded. The amounts of ^{14}C in ethanol soluble and insoluble fractions are given in Table 3.6.3. It is evident that grain cultured with an embryo had more ^{14}C in the ethanol soluble fraction but there was no effect of the removal of the embryo on the incorporation of ^{14}C into the insoluble fraction. Slicing the grain transversely resulted in a higher amount of ^{14}C in the soluble fraction but slicing longitudinally had the opposite effect. However, in both cases incorporation of ^{14}C into insoluble fraction was reduced.

In the first experiment (Table 3.6.3), a partially sterile culture system was used (glass-ware and sucrose solution were pre-sterilized but grains were cultured in the laboratory without any surface sterilization). To compare culturing by the above method with culturing the grains in laminar flow cabinet under completely sterile conditions, ten depericarped grains without embryo were cultured individually (as described earlier) in the laboratory and a comparable set of 10 grains (surface sterilized for 30-60 s in NaOCl solution, 1% available Cl, before dissection) were cultured in the laminar flow cabinet. Grains cultured in the laboratory were transferred to the laminar flow cabinet

Table 3.6.3

Effect of grain manipulation, and presence or absence of the embryo on the uptake of radioactive sucrose (100 gl^{-1} ; specific activity 10.73 $\mu Ci\ g^{-1}$ sucrose) and its conversion into ethanol-insoluble material during a 10 h period by 20 days old wheat grains.

Grain manipulation	With or without embryo	Incorporation of ^{14}C[mg sucrose equiv. $grain^{-1}$] into: Soluble pool	Insoluble fraction	Total
1. Grain without outer pericarp and the crease tissue	with	0.87	0.24	1.11
	without	0.64	0.25	0.89
	mean	0.76	0.25	1.00
2. As for (1) sliced longitudinally	with	0.76	0.14	0.90
	without	0.61	0.20	0.80
	mean	0.68	0.17	0.85
3. As for (1) but grain sliced transversely	with	0.84	0.21	1.04
	without	0.94	0.17	1.12
	mean	0.89	0.19	1.08
Overall means	with	0.82	0.20	1.02
	without	0.73	0.21	0.94
Least significant difference				
Grain manipulation		0.09	0.05	0.11
With or without embryo		0.08	n.s.	n.s.
Interaction		0.13	n.s.	0.16

to avoid the differences in temperature (temperature in the laminar flow cabinet 23-25°C). After 24 h of culture, grains were analysed for ^{14}C in the ethanol soluble fraction, in starch, and in the insoluble residue, and the values are given in the following tabulation.

Grains cultured in	Incorporation of ^{14}C[mg sucrose equiv. grain^{-1} day^{-1}] into:			
	Soluble fraction	Starch	Residue	Total
Laminar flow cabinet-sterile conditions	0.901	1.067	0.125	2.093
Laboratory-partly sterile conditions	0.979	0.869	0.087	1.986
L.S.D.	n.s.	n.s.	n.s.	n.s.

Partitioning of ^{14}C-sucrose into ethanol soluble, starch and insoluble residue fractions was comparable in both types of culture, therefore, in the following two experiments grains were cultured in the laboratory.

Although the effects of the embryo on uptake of ^{14}C-sucrose and its conversion into insoluble material were examined in the first experiment, no attempt was made to analyse separately the amounts of ^{14}C present in starch and in the other insoluble material. Moreover, the embryo at the end of incubation period was discarded. Accordingly the aim of the next experiment was to determine the partitioning of ^{14}C into various fractions of endosperm and embryo over a longer period of grain culture (24 h). The results are presented in Table 3.6.4. Grains with the embryo in place had less ^{14}C in the soluble fraction and also synthesized less radioactive starch than the grains where the embryo had been removed. Grains cut transversely at the base to expose its endosperm cavity to the culture medium absorbed appreciably smaller amounts of sucrose and converted only about one fourth of the total amount of sucrose absorbed into starch. Grains with embryos, sliced longitudinally into two halves had 38% more sucrose than comparable intact grains (compare treatments 3 and 1, Table 3.6.4) yet these grains synthesized only 78.4% of the amount of starch synthesized by the

Table 3.6.4

Effect of grain manipulation on the uptake of uniformally labelled sucrose (100 gl^{-1} ; sp. radioactivity 23.65 µCi g^{-1} sucrose) and its conversion into starch and other ethanol-insoluble material during a 24 h period by 21 days old wheat grains. Values in parentheses are per cent amount of radioactive starch of the total ^{14}C-sucrose absorbed by the grain.

Grain manipulation	Incorporation of ^{14}C [mg sucrose equiv. $grain^{-1}$ day^{-1}] into: ETOH-soluble pool	Starch	Insoluble residue	Total
1. Grain without outer pericarp and tissue with embryo	0.61	0.74 (51.7)	0.08	1.43
2. As for (1) but without embryo	0.70	0.81 (50.6)	0.09	1.60
3. As for (1) but sliced longitudinally	0.84	0.58 (39.2)	0.07	1.48
4. Intact grain freshly cut at the base to expose the endosperm cavity	0.42	0.16 (25.4)	0.05	0.63
L.S.D.	0.11	0.19	n.s.	0.22

unsliced grains. The embryo absorbed 102 µg (sucrose equiv.) of sucrose in 24 h and converted about one fifth of this amount into insoluble material.

In conclusion, the results in Table 3.6.4 confirm the earlier observations (Table 3.6.3) that depericarped grain without embryo gives the best results over short-term grain culture and was used in the subsequent experiments.

Substrate response and the effect of PEG 4000 inclusion in the medium

Use of 50 µl of 10% sucrose solution was presumably sufficient to support grain growth for 10 to 24 h in the earlier experiments, but the use of such a low volume of solution for low concentrations of sucrose is likely to result in a depletion of substrate for grain growth. However, incubation in a much greater volume of the solution was likely to result in anaerobic conditions for the grain. To avoid these problems, grains in this experiment were cultured with 50 µl of solutions of ^{14}C-sucrose or on ^{14}C-sucrose solutions osmotically adjusted to -0.82 MPa with the addition of PEG 4000 (details for the preparation of the solutions are given in Section 2.7.2) as in the earlier experiments, but were transferred to fresh solutions every 4 h during the first 12 h of culture and then allowed to stay in the same solutions over the next 12 h of culture. At the end of the culture period the grains were washed to remove apparent free space sugars (Section 2.9) and the amounts of ^{14}C in the ethanol soluble fraction (in the cells) and in the insoluble material were determined as described earlier (Section 2.10.1 and 2.10.2). Data for the grains cultured on solutions of sucrose, and for the grains cultured on osmotically adjusted sucrose solutions were analysed as a whole (Table 3.6.5) and separately for each type of culture medium (Fig. 3.6.3).

The total uptake of sucrose by the grains showed a curvilinear response to concentration of sucrose in both types of solutions (Fig. 3.6.3). The amount of the label in insoluble material increased with an increase in the external concentration of sucrose up to about 200 mMolal and levelled off above this concentration. By contrast incorporation of ^{14}C into the soluble fraction in the cells (presumably sugars and amino acids) increased almost linearly with increase in sucrose concentration in the medium. Addition of PEG 4000 to adjust Ψ_π the external solution close to the values determined for endosperm cavity sap, overall had no

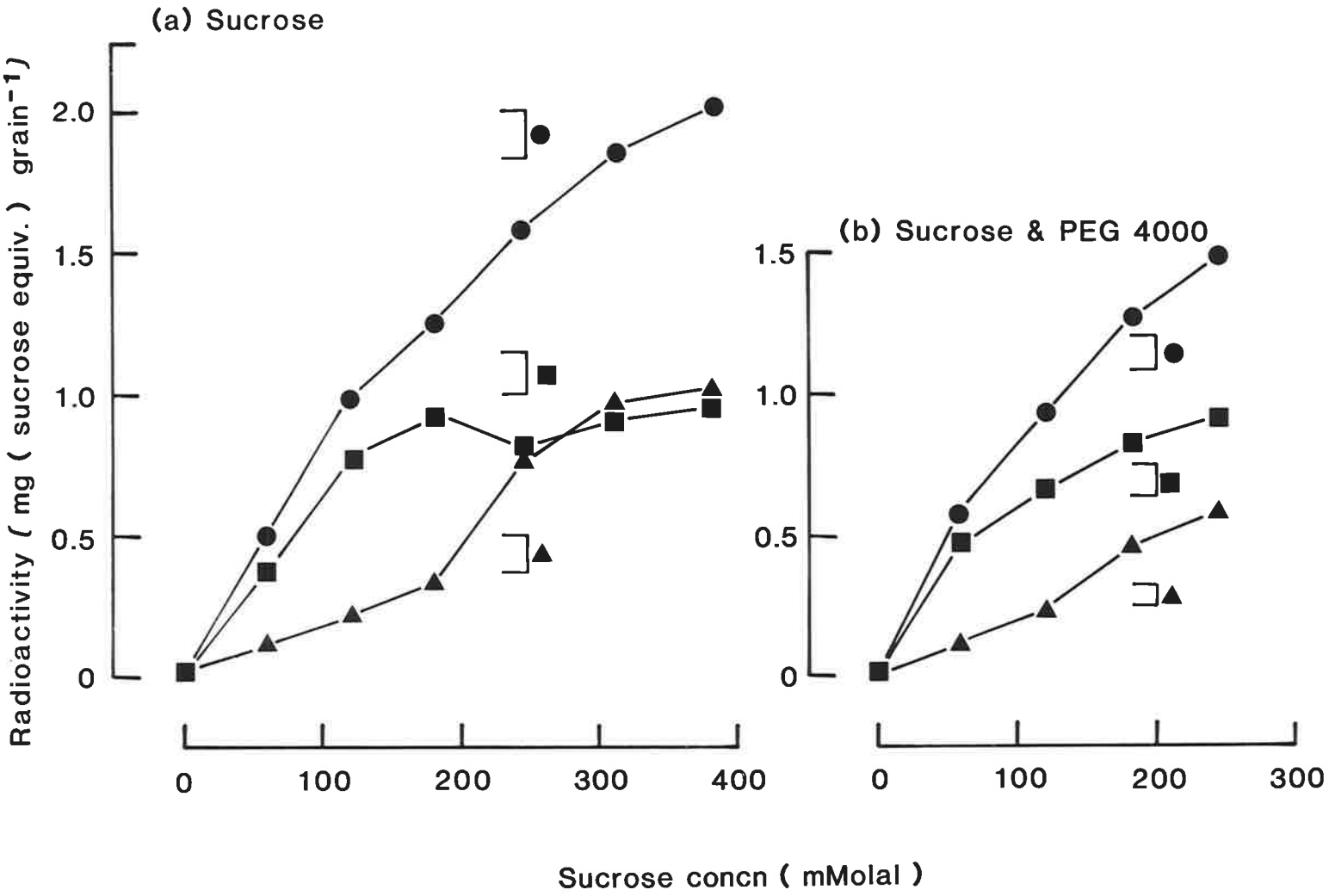
Table 3.6.5

Effect of the addition of PEG 4000 to sucrose solutions (to adjust osmotic potential to -0.82 MPa) on ^{14}C-sucrose uptake and its incorporation into ethanol-insoluble material in 24 h culture by 20 days old grains. (Details in the legend to Fig. 3.6.3)

Sucrose concn (gl^{-1} water) (mMolal in parentheses)	with or without PEG 4000	Incorporation of ^{14}C-sucrose [mg sucrose equiv. $grain^{-1}$] into: Soluble fraction (in cells)	Insoluble fraction	Total
0	without	0.007	0.022	0.029
(0)	with	0.006	0.020	0.026
	mean	0.007	0.021	0.028
20	without	0.115	0.371	0.486
(59.4)	with	0.109	0.464	0.572
	mean	0.112	0.417	0.529
40	without	0.222	0.775	0.997
(120.2)	with	0.228	0.707	0.935
	mean	0.225	0.741	0.966
60	without	0.331	0.915	1.246
(182.7)	with	0.455	0.828	1.283
	mean	0.393	0.872	1.265
80	without	0.773	0.809	1.582
(246.8)	with	0.571	0.916	1.487
	mean	0.672	0.862	1.534
	Least Significant Differences			
Concn		0.053	0.085	0.079
Interaction		0.075	n.s.	n.s.

Fig. 3.6.3

Uptake of radioactive sucrose from solutions of ^{14}C-sucrose (a), or from solutions of ^{14}C-sucrose osmotically adjusted to -0.82 MPa by addition of PEG 4000 (b) in 24 h by wheat grains (20 days after anthesis). Amount of radioactivity in total (●), in ethanol soluble fraction (in cells) (▲), and incorporated into insoluble fraction (■). Each point is mean of 5 replicate grains. Details in text.


(a) Sucrose
(b) Sucrose & PEG 4000
Radioactivity (mg (sucrose equiv.) grain^{-1})
2.0
1.5
1.0
0.5
0
0
100
200
300
400
Sucrose concn (mMolal)

effect on the total uptake of sucrose, or its incorporation into the insoluble fraction (Table 3.6.5).

Measurement of $^{14}CO_2$ released by the cultured grains

The amount of $^{14}CO_2$ released by the grains cultured on ^{14}C-sucrose was determined by absorbing CO_2 continuously onto a concertina of paper saturated with KOH (KOH trap) over the grain culture period. The description of the grain culture assembly, the method of grain culture (Method II) and procedures for processing the samples to determine the amount of radioactivity are given in Sections 2.8.2.2, 2.9 and 2.10. The amount of 30 gl^{-1} KOH solution (75 µl) used to soak the concertina of paper was 7–8 times more than the amount of KOH required to absorb the amount of CO_2 expected to be released by the grain, and the assembly contained about 3 times the amount of oxygen required for the respiration of the grain.

Efficiency of KOH trap

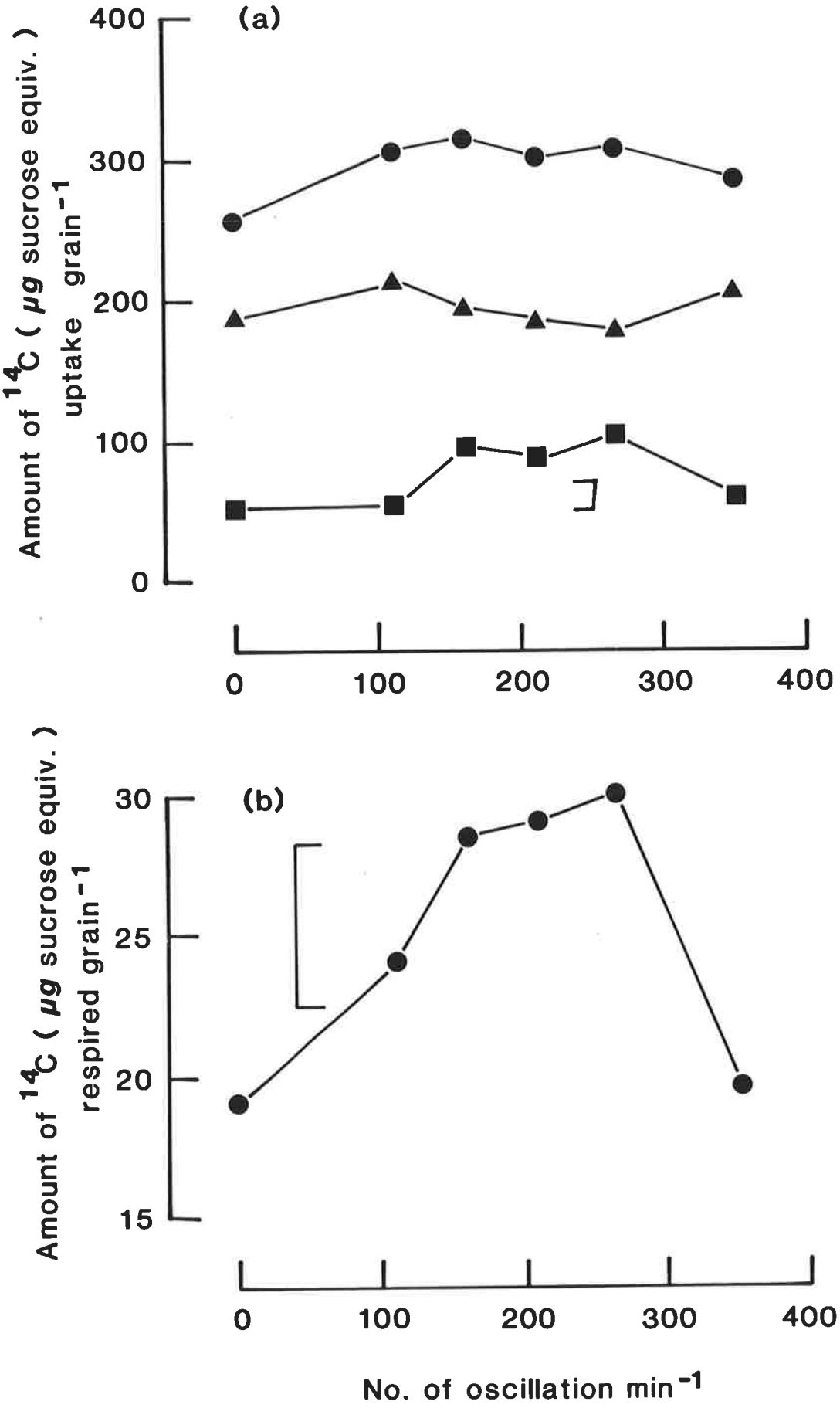
Assuming that grain respiration was 20% of the rate of grain growth at 20°C (approximate value - from Wardlaw et al. 1980 ; Vos 1981), the amount of CO_2 expected to be released by 20 day old grain would be about 4.2 µmoles in 8 h. To check the efficiency of the KOH trap, 100 µl of freshly prpeared sodium ^{14}C-bicarbonate [stock purchased from Amersham Australia Pty. Ltd ; specific activity 56 mCi/mmole] solution (containing 4.2 µmoles of sodium bicarbonate) was added to each assembly and the lid closed immediately. Ten µl of acetic acid (1.745 µmoles μl^{-1}) was injected through the lid of the assembly into the sodium bicarbonate solution, and the syringe needle hole was sealed with a blob of Blu-tack. The assemblies were shaken gently for 10 min, and then 10 µl of 0.1 N HCl was added to the solution through the hole in the lid and the assemblies heated at 70°C for 10 min. The radioactivity entrapped on the paper accounted for 93.4% of the radioactivity originally present in sodium bicarbonate solution. Therefore, the values obtained in the subsequent experiments were corrected for this discrepancy.

Effect of shaking rate on $^{14}CO_2$ evolution

For maintaining a constant supply of oxygen for respiration to the partially submerged grain, and also to enhance the diffision of $^{14}CO_2$ for effective absorption by the KOH trap, shaking of the assemblies may

Fig. 3.6.4

Influence of shaking rate on the total uptake of sucrose (●), and its incorporation into ethanol soluble (▲) and insoluble (■) fractions (a), and amount of radioactive sucrose respired (b) by the grains. Grains (20 days after anthesis) were cultured on osmotically adjusted (-0.82 MPa) sucrose solution [containing 30 gl^{-1} (89.5 mMolal) sucrose plus ^{14}C-sucrose (34.1 µCi g^{-1} sucrose)] for 8 h at 35°C. Each point is the mean of 4 replicate grains.


(a)
Amount of ^{14}C (μg sucrose equiv.) uptake grain^{-1}
400
300
200
100
0
0
100
200
300
400
(b)
Amount of ^{14}C (μg sucrose equiv.) respired grain^{-1}
30
25
20
15
0
100
200
300
400
No. of oscillation min^{-1}

be important, and was tested by culturing the grains with 100 µl of 30 gl^{-1} (89.5 mMolal) sucrose solution at 35°C for 8 h shaken at various speeds (Fig. 3.6.4). Both the amount of $^{14}CO_2$ released and the amount of ^{14}C incorporated into the insoluble fraction was increased with increase in the speed of oscillations and showed a broad optimum around 200 oscillations (1.5 cm length) min^{-1}. By contrast, the amount of ^{14}C in the ethanol soluble fraction of the grain remained relatively constant over all the shaking speeds tested. So a speed of 200 oscillations min^{-1} was used in further experimentation.

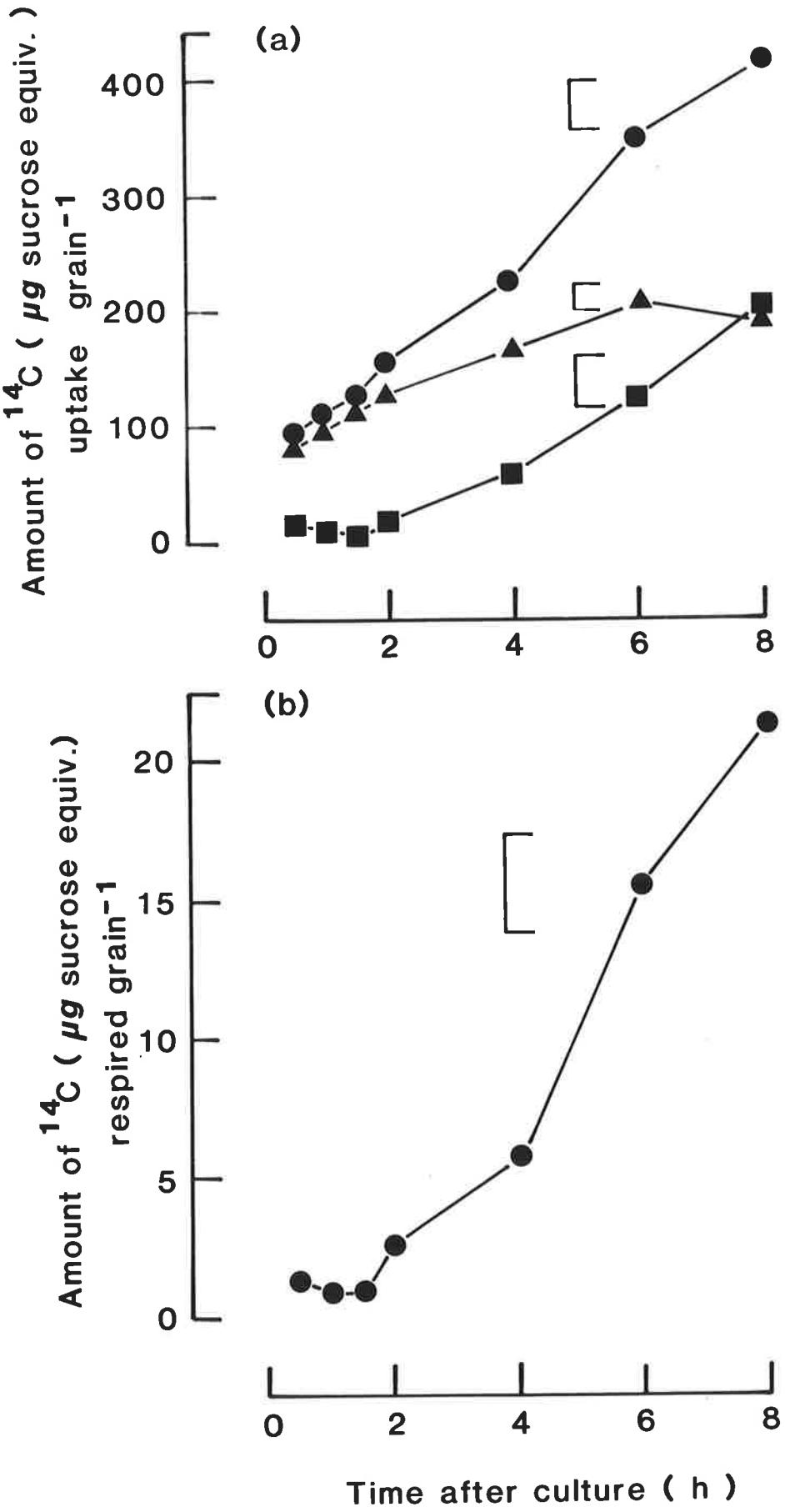
As the grain culture in these experiments was carried out in a laminar flow cabinet under presumably sterile conditions, it was expected that the counts for the papers from the assemblies having sucrose solution but without grain would be the same as for the papers from the blank assemblies (without sucrose solution). However, the papers from the assemblies containing sucrose (without grain) did absorb some radioactivity. The source of this ^{14}C could have been the release of $^{14}CO_2$ as a result of microbial activity before millipore filtration and/or a labelled volatile impurity generated by the addition of acid and heating (Section 2.8.2.2.2). No microbes were found in sucrose solutions at the end of culture period tested by using a general purpose agar medium (Difco Laboratories, Detroit, U.S.A.). Also neither degassing the sucrose solution with N_2 nor even autoclaving it did affect the number of counts detected on the paper in the assemblies with sucrose solution. So, for all the calculations the counts on the paper from assemblies containing sucrose solution (without grain) were substrated as background counts from those assemblies with grain.

Time-course of ^{14}C-sucrose uptake and $^{14}CO_2$ evolution

Total uptake of ^{14}C-sucrose by the grain increased almost linearly over the 8 h period of culture (Fig. 3.6.5a). Extrapolation of this curve to zero time gives a value of 2.1 µl for the apparent free space volume. The amount of label present in the ethanol soluble fraction increased linearly over the first 6 h and did not increase further during the subsequent two hours of culture. The incorporation of label into the ethanol insoluble fraction and the appearance of label in the KOH soaked paper ($^{14}CO_2$ respired by the grain) showed a lag phase of about 1.5 h and increased linearly with time thereafter. After 8 h of culture, about one-half of the total amount of label was in the

Fig. 3.6.5

Time-course of radioactive sucrose uptake (total) (●) and its incorporation into ethanol soluble (▲) and insoluble (■) fractions (a), and amount of radioactive sucrose respired (b) by the grain. Grains (20 days after anthesis) were cultured on osmotically adjusted sucrose solution [-0.82 MPa, containing 30 gl^{-1} (89.5 mMolal) sucrose plus ^{14}C-sucrose 62.2 $\mu Ci\ g^{-1}$)] for various periods at 25°C. Each point is the mean of 4 replicate grains. Details in the text.


(a)
Amount of ^{14}C (μg sucrose equiv.) uptake grain^{-1}
400
300
200
100
0
0
2
4
6
8
(b)
Amount of ^{14}C (μg sucrose equiv.) respired grain^{-1}
20
15
10
5
0
0
2
4
6
8
Time after culture (h)

insoluble fraction and the amount of ^{14}C-sucrose respired by the grain was only 5.4% of the total.

Effect of light on $^{14}CO_2$ released by the grain

The inner (chlorophyllous layer) pericarp of the grain has been reported to be photosynthetically active (Section 1.9), therefore, it is possible that some of $^{14}CO_2$ released by the grain during the culture was refixed by this layer because no precaution was taken to prevent light entering the assembly. Conceivably this could have resulted in an under estimate of the amount of radioactive sucrose respired by the grain. This possibility was tested by culturing the grains by the routine method but in complete darkness. There was no difference in the amount of radioactive sucrose respired by the grain in 8 h cultured on 30 gl^{-1} (89.5 mMolal)sucrose solution at 25°C by the routine procedure or in complete darkness (respectively, 8.7 and 9.2 µg radioactive sucrose, L.S.D. 2.8).

Effect of temperature on ^{14}C-sucrose uptake and its incorporation into starch

To study the effect of temperature on ^{14}C-sucrose uptake and its partitioning into various fractions of the grain two experiments were performed. In the first grains were cultured individually (as described earlier) on ^{14}C-sucrose [containing 30 or 50 gl^{-1} (89.5 or 151.2 mMolal respectively) of sucrose] at various temperatures between 15 and 35°C for 8 h, and measurements were made on the amount of ^{14}C in the apparent free space, in the ethanol soluble fraction, in the insoluble fraction, and on the amount of ^{14}C respired by the grain (Table 3.6.6). The second experiment was a repeat of the first, except that five grains in a sample were cultured by method III (Section 2.8.2.3) and the grains were analysed for ^{14}C present in starch rather than in total insoluble material, and no measurements on amount of ^{14}C-sucrose respired by the grain were made (Table 3.6.7). The results for the grains cultured individually or as a sample of five grains were very similar, except that the grains used for the latter experiment were heavier, absorbed more sucrose and had more label in all the analysed fractions of the grain.

As expected, the amount of ^{14}C washed from the apparent free space of the grains were higher for the grains cultured at 50 gl^{-1} sucrose

Table 3.6.6

Effect of temperature on ^{14}C-sucrose uptake and its incorporation into various fractions of 20 days old b grains. Grains were cultured individually (Method II - Section 2.8.2.2) on 100 µl of 30 g l^{-1} (89.5 mMolal, sp. activity 54.4 µCi g^{-1} sucrose) or 50 g l^{-1} (151.2 mMolal, sp. activity 18.05 µCi g^{-1} sucrose) sucrose solution (osmotically adjusted to -0.82 MPa) for 8 h. Values are mean of 10 replicate grains.

Temp. of incubation (°C)	Sucrose concn in culture medium ($g l^{-1}$ of water)	Dry weight per grain (mg)	Incorporation of ^{14}C[µg sucrose equiv. $grain^{-1}$] into: Apparent free space	Soluble fraction (in cells)	Insoluble fraction	CO_2	Total
15	30	15.9	40.2	100.5	62.4	4.65	208.1
	50	17.0	73.3	164.7	68.5	6.83	313.8
	Mean	16.5	56.8	132.6	65.4	5.74	261.0
20	30	17.7	41.7	98.8	104.2	10.30	253.3
	50	17.7	73.3	197.5	144.4	17.05	448.0
	Mean	17.7	57.5	148.1	124.3	13.67	350.7
25	30	16.2	41.9	111.3	131.0	11.04	296.1
	50	17.9	80.3	233.1	109.1	12.43	435.9
	Mean	17.0	61.1	172.2	120.1	11.74	366.0
30	30	19.0	47.5	135.6	181.9	21.87	388.4
	50	19.2	89.6	260.6	151.3	23.13	515.9
	Mean	19.1	68.6	198.1	166.6	22.50	452.2
35	30	17.0	61.1	161.2	122.0	27.76	374.0
	50	17.8	78.4	261.3	187.2	35.61	564.9
	Mean	17.4	69.7	211.3	154.6	31.68	469.5
Mean over temperatures							
	30	17.2	46.5	121.5	120.3	15.12	304.0
	50	17.9	79.0	223.5	132.1	19.01	455.7
		Least Significant Difference					
Temperature		1.1	6.7	13.1	34.4	2.90	30.9
Concn		0.7	4.3	8.3	n.s.	1.83	19.6
Interaction		n.s.	9.5	18.6	48.6	n.s.	43.7

Table 3.6.7

Effect of temperature on ^{14}C-sucrose uptake and its incorporation into various fractions of 20 days old b grains. Five grains were cultured (Method III - Section 2.8.2.3) on 30 gl^{-1} (89.5 mMolal, sp. activity 9.04 µCi g^{-1} sucrose) or 50 gl^{-1} (151.2 mMolal, sp. activity 5.02 µCi g^{-1} sucrose) sucrose solution (osmotically adjusted to -0.82 MPa) for 8 h. Values are mean of 5 replicate samples.

Temp. of incubation (°C)	Sucrose Concn in culture medium (gl^{-1} of water)	Fresh weight	Dry weight	Starch	Amount of ^{14}C[µg sucrose equiv. $grain^{-1}$] incorporated into: Apparent free space	Soluble fraction (in cells)	Starch
		----- mg per grain -----					
15	30	45.6	20.4	13.9	49.3	130.7	98.1
	50	47.0	20.4	13.8	90.0	204.7	116.2
	Mean	46.3	20.4	13.9	69.6	167.4	107.1
20	30	49.9	22.8	15.3	59.5	154.7	171.7
	50	50.0	22.7	16.0	88.3	241.6	184.4
	Mean	50.0	22.8	15.6	73.9	198.1	178.0
25	30	47.0	20.6	15.0	46.9	192.8	131.9
	50	47.7	20.5	16.3	86.6	285.8	192.8
	Mean	47.7	20.6	15.7	66.8	239.3	162.3
30	30	50.1	21.2	14.7	59.6	224.0	219.9
	50	48.8	21.3	15.5	102.7	320.6	218.6
	Mean	49.5	21.2	15.1	81.1	272.3	219.3
35	30	46.7	21.2	14.3	73.8	218.9	92.4
	50	47.8	21.8	15.2	122.9	331.7	129.0
	Mean	47.2	21.5	14.7	98.3	275.3	110.7
Mean over temperatures							
	30	47.9	21.2	14.6	57.8	184.2	142.8
	50	48.3	21.3	15.4	98.1	276.8	168.2
		Least Significant Differences					
Temperature		1.9	n.s.	n.s.	8.2	14.9	36.8
Concn		n.s.	n.s.	n.s.	5.2	9.4	23.3

than for those cultured at 30 gl^{-1} sucrose at all temperatures of incubation. The amounts in the apparent free space were fairly constant between temperatures 15 and 25°C, but increased significantly at temperatures of 30 and 35°C (Fig. 3.6.7). The amount of soluble ^{14}C in the cells increased linearly between temperatures 15 and 30°C and did not increase by increasing temperature to 35°C. Incorporation of ^{14}C-sucrose into insoluble material (Table 3.6.6) or starch (Table 3.6.7) increased with increase in temperature between 15-25°C, and was either little different at temperatures of 30 and 35°C (Table 3.6.6) or increased up to 30°C, above which there was a substantial reduction with further increase in temperature to 35°C (Table 3.6.7 and Fig. 3.6.7). The amount of ^{14}C-sucrose respired by the grain increased almost linearly over the entire range of temperature and was always higher for the grains cultured at 50 gl^{-1} sucrose than those cultured at 30 gl^{-1} sucrose (Fig. 3.6.6).

Discussion

Although centrifugation of the grains to collect sap from the endosperm cavity is convenient and provides samples in sufficient quantity for compositional analysis and osmotic potential determinations, the interpretation of the data is difficult unless it is certain that the sap is collected only from the endosperm cavity. Ho and Gifford (1984) provide some evidence that this is the case but from the results in Table 3.6.1, it is clear that precautions must be taken to ensure that sap collected by centrifugation is only from the endosperm cavity. The concentration of sucrose (76 mM) in the sap, collected using a compromise time for centrifugation in which sap is most likely to come from endosperm cavity, is very close to the values reported by Jenner (1974a), somewhat higher than the values reported by Ho and Gifford (1984), and lower than the values reported by Radley (1978). However, these small differences could simply be due to differences in the physiological stage of the grains used for this purpose by the various research workers. Together the amounts of sucrose, glucose, and fructose in the sap account only for 76.5% of total sugars which may suggest that there were other sugars in the sap (most likely oligosaccharides - Ho and Gifford 1984).

Water potential (assuming zero turgor pressure for endosperm cavity) of the sap (-1.1 MPa) is very close to values reported for the intact grains by other workers (Barlow *et al.* 1980 ; Brooks *et al.*

Fig. 3.6.6

Effect of temperature on the amount of radioactive sucrose respired by cultured wheat grains as in Table 3.6.6 ; mean of both concentrations.

Fig. 3.6.7

Effect of temperature on the partitioning of ^{14}C-sucrose into the free space (■), starch (●) and ethanol soluble material in the cells (▲) of cultured wheat grains as in Table 3.6.7 ; means of both the concentrations.

Fig.3.6.6

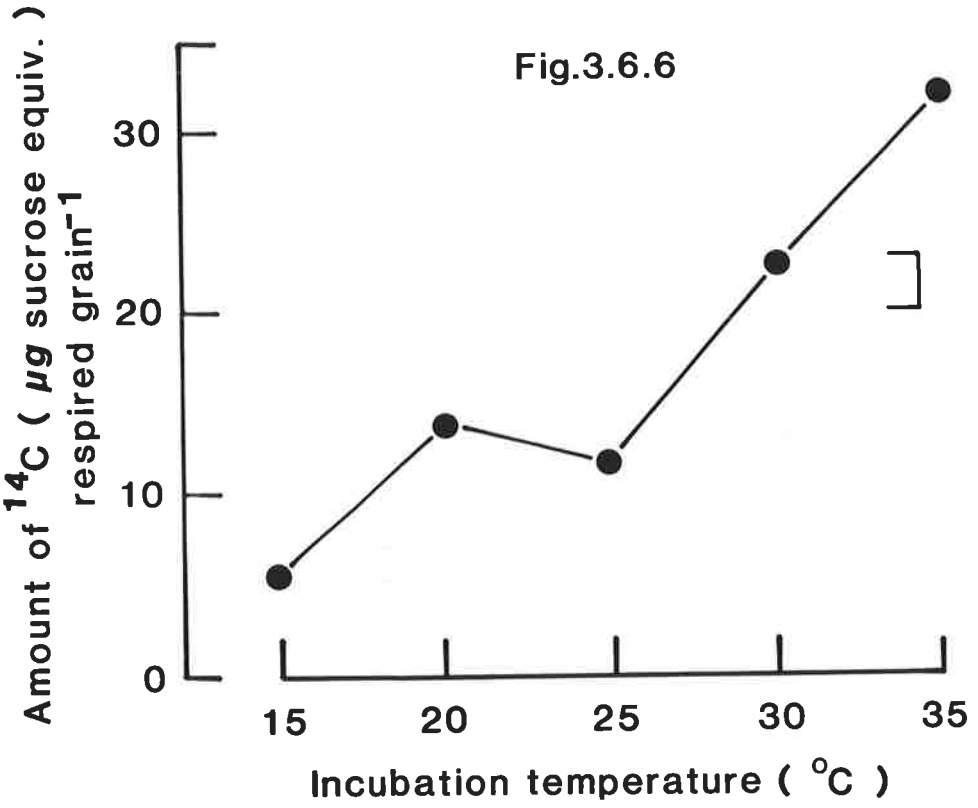
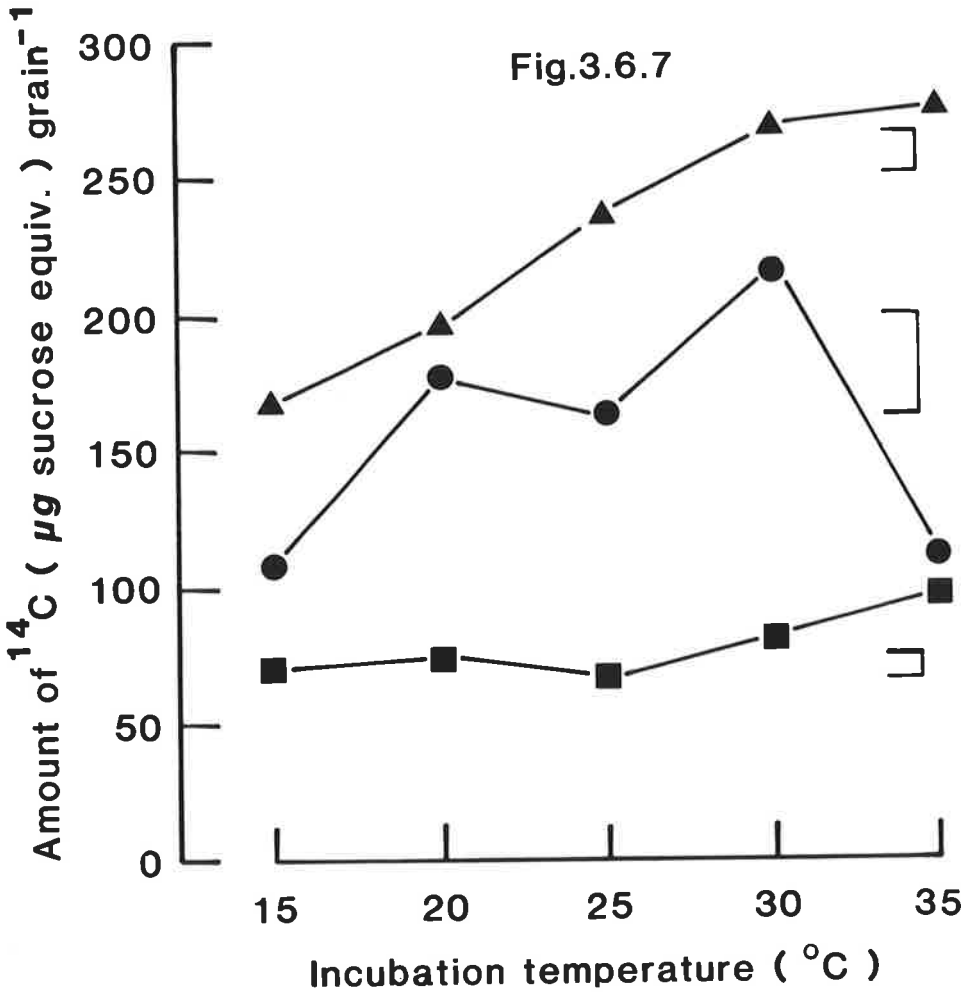


Fig.3.6.7



1982 ; Jenner 1982b) and to the values reported elsewhere in this investigation (Tables 3.2.3 and 3.2.8 - Section 3.2) which may indicate that water potential of endosperm cavity sap *in situ* is reasonably close to that of the endosperm cells. Despite the large amount of sugars in the sap, these sugars make only half the osmolality of the sap and the balance therefore must be due to inorganic or other organic constituents of the sap. Somewhat similar values (0.5 to 0.7 osmolar or -1.22 to -1.67 MPa) have been reported by Smith (1973) for the endosperm liquid of *Phaseolus vulgaris*, and in his attempt to determine the partial contribution made by various inorganic and organic constituents of the liquid to total osmolarity he was able to account for only 76% of total osmolarity by K, Ca, Mg, P, and $N^{+}H_4$ ions, organic and amino acids, and sugars, and concluded that the balance must be due to proteins, hormones, sugar alcohols and unknown acid components.

Estimations of intercellular free space volume by using ^{14}C-PEG 4000 (Fig. 3.6.1) or ^{14}C-sucrose (Fig. 3.6.5a) gives a value of 2 µl or 7.5% of grain water content. This value is comparable to values reported by Ho and Gifford (1984) and lower than the 11-12% figure estimated either by incubating wheat grains in PEG 4000 solution for 24 h (Jenner and Rathjen 1975) or from the amount of ^{14}C-sucrose exodiffused in 90 min (Gifford and Bremner 1981a). As pointed out in the results section the amount of ^{14}C-PEG 4000 taken up by the grains in 24 h gives an over estimate by about 30%, therefore, if the values reported by Jenner and Rathjen (1975) are corrected for this likely discrepancy, the values are very close to the values reported here. Presumably, higher values reported by Gifford and Bremner (1981a) may have resulted from some leakage of ^{14}C from the cells.

For valid interpretations of the results obtained from *in vitro* cultured grains on solutions of sucrose it is important to demonstrate that grains synthesize insoluble material at the rate close to the grains developing on the plant. With the exception of one experiment (results not reported in here) where the incorporation of ^{14}C-sucrose into insoluble material was unexpectedly variable, *in vitro* cultured grains in all other experiments accumulated insoluble material at a rate comparable to grain growth *in vivo*. For example, culture on 60 gl^{-1} (182.7 mMolal) sucrose (Fig. 3.6.3a), the concentration of sugars estimated to be in the apoplast of the grain (Table 3.6.2), has been shown to be optimal for ^{14}C-sucrose incorporation into insoluble material in halved *in vitro* cultured grains (Gifford and Bremner 1981a)

or endosperm slices (Rijven and Gifford 1983). Under these conditions about 0.9 mg of radioactive insoluble material (mostly starch) was produced in 24 h. After taking into consideration the additional amount of non-radioactive material (about 0.2 mg) which must have been synthesised before the intracellular pool of the grain was radioisotopically equilibrated, the resulting value of about 1.1 mg is quite reasonable for the rate of grain growth on the plant. Similarly, from the time course study where the grains were cultured with 30 gl^{-1} (89.5 mMolal) sucrose (Fig. 3.6.5a) between 1.5 and 8 h grains synthesized 29.1 µg radioactive material per h. As the specific radioactivity of sucrose in the cells took at least 6 h to reach equilibrium, the total amount of radioactive insoluble material (radioactive + non-radioactive) produced was probably double this value which would give a rate of 1.4 mg $grain^{-1}$ day^{-1}.

It has been reported earlier (Section 1.8) that movement of sucrose from the apoplast of the grain into the cells of endosperm is either by diffusion and/or metabolically facilitated diffusion. The concentration of sucrose in various parts of the grain (Table 3.6.2) and results from the grain culture experiments seem to support this view. Exposure of the apoplast of the grain to concentrations up to 120 gl^{-1} (381 mMolal) sucrose (Fig. 3.6.5a) resulted in an almost linear increase in the amount of ^{14}C (sucrose equivalents) in the endosperm cells. Assuming that water content of the cultured grains was 26.7 µl (from Table 3.6.2) and did not change during the culture period, the concentration of carbon-14 in endosperm cells was only about one-fifth of the corresponding sucrose concentration in the external medium, suggesting that movement of sucrose from apoplast into the endosperm cells is dependent on the gradient across plasma membrane, which implies a high resistance to this movement (Jenner 1974a).

Different processes related to the formation of dry matter seem to respond differently to an increase in temperature during the grain culture period. Both uptake of ^{14}C-sucrose into the cells (Tables 3.6.6 and 3.6.7) and the amount of ^{14}C-sucrose respired by the grain (Table 3.6.6 and Fig. 3.6.6) increased almost linearly with increase in temperature, whereas the amount of label incorporated into insoluble material either did not increase in the grains cultured at temperatures above 25°C (Table 3.6.6) or the amount of starch synthesized by the grain when cultured at 35°C (Table 3.6.7 and Fig. 3.6.7) was actually

depressed despite the increase in the availability of ^{14}C-sucrose in the cells.

Starch in wheat is synthesized inside the amyloplast, the functioning of which is poorly understood. However, from the distribution of various enzymes involved in starch synthesis between the amyloplast and the cytosol from suspension cultures of soybean, MacDonald and apRees (1983) have speculated that sucrose in the cell may be converted to triose phosphate before its passage through the amyloplast membranes which then may be converted to starch by the concerted action of a number of enzymes inside the amyloplast (also see Jenner 1976a). Apparently then the depression in the production of starch at elevated temperature could either be due to impairment of the transport function of the amyloplast membrane(s) or due to a reduction in the activity of one or all enzymes involved in starch biosynthesis. The activity of the enzymes involved in starch synthesis has been reported to be reduced in leaves of grapevine grown at 35/30°C (Hawker 1982) and in potatoes grown at 30°C (Mangat and Badenhuizen 1971).

Increase in rate of respiration of *in vitro* cultured grains with increase in temperature during incubation period (Table 3.6.6 and Fig. 3.6.6.) is in agreement with the results reported by other workers for the isolated intact grains (Section 1.10). From the linear part of the curve in Fig. 3.6.5b, an estimate of respiration for the cultured grain is 3.22 µg ^{14}C (sucrose equiv.) per h, and taking into consideration that specific activity of the sucrose in the intracellular pool takes about 6 h, the rate of respiration for the cultured grains may be double this value which would give a value of 154.6 µg sucrose $grain^{-1}$ day^{-1}. This value is equivalent to about half the rate of respiration measured in isolated intact grains at 20°C (Evans and Rawson 1970 ; Chowdhury and Wardlaw 1978). Moreover, Nutbeam and Duffus (1978) have shown that O_2 uptake by isolated grains at 30°C in the dark almost doubles when the outer transparent pericarp is removed from the grain, suggesting an increase in the respiration of the grain. However, no information was obtained in this study on the effect of removal of the outer pericarp on total respiration of the grain supplied *in vitro* with ^{14}C-sucrose. Lower consumption of radioactive sucrose for respiration by the cultured grains could be due to the absence of the embryo and the crease tissue, parts of the grain likely to be dependent rather heavily on respiration for energy needs for their metabolic functions.

SECTION 4: GENERAL DISCUSSION

4. GENERAL DISCUSSION

The wheat plant during its life cycle passes through three main developmental stages. In the first or the vegetative phase, leaf growth, root growth and tillering predominate, and the environmental conditions during this phase determine the number of tillers per plant. During the second phase, the reproductive period, which extends from floral induction and initiation of inflorescences to their anthesis and fertilization, the environmental conditions during this phase determine the rate and extent of floral differentiation, and hence the potential number of grains per plant. But it is the conditions during the third or grain filling phase which determine the actual grain yield. High temperatures during early spike development accelerate spikelet differentiation, which may reduce the number of spikelets per ear (Thorne et al. 1968), and high temperatures around anthesis can promote floret sterility and so reduce grain number. However, as the wheat plant has an in-built capacity for yield compensation, some of the negative effects on grain yield as a result of a reduction in grain number can be compensated by increases in the remaining grain weights. High temperature during grain filling, when the other components of yield have been fixed, usually reduce average grain weight, and it was the objective of this study to examine some of the factors which possibly could reduce grain dry weight as a result of high temperature during grain filling.

The main objectives of this investigation were to see: (a) whether or not the rate of grain growth at high temperature is limited by the availability of assimilate supply or is it due to factors operating within, or close to, the grain ; (b) what is the effect of high temperature on starch and protein accumulation in the grain ; (c) the effects of temperature on the uptake of sucrose and its conversion into starch in grains cultured *in vitro*.

Before discussing the effects of temperature on grain growth, it seems necessary to make a comment about the selection of the timing for the imposition of heat treatments. Fertilization in the wheat ear takes place over several days, so any treatment which starts when only the most advanced florets have been fertilized may interfere with fertilization in the later developing florets. It has been shown that when the number of grains per ear is reduced the mean weight in the remaining grains is increased (see Discussion in Section 3.1). Such an

indirect effect of high temperature could present some problems of interpretations in comparing grain growth in ears developing at different temperatures, so it was necessary to allow an interval of a few days between anthesis in the most advanced florets and the imposition of high temperature treatment(s). Day 10 after anthesis as the earliest time for the imposition of heat treatments was selected on the following considerations.

(a) Anthesis in all florets in a spike of cv. Sonora in the present experiments was complete by 5-6 days after anthesis of the most advanced florets, therefore, imposition of high temperature treatment(s) after this time was presumed to have a little or not effect on the total number of grains per ear.

(b) The first appearance of starch granules in the endosperms from the central b floret grains was observed between days 6-8 after anthesis in these florets. Therefore, these grains were expected to accumulate dry matter linearly thereafter.

(c) As grain growth both during and after the imposition of high temperature was under investigation, it was therefore necessary to treat the plants or the ears during early stages of grain growth so that observations during later stages could be made.

With the exception of two treatments (30/25 and 33/25°C) in experiment 1, where the number of grains was significantly reduced as a result of warming ears, the imposition of heat treatments in other experiments either did not reduce grain number or the reductions in grain number were quite small, so it was assumed that the comparisons in grain between the control and warmed ears are not complicated by indirect effects.

Final grain weight is a function of the rate and the duration of grain growth, and both these components are influenced by increases in temperature during grain filling, and will be discussed separately.

4.1 THE RATE OF GRAIN GROWTH

The rate of grain growth is presumably determined by the supply of assimilates and by the rate at which these low molecular weight substrates (sucrose and amino acids) are converted to high molecular weight storage products (starch and protein). Possibly the limitation to the rate of grain growth at high temperature may either operate through the processes related to the delivery of the assimilate to the

grain or through its effects on the processes governing the conversion of assimilate to dry matter, or both.

4.1.1 AVAILABILITY OF ASSIMILATE FOR GRAIN GROWTH

Sucrose and amino acids are the main precursors for grain growth, and the amount of these precursors within the grain at any time does not exceed a day's requirement for growth. Therefore, the grain is continuously dependent for the supply of these precursors from the other parts of the plant (for the sources - see Section 1.7). Under conditions of normal grain growth there is an adequate supply of assimilate for grain filling (Jenner 1982a and references cited therein) and the rate of grain growth is governed by the processes operating within or close to the grain (Jenner and Rathjen 1972a, 1972b; Jenner 1974a, 1976b; Apel 1976; Thornley et al. 1981). Increase in temperature above about 20°C during grain filling has commonly been observed to reduce final grain weight. As high temperature enhances senescence of photosynthetic tissues and also enhances the loss of carbon by respiration, it has been suggested (Sofield et al. 1974; Spiertz 1974; Fischer and Laing 1976) that high temperature reduces final grain weight through reducing the amount of assimilate available for grain growth. Critical evidence in support of this supposition is lacking, however. The hypothesis whether or not the rate of grain growth at high temperature is limited by the availability of assimilate supply for grain growth was tested in the present experiments by warming the ears alone where the effects of temperature on the other parts of the plant are likely to be minimal, and hence eliminating the possibility that temperature may have effected grain growth indirectly through its effects on other parts of the plant.

From various experiments in this investigation it seems reasonable to conclude that a reduction of, or no increase in, the rate of grain growth as a result of ear or whole plant warming during grain filling is not likley to be due to a shortage in the supply of assimilate but is more likely to be due to factors operating within the grain. This conclusion is supported by many reports in the literature (for wheat see - Ford et al. 1976; Wardlaw et al. 1980; Donovan et al. 1983; for rice see - Sato and Inaba 1976; for soybeans see - Egli and Wardlaw 1980), and further evidence to support the above contention is cited as follows.

4.1.1.1 REDUCTIONS IN GRAIN WEIGHTS IN VARIOUS FLORET POSITIONS WITHIN A SPIKELET

Under conditions of limited supply i.e. shading and defoliation, it has been shown (cf. Bremner 1972; Bremner and Rawson 1978) that proportionately greater reductions in grain weight are observed in distal florets in comparison with proximal florets. It has also been shown (Singh and Jenner 1983) that the proportionate increase in grain weight, in detached ears supplied with sucrose above the levels required for normal growth, is relatively greater in distal florets than in proximal ones. Therefore, it would appear that grain weight in distal florets is more responsive to changes in the supply of assimilate than proximal ones. Then, if the effect of temperature on grain growth is mediated through a shortage in the supply of assimilate, it would be expected that proportionate reductions in dry weight should be relatively greater for the grains from distal than proximal florets. But such was rarely the case in the experiments reported in this investigation. For example, in experiment 1, reductions in grain dry weight were evident in all floret positions (Table 3.1.2), and in all the parts of the ear (Fig. 3.1.3), approximately to the same extent. Similar responses were also noticed in experiments 7 (Tables 3.3.2 and 3.3.3), 8 (Tables 3.3.6 and 3.3.7), 11 (Table 3.5.2), and 12 (Table 3.5.7) where it was found that proportionate reductions in grain dry weight for florets a, b or c from the central parts of the ear were very much the same as the reductions in the average grain weights per ear. Some workers (Wardlaw *et al.* 1980) have shown that decreasing the overall requirement for photosynthate, by removing one-third of the grains in an ear, had little effect on the temperature response of the remaining grains. In other words the effect of temperature was not greatly modified by an increase in the supply of assimilate. [However, these responses should be interpreted with caution, as abnormal or unusual responses in the grains remaining after grain removal have been reported (Radly 1978; Jenner 1980b), so these responses may not strictly represent changes in the photosynthate alone]. Lastly, the proportionate reductions in the rates of grain growth in the b and c grains in experiment 4, and also the relative increases (observed mainly for the ears cultured with 20 gl^{-1} of sucrose) in the rates of grain growth for b and c grains in experiment 8 to warming were very much similar (Fig. 3.3.4). From the foregoing, it could be adduced that similar relative

reductions in grain weights in various floret positions seem to support the possibility that the supply of assimilate may not be limiting for grain growth at high temperature. And furthermore it is evident that whatever the mechanism by which high temperature reduces grain growth, it has a similar effect on all the grains within a spikelet, or grains in the different parts of the ear.

4.1.1.2 AMOUNT OF SUCROSE IN THE GRAIN

Control of deposition of starch by intracellular level of sucrose has been demonstrated in the developing wheat grain (Jenner 1970; Jenner and Rathjen 1978). The influx of sucrose into the grain is not directly related to the supply of sucrose to the ear and it has been proposed that the transport of sucrose is restricted in the last stages of its passage through the ear into the grain (Jenner 1976b). However, the sucrose concentration in the grain is alterable and the various levels that can be achieved with the method of culturing ears vary in parallel with production of starch (cf. Singh 1982). Sucrose is the main form of sugar transported to the grain (Section 1.7). In testing whether or not the rate of dry matter accumulation at high temperature was limited by the supply of assimilate, the assumption made in this investigation was that the effect of temperature on rate of grain growth must operate by changing the levels of assimilate (sucrose) within the grain.

Effects of warming ears on the absolute amounts and the concentrations of sucrose and other soluble sugars (excluding sucrose) in the grain and their relationship to the rate of dry matter accumulation have been considered elsewhere (Sections 3.2 and 3.3), so only the general features of the response will be discussed here. Warming the ears, in all experiments, resulted in a significant decrease in both the absolute amount and the concentration of sucrose in the grain. However, this reduction in the concentration was temporary, and soon after the warming treatments were finished, the sucrose concentration in the grain from the warmed ears was either comparable to (expts. 4,6,7), or even higher than, the sucrose concentration in the grains from the control ears (expt. 8). The amount of sucrose in the grain at a given time is presumably the balance between utilization and supply, so these results would suggest that either the supply of sucrose to the grain was reduced as a result of warming the ears or the utilization of sucrose was faster at high temperature. Out of the two

alternatives the latter is more likely and is supported by observations in this investigation and by the reports in the literature. Movement of ^{14}C-assimilate from the flag leaf to the ear has been shown (Ford et al. 1976; Wardlaw et al. 1980) to be enhanced by raising the temperature of the ears. This observation is supported by the results in experiment 6, where warming the ears at either humidity had little effect on the concentration of sucrose in the rachis, and the concentration of sucrose in the bracts was increased significantly as a result warming. Moreover, the sucrose concentration in the grain during the period of ear warming was reduced, in experiment 8, even when the ears were cultured on various levels of sucrose in the culture medium where the sucrose supply was presumably non-limiting. Further as the concentration of sucrose in the grains from the warmed ears returned soon after the warming treatments were over to levels as high, or even higher than, those in the grains from the control ears, these observations might suggest that it was the utilization of sucrose in the grain which was enhanced by warming. Similar observations have been reported by other workers (Robertson et al. 1962; Jenner 968). However, the enhanced utilization was not accompanied by an increase in the rate of grain growth in most of the experiments. It seems likely that this is due to an increase in the respiration rate of the grain at high temperature which has been reported by many other workers (Section 1.10), and was also observed for the grains cultured *in vitro* (Fig. 3.6.6).

Sucrose concentration in the grains from ears cultured at various levels of sucrose increased with an increase in sucrose level in the external medium, and the internal concentrations in the warmed ears were comparable with those observed in the grains from control ears cultured at the same levels of sucrose. Despite the comparable sucrose concentrations in the grains from control and warmed ears, the rate of grain growth responded differently in the two types of ears to variation in sucrose levels in the external medium. The rate in the control ears increased almost linearly with increase in sucrose levels in the medium (or in the grain) but there were no significant differences in the rates of growth of the grains growing in the warmed ears cultured at any level of sucrose. These observations strongly support the view that slower grain growth in the warmed ears is not likely to be due to a shortage in the supply of sucrose. An alternative explanation comes from experiment 9, where isolated endosperms from the control and warmed ears (at high or low humidity) were cultured on solution of ^{14}C-sucrose. Although the

three types of endosperms had the same amount of soluble-^{14}C in the cells, endosperms from the warmed ears synthesized less radioactive starch than the endosperms from the control ears. This suggests that the process of sucrose uptake by the endosperm cells and the processes involved in the conversion of sucrose to starch have different susceptibilities to high temperature. Observations from the experiments where isolated endosperms were cultured on ^{14}C-sucrose at various temperatures between 15 to 35°C support this conclusion. It is clear from Fig. 3.6.7 that uptake of ^{14}C-sucrose into the cells increased almost linearly with increase in temperature up to 35°C, whereas sucrose to starch conversion displayed a broad optimum around 25°C and was significantly inhibited at a temperature of 35°C. These observations would further support the idea that high temperature seems to have a direct deleterious effect on the physiological and/or biochemical processes producing starch.

4.1.2 EFFECT OF TEMPERATURE ON GRAIN SIZE AND WATER RELATIONS OF THE GRAIN

The capacity of the grain to accommodate dry matter is primarily determined during the early stages of grain growth, and is a function of the number of cells per endosperm and the volume of each cell (Section 1.6.2). Although no measurements on endosperm cell numbers were made in this study, effects on this attribute appear to be unlikely. Firstly, in all experiments, ear or whole plant warming treatments were not carried out until day 10 after anthesis (or thereafter) by which time a substantial proportion of endosperm cell number has already been determined (about 70% of total cell number - see Fig. 1 in Singh and Jenner 1982b), so elevation in temperature after this stage is not likely to have a large effect on endosperm cell number. Secondly, it has been shown in many reports that although an increase in temperature during the early part of the cell division phase (Wardlaw 1970; Singh 1982), or throughout the cell division phase (Hoshikawa 1962; Radley 1976) accelerated the rate of cell division there was no effect on the total number of cells produced.

Warming significantly reduced the water content of the grains. But this reduction in water content of the grain did not appear to be simply explained by a change in the water - or osmotic potential of the grain

(expts. 5,6). As the changes in water content of the grain closely parallel the changes in the volume of the grain (Radley 1976), and assuming that there was no reduction in endosperm cell number, then it was the cell volume which was reduced in the present experiments. May and Buttrose (1959) found a significant correlation between endosperm volume and total starch granule volume but it is debatable whether the former controls the latter or vice versa. If the former is the case, it is possible that a reduction in the volume of the endosperm could impose a physical constraint on the grain for the accumulation of dry matter. Radley (1976) has reported that raising temperature of the ear from 15 to 25°C significantly reduced water content of the grains (also see Ford et al. 1976). From these and microscopic observations on the aleurone layer of the endosperm, she suggested that an increase in temperature during the early stages of grain growth accelerated the differentiation of the endosperm tissues, hence not allowing enough time for cell expansion to take place, thereby reducing the cell volume and the capacity of grains to accumulate dry matter. In the present study, in the experiments where water content of the grain was still increasing at the time of imposition of high temperature treatments (10 days after anthesis), it is possible that a part of the reduction in water content of the grain was due to accelerated development of the grain and hence reducing the time for cell expansion. However, this response was apparently reversible, at least in some experiments (Section 3.2), and reductions in water content were observed even when the heat treatments were employed after the water content in the grains had reached a plateau. Such a response cannot simply be explained on the basis of acceleration in the development of the grain because in these cases a small amount of water is actually lost from the grain as a result of high temperature. Although the mechanism by which water is lost from the grain is enigmatic (see Discussion in Section 3.4 for details) it is obvious that the cell expansion phase in wheat grain is dynamic to a certain extent and is highly sensitive to temperature conditions.

4.2 EFFECTS OF TEMPERATURE ON THE DURATION OF GRAIN GROWTH

Although Welbank et al. (1968) have reported that there is a marked decrease in the duration of grain growth in wheat crops in the field with an increase in light intensity, these observations are not supported by many other reports in the literature. For example, Sofield

et al. (1974) and Spiertz (1977), in their controlled environment studies have shown that a 2-3 fold decrease in light intensity had not effect on the duration of grain growth. Therefore, it is possible that the effect of light intensity shown by Welbank and co-workers may be due to covariation of temperature and light intensity. Moreover, it is clear from the information presented in Table 4.1 that despite differences in environmental conditions employed, and differences in the physiological stages used for calculating the duration of grain growth by various workers, the reductions in the duration of grain growth for a 1°C increase in temperature (weighted mean) throughout grain growth were fairly constant. On average for a unit increase in temperature there was a reduction of about 3 days in the duration, which would suggest that temperature during the grain filling phase is an important factor determining the duration of grain growth.

In all the experiments reported in this investigation high temperature accelerated the development of the grain and hence curtailed the duration of grain growth. As increase in temperature of the air results in a concomitant reduction in relative humidity in the air it is possible that humidity, in addition to the effects of temperature, may have influenced the development of the grain. Conclusive evidence to discard this possibility is not available from the experiments presented here. But a tentative conclusion which can be drawn from the observations in experiment 6 is that the effect of temperature on the development of the grain seems to be independent of the humidity of the warm air (also see Discussion to Section 3.2). Ford et al. (1976) in their ear warming experiments have reported that altering the humidity by 4.7 mb at a constant temperature had no effect on the ear growth or senescence. A more important question, however, is whether temperature affected grain development through its effects on the processes operating within, or close to the grain, or indirectly through its effects on the processes operating in other parts of the plant. As grain development was enhanced regardless whether ears alone or whole plants were warmed, it would appear that temperature influences grain development through the proceses operating within the grain and/or in the other parts of the ear rather than through its effects on other parts of the plant.

Although the frequency of the sampling during grain growth in most of the experiments does not permit precise evaluation of the effect of

Table 4.1

Reduction in the duration of grain growth with increase in temperature during grain filling phase in wheat.

Grain growth period	*Change in duration (days C^{-1} increase in temperature)	Temperature range and experimental conditions	Source
Linear period of grain growth	-3.1	**14.9-20.7, Field	Gallagher et al. (1976), Table 1
7 days after anthesis to maturity	-2.9	**12-22, Phytotron	Bagga and Rawson (1977), p.884 & Fig. 2
Linear period of grain filling	-2.4	**11.7-26.7, Phytotron	Sofield et al. (1977a), Fig. 7, Expts.I & II
Anthesis to maximum grain weight	-3.6	**10-25, Phytotron	Spiertz (1977), p.196
Heading to ripe	-4.6	***12-20, Field	Konturri (1979), Fig. 10
Flowering to physiological maturity	-3.1	**15.8-27.7, Field	Wiegand and Cueller (1981), Fig. 1 & Table 2

* Calculated by linear regression over the temperature range

** Weighted mean temperature

*** Approximate values

temperature on the duration of grain growth, it is possible to calculate the effect of temperature on the development of the grain by assuming that the number of degree-days required for the completion of grain growth is the same for the grains in the control as well as warmed ears (or plants). The calculated reductions in durations for some of the experiments in this study are given in the following tabulation:

Experiment number	Temperature differential day/night (°C)	Duration of warming (between days after days)	Accumulated temperature increase above ambient in degree-days	Calculated reduction in duration (days)
4	33/35	10–16	64.5	4.8
5	30/25	10–17	63.0	4.7
6	33/25	15–21	64.5	4.8
7	30/25	10–17	63.0	4.7
8	30/25	10–17	63.0	4.7
9	33/25	10–16	64.5	4.8
11	33/25	10–20	107.5	8.1
12	27/22	10–30	120.0	9.0

The calculated values for reduction in duration are consistent with direct observations. For example, in experiments 11 (Fig. 3.5.1) and 12 (Fig. 3.5.6) grains in the warmed plants ceased to accumulate total dry matter, in all cultivars, between 5–10 days earlier than those in the control plants. These calculated reductions in the duration in grain growth are also reasonably close to values estimated for the acceleration of grain development in the warmed ears based on criteria other than dry matter accumulation. For example, in experiment 4, grains in the warmed ears matured at least by 4–5 days earlier than the grain in the control ears (from chlorophyll measurements in the pericarp - Fig. 3.2.2). However, there are two important points which need careful consideration. Firstly, although thermal time (number of degree-days) required for the maturation of grain between the temperature range of

15-25°C seems to be constant (Bagga and Rawson 1977; Davidson and Campbell 1983), there are many reports (Gallagher et al. 1976; Sofield et al. 1977a; Spiertz 1977) where the number of degree-days required for the grain to reach maturity decreases almost linearly with increase in temperature. So, the assumption made earlier for calculating the reductions may not necessarily be true. Secondly, an increase in temperature during grain filling does not have the same effect on the durations of starch and protein accumulation, so apparent earlier termination of total dry matter accumulation in the warmed plants is mostly due to a dominant effect of temperature in reducing the accumulation only of starch.

4.3 EFFECT OF RELATIVE HUMIDITY ON GRAIN WEIGHT RESPONSE

It is well known that atmospheric humidity strongly influences transpiration and hence the water-use efficiency of the crop plants, and it may also affect the economic yield of the crops. O'Leary and Knecht (1971) found that high relative humidity (95-100%) reduced transpiration and increased water-use efficiency in bean plants compared to plants kept at lower humidities (35-40% and 70-75%). They also reported that the bean yield in low humidity (35-40%) was only 60% of that at the intermediate humidity, and there were no reductions either in growth or yield by continuous relative humidities near saturation. Hoffman (1973) in 9 crop species has shown that increasing relative humidity from about 40% to 90% increased yield (marketable product) in all the crops. The effects of relative humidity on grain yield in wheat are contradictory, and the reports recording the effects of relative humidity on the rate of grain growth and development are rare. Hoffman (1973) observed a 21% increase in grain yield with increase of humidity from 41 to 90%. Campbell et al. (1969), however, found no appreciable influence of raising the relative humidity from 60 to 80% on grain or straw production. In the present study (expts. 5,6 and 9), warming the ears at high humidity reduced total ear grain weight and individual grain weight to a smaller extent than warming the ears at low humidity, and at least in two (expts. 5 and 6) of the three experiments high humidity, compared to low, seems to have had a smaller effect on the rates of grain growth. How humidity influences the rate of grain growth is not clear from the observations in this investigation. High humidity effects at high

temerature do not seem to be mediated through changes in the water relations of the grain or the bracts, or through the amounts of sugars in the grain (also see the Discussion to Section 3.2). Evidently, the reduction in the amount of water in the grain was relatively smaller in the grains from the ears warmed at high humidity in comparison to the ears warmed at low humidity (Fig. 3.2.4a), which may explain the above response.

4.4 EFFECT OF HIGH TEMPERATURE ON STARCH GRANULE NUMBER AND SIZE

Warming the plants during the early part of linear grain growth significantly reduced the number of starch granules, an effect which was evident only at the later stages of grain growth. The reduction in granule number was confined to B-type granules and high temperature virtually had no effect on the number of A-type granules. A-type granules are initiated during early stages of grain growth and continue to grow well after the time of their initiation (Section 1.4.2.1.2). Thus although high temperature treatments reduced starch synthesis late in grain development and did not affect the number of A-type granules, it might well have affected their growth (see Discussion in Section 3.5). The observations made in this study support the observations made by other workers (Hoshikawa 1962; Moss 1963) that A-type granules in the grain from the plants grown at high temperature appeared smaller than in the control plants. The effect of high temperature on the number of B-type starch granules closely resembles the effects of mineral deficiency (Buttrose 1958), shading (Brocklehurst et al. 1978), and water stress (Brocklehurst et al. 1978; Brooks et al. 1982). Since the number of A-type starch granules under various stress conditions remains relatively unaffected while it is the number of B-type granules which is affected, Buttrose (1960) has suggested that the number of A-type granules is genetically fixed whereas the number of B-type is dependent on the prevailing environmental conditions during grain growth. Although work by Gleadow and co-workers (1982) have shown that there are differences in the number of A-type granules among the cultivars having different grain weight at maturity, critical evidence to support Buttrose's claim is lacking. No inference in this regard can be drawn from the present work, as no significant differences in the number of A-type granules among the cultivars were found. Alternatively, the relative stability in the number of A-type granules to adverse

environmental conditions reported by various workers could simply be due to the fact that the number of these granules is determined very early in grain's development and environmental stress in these reports did not develop early enough or was not severe enough to reduce the number of A-type granules.

4.5 INDEPENDENT REGULATION OF STARCH AND PROTEIN ACCUMULATION IN THE GRAIN

It is well known that the final concentration of nitrogen in the grain is generally higher at high temperature (Sofield et al. 1977b), and examples of nitrogen concentration increasing under unfavourable conditions of grain growth are quite common (see introduction to Section 3.5). There are several reports in the literature where a significant increase in nitrogen in the grain occurred without significantly affecting grain weight (Konovalov 1966; Jenner 1979, 1980; Radley and Thorne 1981; Thorne 1981). In the present study, it was evident that warming the ears (expts. 7 and 8) or whole plants (expts. 11 and 12) during the grain filling period significantly reduced total grain dry weight but had no effect on protein content of the grain. The above reports and these observations support the hypothesis that the processes regulating the accumulation of starch and protein in the grain are independent of each other (also see Barlow et al. 1983).

Concerning the causes of this differential response of starch and protein accumulation, it is relevant to recall that, in experiment 11, the rate of protein deposition in all the cultivars was accelerated relatively more than was total dry matter accumulation (Table 3.5.1), and in experiment 12 in cvv. Sonora and WW15 the total dry matter accumulation rate during the linear phase of grain growth was not altered by a change in the temperature, whilst the rate of protein deposition was strongly enhanced (Table 3.5.6). Furthermore, it can also be derived from the data of Sofield et al. (1974 their Fig. 1) that the accelerating effect of elevated temperature on nitrogen (protein) accumulation was maintained up to higher temperature than the temperature acceleration of total dry matter accumulation rate. From this report and the results in this investigation, it could be inferred that protein deposition might show a higher temperature optimum than starch accumulation, and that the high temperature response in the rate of total dry matter accumulation is due mainly to the lower optimum for

starch. One other important observation from the experiments in Section 3.5 is the effect of high temperature on starch and protein accumulation during the post-temperature treatment period. High temperature treatment, in experiment 11, had no effect on the rate of protein deposition whereas the rate of non-protein dry matter (mostly starch) was apparently reduced. Similar response was observed in cvv. RC-81-429 and Kite in experiment 12, but in cvv. Sonora and WW15 the temperature acceleration of protein deposition rate and a reduction in the duration were counterbalanced because the final grain protein did not differ between temperature treatments. However, cases where there is no such a counterbalance have been reported in the literature. For instance in experiments of Sofield et al. (1977b) final nitrogen content in the grain was considerably lower at high temperatures. However, the experiments reported here differ from those reported by Sofield and co-workers in that here high temperature treatments were imposed only during a part of the grain filling whereas in their experiments high temperatures were maintained from a few days after anthesis until maturity.

Starch and protein accumulation in the wheat grain differs in at least two important respects.

1. The influx of sucrose into the grain is restricted during the final stages of its passage from the ear into the grain (Jenner 1976b), whereas parallel studies on the transport of amino acids have not shown such restrictions to the inflow of amino acids into the grain. For example, Barlow et al. (1983) have shown that by culturing detached ears of wheat at various levels of L-glutamine, it is possible to achieve higher levels of both soluble nitrogen (amino acids) and protein in the grain above the levels normally observed in the grains from the intact plant, suggesting that protein synthesis under conditions of normal grain growth is substrate limited.

2. Starch and protein accumulation, within a cell, are spatially separated. Starch accumulates inside the amyloplast, for which the passage of the sucrose (or derivatives) through a double membrane would seem to be a pre-requisite before its conversion into starch. Protein synthesis, on the other hand, occurs on the rough endoplasmic reticulum for which no such additional transport step for amino acids is required. Alternatively, the synthesis of starch takes place inside the envelope, the functional integrity of which would appear to be important, whereas the enclosure of protein in a membrane is a post-synthesis process

(Bechtel et al. 1982; Campbell et al. 1981) and also the storage of protein seems to involve less anatomical organisation than starch.

To explain the increase in the rate of protein synthesis during the period of high temperature, it is possible that high temperature increased the availability of amino acids (by enhancing the senescence of leaves) and also raised the rate of conversion into protein. Why the duration of protein accumulation was relatively less affected as a result of warming needs another kind of explanation.

Chloroplasts and amyloplasts are homologous in their function in many ways ; are interconvertible (in some species, under certain circumstances), and have a common origin as proplastids (see Whatley 1978 for details). Moreover, recently Scott et al. (1984) by using restriction-enzyme digestion and renaturation kinetics techniques have shown that the DNA of potato-tuber amyloplasts and of potato-leaf chloroplasts is essentially the same which would further support Whatley's suggestions. As already reported in Section 1.10, the chloroplast membrane is the most sensitive to high temperature, the mitochondrion less so, and the plasma membrane the least sensitive. If the situation for the amyloplast membranes is similar to that of the chloroplast membranes, then one possible explanation for the differential response of starch and protein accumulation in the grain during the post-treatment period could be that the membrane system of amyloplast and the membrane(s) involved in the synthesis and storage of protein have different susceptibilities to high temperature.

4.6 CONCLUSIONS

1. Both the rate and the duration of grain growth are affected by elevation in temperature during the grain filling phase, and temperature influences these attributes most likely due to its direct effects on processes operating within, or close to the grain.
2. Final grain weight is reduced as a result of warming for a brief period during grain filling because there is no compensatory increase in the rate of grain growth at high temperature to balance the reduction in the duration of grain growth.
3. Reduction in final grain weight at high temperature seems not to be mediated either through its effects on the water relations of the grain or the other parts of the ear, or through the effect of

temperature on the availability of assimilates for grain growth. It is more likely due the effects of temperature on the processes governing the capacity of the grain to accommodate dry matter and possibly through more direct effects of temperature on the processes related to the conversion of sucrose into starch.

4. The main constituents of mature grain weight, starch and protein, respond differently to increases in temperature during grain filling.
5. Different processes operating in the grain seem to have different temperature optima. For example, the uptake of sucrose by the endosperm cells and respiration have higher optima than the processes related to the conversion of sucrose into starch.
6. High temperature during the grain filling phase not only reduces grain yield, but also affects the composition of the grain which determines the quality of the flour.

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