Effects of Zinc Nutrition and High Temperature on the Growth, Yield and Grain Quality of Wheat (*Triticum aestivum* L.)

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ABSTRACT

Wheat production is the largest enterprise within the Australian grain industry, with an annual gross value of production of approximately $4 billion. However high temperature stress (>35ºC) and zinc (Zn) deficiency in soils are a frequent occurrence across the Australian wheat belt and represent two of the most important environmental limitations to wheat production and grain quality. The work presented here has shown for the first time that Zn nutrition can provide wheat plants with a level of tolerance to high temperature stress.

Field trials, along with controlled environment studies, showed that supplementary Zn nutrition improved photosynthetic activity during a high temperature event, by stabilising chlorophyll initial fluorescence, Fo. Since increases in Fo under heat stress are associated with an increase in lipid fluidity of the thylakoid membranes at high temperature, the results suggested that adequate Zn fertilisation could preserve membrane integrity during heat stress. Electron microscopy confirmed this hypothesis, and showed that adequate Zn nutrition could maintain the integrity of a number of cellular membranes during high temperature, including the tonoplast, chloroplast envelope and the thylakoid membranes.

Measurements of canopy temperature depression showed an improvement in the evaporative cooling of the canopy with supplementary Zn nutrition in the Zn inefficient varieties, suggesting better soil water extraction under warm conditions. Supplementary Zn nutrition also increased the kernel weight of plants grown under warm conditions in the field, however this was unrelated to the improvement in photosynthetic activity. Nevertheless, results from both controlled environment and field experiments demonstrated that the detrimental effects of low Zn availability and high temperature on the yield of Zn inefficient or thermosensitive wheat varieties will be most damaging when these stresses occur in combination.

Analysis of protein composition showed that supplementary Zn fertilisation increased

the glutenin:gliadin ratio in the grain. This suggests that Zn fertilisation may improve the bread-making quality of wheat under conditions of Zn deficiency. The results also showed a negative association between grain Zn concentration and the number of days over 35ºC during grain filling, which suggests that the negative effects of high temperature stress on grain protein composition will be compounded when plants are grown on soils of low Zn availability.

This thesis represents a valuable contribution to the understanding of the relationships between micronutrient supply and environmental stress. Further studies should be undertaken to establish whether the protective effect of Zn on the photosynthetic apparatus will be maintained under consecutive heat stress events, to determine the ways in which Zn ions stabilise and protect bio-membranes under heat stress and to confirm the positive effects of Zn on grain protein composition and baking quality.

DECLARATION

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by any other person, except where due reference has been made in the text.

I give consent to this copy of my thesis, when deposited in the University Library, being available for loan and photocopying.

……..Date……

Alison W Graham

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CHAPTER 1

GENERAL INTRODUCTION

The production of wheat (*Triticum aestivum* L.) for human consumption is the largest and most important enterprise within the Australian grain industry. Wheat production comprises over 50% of total grain production in Australia, with the majority processed into human food products (Panozzo and Eagles 1998). Australia produces approximately 20 million tonnes of wheat per annum, of which over two-thirds is exported. On average the wheat industry contributes approximately $4 billion in export revenue to the Australian economy each year (AWB Limited 2001, Department of Foreign Affairs and Trade 2004).

Although only a relatively small producer of wheat by world standards (accounting for around 3-4% of annual world production), Australia accounts for approximately 16% of the world's wheat trade. In 2001/02 Australia exported 15.9 million tonnes of the 24.5 million tonnes of wheat it produced, making it the world's second largest exporter of wheat after the USA (AWB Limited 2002). Export sales of Australian wheat are supported by highly targeted international marketing programmes, which have allowed Australia to maintain its strong competitive position in the international grain market. Various international customers commit the industry to providing quality wheat suitable for a variety of end-product uses.

Grain quality, in addition to grain yield, is therefore of prime importance to the Australian wheat industry. Quality in wheat can be defined as the ability of the grain to meet the requirements of the processor, and is evaluated by several variables including grain size, protein content and composition and starch composition (Panozzo and Eagles 1998). Both genotype and the environment in which the crop is grown, as well as the interaction between the two, have been found to affect the grain yield and quality of wheat.

Temperature and nutrition are two of the major components of environmental variation, and together provide significant limitations to successful crop production. Mineral nutrients are essential for plant growth and development through their fundamental roles in plant metabolism, while high temperature is prominent among the chief ecological factors that determine crop growth and productivity (Al-Khatib and Paulsen 1999). Many physiological processes in plants are impaired by high temperature stress, including photosynthesis, enzyme activity, membrane stability and ultimately growth (Nguyen and Joshi 1992).

Elevated temperatures are a major cause of yield and quality loss in cereal crops throughout many of the world's cereal growing areas, including North America, India and France, as well as Australia (Nguyen *et al.* 1989, Wardlaw *et al*. 1989a, Mullarkey and Jones 2000). Despite the fact that wheat is grown as a winter season crop in southern Australia, the occurrence of heat stress (maximum daily temperatures above 35°C) is a frequent phenomenon across the Australian wheat belt during the growing season, particularly during the grain filling period (Blumenthal *et al.* 1991a). Since wheat is a crop that is adapted to cool, moist growing conditions, and has an optimum temperature for grain growth of approximately 15°C (Paulsen 1994), it is not surprising that high temperature has been found to be one of the major environmental factors which limit both the quantity and quality of wheat production in Australia.

High temperature stress is often accompanied by a number of other environmental stress factors, including water stress, high solar irradiance and wind. While the interactions of heat stress and these other stresses have generally received a reasonable amount of discussion in the literature, the interaction of heat stress and nutritional stress (with the exception of nitrogen) seems to have received little attention. Australian soils are notoriously deficient in many of the micronutrients essential to plant growth, and zinc (Zn) is no exception. Zinc deficient soils are widespread throughout Australia, and they commonly occur in areas where crop plants are also subjected to heat stress (Donald and Prescott 1975, White and Zasoski 1999, Cakmak 2000).

Zinc is involved in a wide range of physiological processes within the plant cell, and

several of these are also associated with tolerance to high temperature stress. Zinc plays a key role in the maintenance of photosynthetic activity (Brown *et al*. 1993), the preservation of membrane integrity (Bettger and O'Dell 1981, Welch *et al*. 1982, Cakmak and Marschner 1988a) and the continuance of enzyme activity (Jyung *et al*. 1972, Seethambaram and Das 1985, Cakmak and Marschner 1988b), as well as being an important factor in a plant's defence against reactive oxygen species, which proliferate under various stress conditions, including heat stress (Cakmak and Marschner 1993, Cakmak *et al*. 1997, Yu *et al*. 1998, Obata *et al*. 1999). This suggests that adequate Zn nutrition may be important for maintaining plant productivity in high temperature environments.

There is little information concerning the relationship between Zn nutrition and heat stress available in the literature, and the interaction does not appear to have been studied before in plants to any depth. Therefore the present study was designed to investigate the possible role of Zn in providing thermotolerance to wheat plants. As part of this study, the physiological responses of wheat to Zn supply and high temperature during early vegetative growth and during grain filling were evaluated, and the effects of these two stresses on grain yield and quality were also examined. A secondary aim of the research programme was to investigate some of the genotype by environment interaction responses of wheat to Zn fertilisation under field conditions. Finally a preliminary analysis of some of the mechanisms by which Zn may provide wheat seedlings with tolerance to high temperature stress was conducted in two bread wheat cultivars differing in Zn efficiency.

CHAPTER 2
LITERATURE REVIEW

2.1 Introduction

The twin stresses of zinc (Zn) deficient soils and extreme high temperature often occur in combination throughout many of the world's cropping areas, and yet the possible interaction between these two stress factors has been largely overlooked. This review describes current knowledge of the independent effects of high temperature stress and Zn nutrition on the growth, grain yield and grain quality of wheat. The physiological responses of plants to heat stress are discussed, together with the genetic variation in these responses that exists between wheat cultivars. Consideration is given to the various roles of Zn as an essential plant nutrient, and some of the better-understood mechanisms responsible for genotypic variation in Zn efficiency are described. Particular attention is given to the effects of environment, genotype and the interaction between genotype and environment on grain yield and grain quality. Finally, the possible role of Zn in the provision of thermotolerance to plants under heat stress is addressed.

2.2 Heat stress and crop productivity

2.2.1 The cropping environment of southern Australia

Production of agricultural crops in southern Australia is very much influenced by the range of temperatures that occur during crop growth. Temperature is a significant factor that cannot be modified conveniently in broad scale agriculture by human intervention, and successful crop production has come to rely on matching plant growth patterns to the prevailing temperature regime (McDonald and Gardner 1996). Recent plant breeding techniques have assisted this to some extent, with the selection and development of crop cultivars that are well adapted to the diverse temperature ranges that exist throughout Australia. However high temperatures continue to impose limitations on crop yield, especially in wheat and other cereal crops, which are adapted to much cooler conditions.

The cropping environment of southern Australia is characterised by a Mediterranean-type climate, with cold, wet winters, followed by rising temperatures during anthesis and grain filling. Crops in these environments are therefore often subject to terminal heat stress. Attempts to maximise yields within such an environment have resulted in the identification of 'critical periods' for the growth and development of crops, which are largely defined by temperature and available soil moisture (Beech and Norman 1966, Kohn and Storrier 1970). However even if sowing takes place at the recommended time and crop growth falls within these critical periods, unavoidable high temperature events (maximum daily temperatures above 30°C) are often still experienced, particularly during grain fill, in many of the cereal growing regions of southern Australia (Table 2.1, Figure 2.1).

It has been estimated from a number of field trials and controlled environment studies that there is a reduction in the yield of wheat of 10-15% per year in both Australia and the US resulting from above optimum temperatures during grain filling (Wardlaw and Wrigley 1994). Increased temperatures between stem elongation and anthesis in wheat tend to reduce grain number, while high temperatures during grain filling reduce grain size. These yield reductions can be largely attributed to the twin effects of increased developmental rate and shortened developmental duration, which occur as a result of high temperature stress. Furthermore high temperature events following anthesis also affect wheat grain quality, reducing starch content and altering protein composition.

2.2.2 Growth responses to heat stress in wheat

2.2.2.1 Pre-anthesis growth

The optimum day/night temperatures for the pre-anthesis growth and development of wheat have been found to range from 15/10°C to 18/13°C (Wardlaw *et al.* 1989a). Temperatures above this optimum result in both an acceleration of plant development and a reduction in vegetative stage duration. Increases in the rate of plant development result in a rapid transition from one developmental stage to the next, and this causes a reduction in organ size and consequently an overall reduction in plant size. A shortened duration of vegetative growth is associated with a reduction in tiller and spike number per plant, and

spikelet and kernel number per spike (Marcellos and Single 1972, Sofield *et al.* 1977, Wardlaw *et al.* 1989a).

Table 2.1. Mean number of days with a maximum temperature of above 30°C, 35°C and 40°C recorded at a number of sites throughout the cereal growing areas of southern Australia. Averages were calculated from a minimum of 60 years of climatological data (Bureau of Meteorology 2002).

Location	Latitude (S)	Mean number of days with a maximum temperature of above								
		30°C			35°C			40°C		
		Oct	Nov	Dec	Oct	Nov	Dec	Oct	Nov	Dec
Victoria										
Horsham	36°65’	1.5	4.9	9.6	0.1	1.3	3.2	0.0	0.1	0.3
Birchip	35°98’	1.9	5.9	11.2	0.2	2.1	4.6	0.0	0.2	0.7
Walpeup	35°12’	3.4	8.0	14.2	0.6	2.5	5.8	0.0	0.5	1.1
New South Wales										
Wagga Wagga	35°05’	0.6	4.3	13.8	0.1	0.9	3.9	0.0	0.1	0.3
Gunnedah	30°98’	4.0	11.1	20.5	0.2	1.9	6.1	0.0	0.2	0.2
Narrabri	30°34’	8.0	15.9	24.3	0.9	4.2	11.3	0.0	0.3	0.7
South Australia										
Keith	36°10’	2.4	6.2	10.0	0.2	1.9	4.0	0.0	0.3	0.6
Lameroo	35°33’	3.0	7.5	12.2	0.4	2.5	5.0	0.0	0.3	0.8
Kadina	33°96’	3.5	7.8	11.4	0.4	2.6	4.9	0.0	0.3	0.6
Minnipa	32°84’	5.2	9.8	13.4	1.2	3.8	6.2	0.1	0.5	1.2
Western Australia										
Narrogin	32°93’	1.3	5.1	12.3	0.1	1.0	3.7	0.0	0.0	0.1
Wongan Hills	30°89’	4.9	11.4	21.1	0.8	3.5	9.5	0.0	0.1	1.8
Geraldton	28°80’	3.5	6.9	11.1	0.8	2.5	5.5	0.0	0.2	1.4

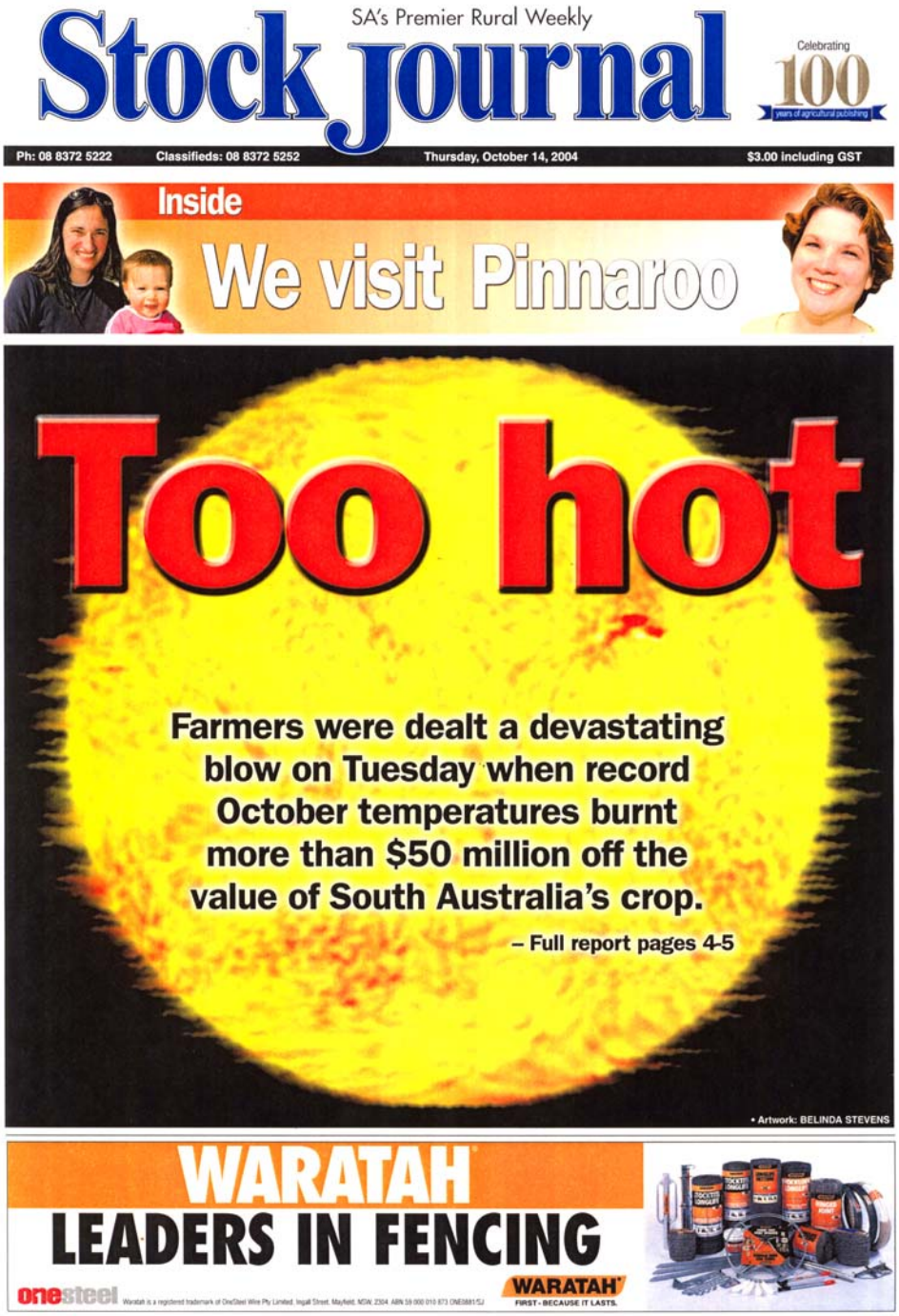


Figure 2.1. Despite all attempts to sow and produce crops within the best possible conditions, headlines such as this are still common during grain filling in many regions of southern Australia. (Reprinted courtesy of Rural Press Limited 2004).

High temperatures *during* anthesis significantly reduce the grain number of wheat plants at maturity, particularly when combined with high humidity (Tashiro and Wardlaw 1990a). This may be due to disrupted fertilisation of the embryo sac, which normally occurs within a few hours of pollination, or to abnormal ovary development resulting in impaired growth of the pollen tube (Saini *et al*. 1983). High temperatures experienced up to 3 days after anthesis have been found to induce parthenocarpy, abortion and shrinking of wheat kernels, while high temperatures from 6 to 10 days after anthesis cause notching, splitting and chalking of kernels (Tashiro and Wardlaw 1990a). Parthenocarpic kernels are caused by changes in hormone levels in the florets, while abortion and shrinking of the grain can be caused by abnormal cell division. Notching and splitting of kernels may be attributed to the differential growth of cells within the grain, while chalky kernels are related to variations in the starch and protein composition of the grain (Paulsen 1984).

2.2.2.2 Post-anthesis growth

An average post-anthesis temperature of 15°C is close to optimum for the attainment of maximum kernel weight (Chowdhury and Wardlaw 1978), with a general reduction in yield per ear of 3-4% for each 1°C rise above this optimum (Wardlaw *et al*. 1989a). As temperatures increase, even in the low temperature range (10-15°C), there is a reduction in the duration of grain filling, but at these temperatures the shorter duration is compensated for by an increased rate of grain filling, with little change in kernel weight at maturity. At higher temperatures however, the reduction in the duration of the grain filling period is no longer compensated for by an increase in rate, resulting in a reduction in grain weight, and therefore grain yield (Sofield *et al*. 1977, Jenner 1994). Even when water supply is non-limiting, as in irrigated wheat, yields of late plantings have been found to be low, with hastened crop development due to high temperatures during grain filling being a contributing factor (McDonald *et al*. 1983).

Starch is the major storage component in the wheat grain, comprising 65-70% of its dry weight (Morell *et al.* 1995). Starch consists of two types of carbohydrate polymer, amylose and amylopectin, and is deposited in the endosperm as discrete granules. Two types of granules, the larger, lenticular A-type granules, and the smaller, spherical B-type granules, predominate in the mature grain.

Reduced starch content accounts for most of the reduction in grain dry matter at high temperatures (Jenner 1994). This lower starch content has been shown to be due to the

reduced activity of both ADP-glucose pyrophosphorylase (AGP) and soluble starch synthase (SSS), two of the enzymes responsible for the conversion of sucrose to starch. Both of these enzymes are susceptible to heat inactivation at relatively low temperatures and may also have a low optimum temperature for maximum activity (Jenner *et al*. 1993, Keeling *et al*. 1994, Greene and Hannah 1998). This results in a reduction in the rate of starch synthesis during grain development or a failure of the rate of starch synthesis to increase sufficiently to compensate for the shorter grain filling duration (Rijven 1986, Denyer *et al.* 1994).

2.2.3 Quality responses to heat stress in wheat

Several variables contribute to wheat processing quality, two of the most important of which are protein content and composition, along with starch composition. AWB Limited segregates its wheat products into a total of seven milling grade classifications, which are based primarily on genotype and protein content (AWB Limited 2001). These characteristics determine the suitability of the flour for the production of various food products (Table 2.2).

Protein quantity is not the only factor that governs wheat quality for baking; the types of protein and their varying amounts are also important. The unique cohesive-elastic properties of doughs made from wheat flour are primarily due to the properties of the gluten proteins, which make up about 85% of the endosperm storage proteins (Kasarda *et al.* 1971). Gluten proteins fall into two broad groups for classification purposes – the gliadins, which are soluble in aqueous alcohol, and the glutenins, which are not. Each of these gluten proteins play a unique role in dough formation: the monomeric gliadin proteins are responsible for the extensibility of the dough, allowing it to rise during fermentation, while glutenin is a complex polymeric protein comprised of component subunits which give elasticity to the dough, preventing it from becoming over extended and from collapsing during fermentation or baking (Austin 1986).

Table 2.2. Classifications of AWB Limited's wheat products, based primarily on genotype and protein content (AWB Limited 2001).

Classification	Protein level	Products
AWB Prime Hard Wheat	≥ 13 %	High volume European breads Chinese-style yellow alkaline noodles
AWB Hard Wheat	≥ 11.5 %	European pan and hearth breads Middle Eastern style flat breads Chinese-steamed products
AWB Premium White Wheat	≥ 10 %	Middle Eastern flat and pocket breads Indian tandoori bread Asian baked products and noodles
AWB Standard White Wheat	< 10 %	European style loaf breads and rolls Middle Eastern and Indian breads Steamed breads Instant noodles
AWB Soft Wheat	≤ 9.5 %	Biscuits, cakes, pastries, snack foods and steamed buns
AWB Noodle Wheats (specific soft grained varieties)	9.5 – 11.5 %	Udon white salted noodles Chinese-style yellow alkaline noodles
AWB Durum (specific durum varieties)	10 – 13.5 %	Wet and dry pasta products North African couscous Middle Eastern flat breads

As the maximum daily temperature increases during the grain filling period, so too does the proportion of protein relative to starch within the grain endosperm. This phenomenon has been observed in both controlled environment (Kolderup 1975, Bhullar and Jenner 1985, Blumenthal *et al*. 1991a, Stone *et al*. 1997, Corbellini *et al*. 1997) and field studies (Randall and Moss 1990, Blumenthal *et al*. 1991b). In addition, statistical models, developed using historical grain and climate data from throughout South Australia for the years 1971-1991, showed that the number of days in October with a maximum temperature of above 30°C were positively correlated with an increase in wheat grain protein levels (Correll *et al*. 1994).

To some extent protein content and composition is genetically determined, since wheat cultivars differ in their capacity to accumulate protein, particularly the high molecular

weight protein subunits of glutenin (Payne 1987). However the environment has been shown to be the major determinant of both grain protein quantity and quality. From a study of 204 lines from eleven Interstate Wheat Variety Trials, Stoddard and Marshall (1990) concluded that genotype accounted for only 3-13% of the variance in grain protein concentration, with the major contribution being from environmental factors, including site. Environmental factors affect the rate of accumulation of the different seed storage protein subunits (Timms *et al*. 1981, Marchylo *et al*. 1990, Randall and Moss 1990), and as a result, the same cultivar grown in two different locations, or even in the same location under different management strategies, may produce grain with different breadmaking qualities (Borghi *et al*. 1995).

2.2.3.1 Protein content

Under good growing conditions starch and protein are deposited in the grain simultaneously. Under conditions of high temperature however, yield is depressed and grain protein concentration is elevated. Bhullar and Jenner (1985) found that a reduced number of B-type starch granules were formed in the grain at high temperature, and they concluded from this that the increase in grain protein as a result of high temperature is primarily a result of reduced starch content in the grain, rather than an increased amount of protein per grain. Later studies have shown that as temperatures increase beyond 30°C both sucrose and protein deposition in the grain is reduced due to the shortened duration of grain filling, but sucrose deposition is reduced more so than protein deposition (Sofield *et al.* 1977, Bhullar and Jenner 1985, Jenner *et al*. 1991, Stone and Nicolas 1996a). This problem is then amplified as high temperatures suppress the conversion of sucrose to starch within the grain (Jenner *et al.* 1991).

2.2.3.2 Protein composition

Observations of crop statistics, together with field and glasshouse experiments, have indicated that as maximum daily temperatures increase up to 30°C, there is a general increase in dough strength, associated with the increase in grain protein content (Randall and Moss 1990, Blumenthal *et al*. 1991b, Stone *et al*. 1997). As dough strength increases so too does breadmaking quality. High dough strength is associated with a long development time, a slow rate of breakdown and a high resistance to extension, while

doughs with very short development times and low resistance to extension generally perform poorly in breadmaking (MacRitchie *et al*. 1990). A decline in dough strength has been found to occur in response to just a few days of maximum temperatures above 32°C, despite the fact that protein content may continue to increase (Finney and Fryer 1958, Blumenthal *et al*. 1991a, Wrigley *et al*. 1994, Borghi *et al*. 1995). This weakening of dough properties occurs as a result of changes in protein composition associated with high temperatures during grain filling, with these doughs having lower extensibility than doughs from grains of similar protein content produced at lower temperatures (Archer and O'Brien 1987).

Seed storage proteins in wheat can be broadly classified into four groups: the albumins, which are soluble in water; globulins, which are insoluble in water but soluble in dilute salt solutions; gliadins, which are insoluble in water and dilute salt solutions but are soluble in 70% ethanol; and glutenins, which are insoluble in 70% ethanol but are soluble in dilute acid or alkali solutions (MacRitchie *et al.* 1990). The gliadins and glutenins together form the gluten, which possesses the unique visco-elastic properties of doughs produced from wheat flour. Glutenins are further divided into high molecular weight (HMW) and low molecular weight (LMW) glutenin subunits, based on their separation by polyacrylamide gel electrophoresis.

The ratio of gliadin to glutenin proteins in wheat is generally reported to increase in response to high temperatures during grain filling (Blumenthal *et al*. 1991a, 1993, Stone *et al*. 1997), and increases in gliadin:glutenin ratios are known to result in reduced dough strength (Wrigley 1994). These increases in the gliadin:glutenin ratio have been found to be influenced by both genotype and environment, particularly the timing of heat stress. Stone and Nicolas (1995a) in a survey of 75 wheat genotypes, found that some varieties showed an increase in gliadin:glutenin ratios when heat-treated early in grain filling and a decrease when exposed to heat stress later, while the opposite was found to occur in other varieties.

Some authors have suggested that the increase in the gliadin:glutenin ratios observed as a result of heat stress occurs because the accumulation of glutenin decreases more than the accumulation of gliadins (which also decrease) (Graybosch *et al*. 1995, Stone *et al*. 1997).

This conclusion was based on the observation that gliadins increased as a proportion of total flour protein, but their accumulation on a per kernel basis was found to decrease in response to increased temperature. In general, gliadin accumulation within the wheat endosperm has been found to be less sensitive to heat stress throughout the grain filling period than glutenin accumulation (Stone and Nicolas 1996a).

Blumenthal and co-workers (1995a, 1998) offer another explanation for the loss of dough strength as a result of high temperature. They observed a decrease in the size of glutenin polymers in the mature grain in response to a heat stress treatment, and suggest that this may be due to the heat sensitivity of the enzymes involved in the formation of the disulphide bonds which link the glutenin polymers, such as protein disulphide isomerase. High temperatures therefore restrict the formation of the complex protein aggregates responsible for superior dough mixing properties (Corbellini *et al*. 1997).

The number and size of subunits within the glutenin polymer is also affected by high temperature stress, resulting in a decrease in grain quality. Huebner and Wall (1976) were among the first to show that the ratio of HMW to LMW subunits of glutenin could be used to indicate baking quality. An increased proportion of HMW glutenin subunits results in a higher proportion of elastic high molecular weight polymers within the total amount of glutenin, which in turn produce flours with increased dough strength (Gupta *et al*. 1993). Heat stress has been found to restrict the synthesis of these HMW subunits of glutenin, with the resulting flours having weaker dough properties (Blumenthal *et al*. 1994, Wrigley 1994, Stone and Nicolas 1996a, Corbellini *et al*. 1998). Stone and Nicolas (1996a) found that the synthesis of the intermediates of sodium dodecyl sulphate (SDS)-soluble polymers (containing mostly LMW glutenin subunits) continued unimpeded during heat stress, while the synthesis of the intermediates of the SDS-insoluble polymers (containing mostly HMW glutenin subunits) was reduced, resulting in a reduction in the percentage of SDS-insoluble polymer in total polymer.

It is now known that the HMW subunits of glutenin have both quantitative and qualitative effects on breadmaking quality, with variation in baking quality correlated with allelic variation in HMW subunit composition (Payne 1987). The loci encoding the HMW glutenins are designated *Glu-A1*, *Glu-B1* and *Glu-D1* for their positions on the long arms

of chromosomes 1A, 1B and 1D, respectively. Correlation studies have indicated that strong or weak dough properties are associated with the combination of specific HMW subunits. For example, the alleles of the D genome (encoding either the 5+10 subunit combination or the 2+12 subunit combination) have been found to provide much contrast in breadmaking quality. There is a general tendency among Australian wheat varieties for cultivars with the *Glu-D1a* allele (coding for subunits 2+12) to have a lower gliadin:glutenin ratio and stronger doughs than those cultivars with the *Glu-D1d* allele (coding for subunits 5+10) (Wrigley *et al.* 1982, Blumenthal *et al.* 1995b, Stone and Nicolas 1996b). Furthermore it has been shown that wheats having the *Glu-D1a* allele tend to be more sensitive to the dough weakening effects of heat stress, when compared with those with the *Glu-D1d* allele (Blumenthal *et al.* 1995b, Panozzo and Eagles 2000). Gupta *et al.* (1996) observed that cultivars with the *Glu-D1d* allele accumulate large polymers more quickly than those with the *Glu-D1a* allele, and it may be hypothesised that this gives these cultivars an advantage when protein deposition coincides with a period of high temperature stress.

2.2.4 Effects of variation in episodes of heat stress

The timing and duration of high temperature stress during grain filling has been shown to influence both the quantity and quality of wheat production significantly. In general, these studies on the effects of post-anthesis temperature have fallen into one of two categories: (1) responses to sustained periods of moderately high temperature (25-32°C) (eg. Chinoy 1947, Wardlaw *et al*. 1980, 1989a); and (2) responses to shorter periods of very high temperature (>32°C) (eg. Finney and Fryer 1958, Randall and Moss 1990, Blumenthal *et al*. 1991a,b, Stone and Nicolas 1994, 1995b).

Sustained periods of moderately high temperature from early in grain fill until maturity have been found to have their greatest influence on grain yield, through a reduction in final grain weight due to the reduced duration of grain filling (Chinoy 1947, Marcellos and Single 1972, Sofield *et al*. 1977, Wardlaw *et al*. 1980, 1989a, Tashiro and Wardlaw, 1989). Grain quality is generally not reduced by these moderately high temperatures since the increase in grain protein content caused by the shorter duration of grain filling usually

results in increased dough strength and therefore enhanced breadmaking quality (Sosulski *et al*. 1963, Randall and Moss 1990, Borghi *et al*. 1995). Short periods of very high temperature (>32°C) during the grain filling period however have been found to have a significant effect on *both* grain yield and protein composition, although this is dependent on the duration and timing of the heat stress, as well as the genotype.

2.2.4.1 Duration of heat stress

Stone and Nicolas (1998b) found that a high temperature treatment (40/19°C day/night) imposed for as little as 1 day reduced mature individual kernel mass by 14% in a heat sensitive variety and by 5% in a heat tolerant variety. However, each additional day of heat treatment after the first (up to 10 days) reduced kernel mass by the same amount in both varieties (1.6%). It is suggested that this may be due to a reduction in yield potential, so that 1 day of heat treatment may reduce the capacity of grains to accumulate dry matter to the extent that responses to subsequent high temperature events are lessened, as they occur against a lower potential for yield. The greatest damage to yield potential therefore occurs during the initial exposure to high temperature; however, it remains unknown whether greater damage will occur when heat stress events are intermittent, as occurs in the field, rather than as one uninterrupted sequence. Stone and Nicolas (1998b) also found that, for a given duration of heat treatment, the difference in response of the two varieties was constant (9%), indicating that the varietal difference in heat tolerance was maintained for both brief and extended periods of very high temperature.

2.2.4.2 Timing of heat stress

Some conflicting evidence exists concerning the time at which the heat stress occurs during grain fill and the resultant effect on grain weight. Stone and Nicolas (1995b) suggest that mature individual kernel mass is most sensitive to heat stress applied early in grain filling, and becomes progressively less sensitive throughout the grain filling period. This conclusion is supported by Panozzo *et al*. (1998), who found that heat stress applied at 0-14 days after anthesis (DAA) produced a more significant effect on grain yield and kernel weight than heat stress applied at either 15-28 or 29-42 DAA. Other authors, however, have observed that the effects of the timing of the heat stress event differ, depending on its duration. Corbellini *et al*. (1997) found that a heat treatment of 10 days

duration produced a greater reduction in grain weight when applied from 7 or 17 DAA than when applied from 27 DAA. However a heat treatment of only 5 days duration had a greater effect on grain weight when applied from 27 DAA, than when applied earlier in grain filling. Genotypic effects are also important, with some cultivars showing no reduction in grain weight in response to a period of high temperature (Stone and Nicolas 1995a).

Protein accumulation and composition, and therefore dough quality, are also affected by the timing of heat stress during grain filling, and again the response varies among genotypes. Randall and Moss (1990) found that a heat treatment applied at 36 or 50 DAA had a more detrimental effect on dough strength than a heat treatment applied at 20 DAA. However, Stone and Nicolas (1996a) and Panozzo *et al*. (1998) both found that heat treatments applied early in grain filling had a greater effect on protein accumulation (as related to increased gliadin synthesis and reduced dough strength) than those applied in the later stages. Stone and Nicolas (1996a) conclude that cultivars vary in both the sensitivity of protein accumulation to heat stress, and the stage during grain filling at which maximum sensitivity to heat stress occurs.

2.2.4.3 Rate of temperature increase

Stone and Nicolas (1995c) showed that the individual kernel mass of a heat sensitive variety was reduced by a rapid increase in temperature from 20 to 40°C to a much greater extent than a gradual (6°C h^{-1}) increase over the same temperature range. For a heat tolerant variety however, there was no difference between the sudden and gradual heat treatments. They concluded that a gradual rise in temperature, which closely resembles field conditions, improves the thermotolerance of some varieties of wheat to short periods of heat stress, as this is a result of a short-term acclimation to high temperature that does not occur with a sudden exposure to heat stress. In addition, Stone and Nicolas (1998a) found that acclimation to heat stress under a gradual increase to high temperature can also reduce the effects of heat stress on fractional protein accumulation, and consequently grain protein composition at maturity. As with grain yield, the ability of protein accumulation to acclimate to high temperatures varied between genotypes.

2.2.4.4 Cumulative hours of heat stress

Blumenthal *et al*. (1991a,b) found that reduced dough strength was related to the number of hours over 35°C during grain maturation, and this was attributed to an increase in gliadin synthesis at the expense of glutenin synthesis. Graybosch *et al*. (1995) also found that protein quality (as measured by SDS sedimentation volume as an indication of dough strength and loaf volume) was related to cumulative hours above 32°C, and additionally, they observed that optimal protein quality was produced with exposure to less than 90 hours of temperature greater than 32°C. These authors also discovered that hours of high temperature above 32°C during grain fill was associated with SDS volume in a curvilinear fashion - increasing temperature stress initially had a positive influence on loaf volume and baking quality, but when the stress exceeded 90 hours there was a strong negative influence on baking quality (Peterson *et al*. 1998).

2.2.5 Physiological effects of heat stress

Many important physiological and biochemical processes in plants are impaired by heat stress, resulting in a decrease in growth, yield and grain quality of crop plants. Each plant species has its own temperature range for optimal function, with temperatures outside of this optimum being inhibitory to cellular metabolism and plant growth (Burke 1990). This species-specific temperature range has been referred to as the thermal kinetic window (TKW), and is defined as " the range of plant temperatures at which the apparent Michaelis constant, Km, is at or below 200% of the minimum observed value" (Burke *et al*. 1988). Temperature above those of the TKW induce changes in a number of physiological processes in plants, including photosynthesis, membrane integrity and enzyme stability (Nguyen and Joshi 1993).

2.2.5.1 Photosynthesis

The photosynthetic apparatus of plants is very sensitive to temperature, and is usually damaged before the visual symptoms of high temperature are manifested (Berry and Björkman 1980). In a survey of bread wheat cultivars obtained from worldwide regions of production, Al-Khatib and Paulsen (1990) found that a high temperature treatment of 32/27ºC day/night for 2 weeks from 14 DAS, or continuously from anthesis until maturity,

reduced mean photosynthetic rates at both the seedling and grain filling stages of growth. Aspects of both the light and dark reactions of photosynthesis are inhibited by high temperature, including photosystem II (PSII) activity, photophosphorylation capability and carbon dioxide fixation (Berry and Björkman 1980).

Photosystem II activity

The thylakoid membrane and reactions associated with it are particularly sensitive to high temperature (Santarius 1975, Smillie 1979, Berry and Björkman 1980). A number of studies have shown that the decline in photosynthesis in C_3 plants as a result of high temperature stress is primarily due to specific inactivation of the PSII complex on the thylakoid membrane, resulting in a decrease in the electron transport activity of the chloroplasts (Berry and Björkman 1980, Al-Khatib and Paulsen 1989, Mishra and Singhal 1993, Takeuchi and Thornber 1994). High temperatures result in an increase in thylakoid membrane permeability and associated alterations to PSII conformation due to hyperfluidity of the membrane lipid matrix and related changes in lipid-protein interactions (Berry and Björkman 1980, Gounaris *et al*. 1984).

PSII is a pigment-protein complex in which light energy is used to drive the transport of electrons and the oxidation of water to molecular oxygen. Several studies have demonstrated that the heat inactivation of the PSII complex is initiated by the destruction of the manganese cluster that catalyses the oxidation of water to molecular oxygen (Santarius 1975, Nash *et al.* 1985, Thompson *et al.* 1989, Mamedov *et al.* 1993, Enami *et al*. 1994). Other high temperature damage to PSII involves the denaturation of certain functional proteins (Thompson *et al*. 1989), a dissociation of the light-harvesting chlorophyll a/b binding proteins associated with PSII (LHCII) from the PSII core complex (Armond *et al.* 1980, Gounaris *et al.* 1984, Sundby *et al*. 1986) and an inhibition of electron transfer between the plastoquinone molecules Q_A and Q_B at the acceptor site (Cao and Govindjee 1990).

The mechanism of PSII inactivation under high temperature stress is somewhat more complex in mature plants compared with young plants, since high temperature injury in mature leaves is superimposed on senescence processes. Harding *et al.* (1990a) found that in the leaves of maturing wheat high temperature accelerates the breakdown of thylakoid components and induces an imbalance between component reaction rates. An imbalance

between PSII and cytochrome f/b_6-mediated activity is most damaging to intersystem electron transport capacity.

ATP generation

Photophosphorylation, the process by which ADP is phosphorylated to produce ATP, is also progressively inhibited at temperatures above 35°C. Stidham *et al.* (1982) found that high temperatures cause an uncoupling of electron transport that may result from an increased thylakoid permeability to protons. This causes a decrease in ATP production required for the regeneration of the CO_2 acceptor.

CO_2 fixation

High temperatures also affect other aspects of the photosynthetic process. The actual fixation of CO_2 is also decreased with increasing temperatures, as a result of the inhibition of the enzyme which catalyses the first step of CO_2 fixation, ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) (Weis 1981, Kobza and Edwards 1987). In addition, while the affinity of Rubisco for CO_2 is reduced with increasing temperature, the oxygenase activity of the enzyme appears to be enhanced, resulting in increased photorespiration and decreased net photosynthetic efficiency (Ogren 1984). Activation of Rubisco in the light is regulated by another stromal enzyme, Rubisco activase, and it is this enzyme which is inactivated at high temperatures, leading to decreased activation of Rubisco and therefore decreased CO_2 fixation (Feller *et al.* 1998).

Respiratory depletion of photosynthetic products

High temperatures also appear to limit the yield of crops by increasing the requirements of the maintenance component of dark respiration. This occurs because mitochondria are very stable at high temperatures in most plant species, and their activity increases over most of the temperature range to which plants are exposed (Paulsen 1994). As temperatures increase, maintenance dark respiration will consume a much larger proportion of the assimilate produced by photosynthesis, leaving little available for the synthesis of new growth. This may eventually increase to the point where the maintenance respiration requirement is equal to the gross photosynthetic product (McDonald and

Gardner 1996). In wheat, this increase in respiratory loss has been shown to account for approximately 25% of the reduction in yield at high temperature (Wardlaw *et al*. 1980).

2.2.5.2 Membrane integrity

One of the primary sites of physiological injury to plants by heat is thought to be the membrane (Raison *et al.* 1980, Blum 1988), with exposure to temperatures beyond that normal for growth resulting in membrane instability and irreversible changes in membrane structure (Nguyen and Joshi 1993). Changes in membrane fluidity during high temperature stress can result from either reorientation of membrane components, or from changes in lipid composition (Suss and Yordanov 1986). This can reduce the ability of the plasmalemma to retain solutes and water. A cell membrane system that can remain functional during heat stress may therefore be central to the adaptation of plants to high temperature (Raison *et al.* 1980).

Furthermore, it is not only a cell's plasma membrane that is subject to injury during heat stress; the membrane that surrounds the vacuole, the tonoplast, can also be affected (Weigel 1983). The tonoplast is relatively heat stable when compared with the photosynthetic membranes (Weigel 1983), however under heat shock conditions the integrity of this membrane can also be affected, and its permeability has been shown increase (Santarius *et al.* 1991).

Damage to the chloroplast by high temperature is largely due to detrimental effects on the envelope membranes (Bauer and Senser 1979, McCain *et al.* 1989) and the thylakoid membranes (Krause and Santarius 1975, Armond *et al.* 1980). Lesions in the chloroplast envelope caused by short periods of very high temperature result in a loss of chloroplast contents, including water and stromal enzymes (Bauer and Senser 1979, McCain *et al.* 1989). Damage to the thylakoid membranes primarily results in the inactivation of water splitting PSII activity, but also in an uncoupling of ATP-forming photophosphorylation, and changes to H^+ uptake into the thylakoids (Krause and Santarius 1975, Al-Khatib and Paulsen 1989). As discussed above, the thylakoid membranes, particularly the PSII component, are one of the most heat sensitive sites within the cell, sustaining damage before stomata malfunction, denaturation of the stromal enzymes or the loss of cell compartmentation integrity (Thebud and Santarius 1982, Al-Khatib and Paulsen 1989).

Electron microscopy of chloroplast ultrastructure has revealed that high temperature-induced inhibition of PSII activity is associated with an increase in thylakoid luminal volume, a progressive unstacking and rearrangement of the grana and a degradation of the light-harvesting chlorophyll *a/b*-binding proteins associated with PSII (LHCII), which are components of the thylakoid membrane (Gounaris *et al.* 1983, Sayed *et al.* 1986, Xu *et al.* 1995). It has been suggested that these structural alterations in the thylakoid membrane result from changes in lipid-protein interactions associated with increased lipid fluidity at high temperature, causing disruption of the supramolecular organisation of PSII (Berry and Björkman 1980). Furthermore there are observations that the close lipidic environment of PSII is of structural importance (Webb and Green 1991) and that changes in membrane lipid composition are associated with changes in PSII thermostability (Thomas *et al.* 1986, Mishra and Singhal 1992, Mamedov *et al.* 1993).

2.2.5.3 Enzyme stability

As discussed above, the TKW for a particular species is sometimes used to indicate the bounds of thermal stress in plants, since this is the temperature range for optimal enzyme function (Burke 1990). The TKW for wheat has been shown to be in the range of 17.5 to 23°C, based on the Km of glyoxylate reductase (Burke *et al*. 1988). It is interesting to note that the ability of plants to withstand high temperatures is somewhat regulated by soil water availability. Irrigated plants have been shown to have the ability to maintain their leaf temperatures within their TKW for optimal enzyme function, whereas rainfed plants did not have this ability. However this is only possible for crops growing in semi-arid environments with low humidity; plants growing in a more humid environment may not be able to maintain their foliage within the appropriate thermal range due to restricted transpirational cooling (Upchurch and Mahan 1988, Burke 1990).

The activity of several enzymes involved in starch and protein synthesis has been found to decrease considerably with increasing temperature stress, leading to a reduction in grain weight and quality. The various roles of these heat sensitive enzymes have been discussed above, the most important of which appear to be soluble starch synthase, responsible for the conversion of sucrose to starch, and protein disulphide isomerase, responsible for the formation of the disulphide bonds which link together the glutenin polymers. Nitrate reductase, an enzyme important in regulating N metabolism, has also been found to

decrease in activity in wheat plants subjected to increasing high temperature (Al-Khatib and Paulsen 1984). Furthermore, as noted above, the activity of Rubisco is also down regulated at high temperatures due to the inhibition of Rubisco activase, resulting in a decrease in overall dry matter production.

Photosynthetic activity is further reduced under high temperature conditions as a result of decreased chlorophyll biogenesis. The synthesis of two precursor molecules of chlorophyll, 5-aminolevulinic acid and protochlorophyllide, is impaired at high temperature, due to the inhibition of several enzymes involved in the biosynthetic pathway (Feierabend 1977). These enzymes include 5-aminolevulinic acid dehydratase and porphobilinogen deaminase (Tewari and Tripathy 1998), which may be impaired or post-translationally modified as a result of heat stress.

2.2.5.4 Oxidative stress

Reactive oxygen species (ROS), such as the superoxide anion radical ($O_2\cdot^-$), hydrogen peroxide (H_2O_2) and the hydroxyl radical ($\cdot OH$), are by-products of normal cell metabolism that can damage many cellular components, including lipids, proteins and nucleic acids (Mead 1976, Brawn and Fridovich 1981, Fucci *et al.* 1983). The conditions leading to damage caused by ROS are referred to as oxidative stress, which can lead to an inhibition of photosynthesis and respiration, and therefore, plant growth. Plants have evolved well-developed defence mechanisms against these ROS, involving enzymatic and non-enzymatic scavenging systems. Under unstressed conditions, the formation and removal of ROS are in balance. Under stress conditions however, including heat stress, the defence system can be overwhelmed, and is then unable to remove the additional ROS with increased enzymatic or non-enzymatic antioxidant processes (Bowler *et al.* 1992).

Of the non-enzymatic antioxidant constituents, glutathione and ascorbic acid are the most important (Noctor and Foyer 1998), while superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT) and glutathione reductase (GR) are key enzymatic antioxidants. SOD catalyses the first step in the scavenging system of ROS by the dismutation of $O_2\cdot^-$ to H_2O_2 and O_2. H_2O_2 is then broken down to H_2O and O_2 by CAT or APX. GR can also remove H_2O_2 via the ascorbate-glutathione cycle (Fridovich 1986, Scandalios 1993). SOD has multiple isoforms, which are classified by their metal cofactor: copper/zinc (Cu/Zn), manganese (Mn) and iron (Fe). Mn-SOD and Fe-SOD are

structurally very similar, while Cu/Zn-SOD is not related. In higher plants, Cu/Zn-SOD is found mainly in the chloroplasts (stroma and thylakoid-bound) and the cytosol, while Mn-SOD is predominantly in the mitochondrial matrix (Fridovich 1986). Cu/Zn-SOD and Mn-SOD are found among all plant species, while Fe-SOD, which is located in the chloroplasts, was first thought to be characteristic of dicotyledonous plant species only. Recently, however, Kaminaka *et al.* (1999) have reported the isolation of a cDNA encoding Fe-SOD from rice (*Oryza sativa* L.).

Within a cell, the SODs constitute the first line of defence against ROS. The superoxide anion is produced at any location where an electron transport chain is present, and hence O_2 activation may occur in different component of the cell (Elstner 1991), including mitochondria, microsomes, glyoxysomes, peroxisomes, apoplasts and the cytosol. However the chloroplasts are potentially the most powerful source of oxidants in plant tissues (Foyer and Harbinson 1994). Chloroplasts generate highly active singlet oxygen ($^1O_2$) and $O_2\cdot^-$ via direct donation of excitation energy (in the case of $^1O_2$) or electrons (in the case of $O_2\cdot^-$) to oxygen from the photosynthetic electron transport chain (Bowler *et al.* 1992, Foyer *et al.* 1994). This $O_2\cdot^-$ is rapidly dismuted to H_2O_2 and O_2 by SOD that is associated with the thylakoids, while the H_2O_2 produced is quickly scavenged by a thylakoid-bound APX. Superoxide and H_2O_2 that diffuse away from the membrane-associated enzymes can be scavenged in the stroma (Allen 1995). Efficient removal of ROS from chloroplasts is critical, since H_2O_2 concentrations as low as 10μM can inhibit photosynthesis by 50% (Kaiser 1976). ROS have been found to trigger deleterious reactions that involve degradative processes of key chloroplastic components, including thylakoid proteins (Thompson *et al.* 1987, Casano *et al.* 1994), Calvin cycle enzymes (Mehta *et al.* 1992) and membrane lipids (Irigoyen *et al.* 1992).

Tolerance to high temperature stress in plants has been reported to be associated with an increase in antioxidant enzyme expression (Sen Gupta *et al.* 1993a, Foyer *et al.* 1994). Exposure of transgenic leaf discs from mature tobacco (*Nicotiana tabaccum* L.) plants that overexpress chloroplastic Cu/Zn-SOD to oxidative stress conditions for up to 6 hours resulted in very little loss in photosynthetic capacity after they were returned to ambient temperatures (>90% recovery), however when control plants were exposed to the same conditions virtually all photosynthetic activity was lost (Sen Gupta *et al.* 1993b). Furthermore, wheat genotypes with a higher level of SOD and APX activity were also

found to have a greater tolerance to high temperature stress than those genotypes with a lower level of antioxidant activity (Sairam *et al.* 2000).

2.2.6 Genetic variation in tolerance to heat stress

Temperature is one environmental variable that cannot be easily manipulated in the field, and therefore crops are often selected on the basis of their response to the temperature conditions of a particular region (Chowdhury and Wardlaw 1978). Some degree of heat tolerance may therefore already exist in many common wheats, since selection for performance in warm environments will have screened out any genotypes susceptible to high temperature (Rawson 1986). Kumar *et al*. (1995) demonstrated some of the genetic variation in heat tolerance that exists between wheat genotypes in their study of 40 cultivars sown under high temperature conditions. A reduction in several yield components was observed in a number of genotypes, including number of days to heading, tillers per plant, plant height, spikelets per spike, grains per spike and grain per yield plant. However no such response was observed in other, more thermotolerant, genotypes.

When assessing genotypic differences in tolerance to high temperature, consideration must be given to the developmental stage at which the heat stress was imposed, the duration of the heat stress and the criteria used for evaluating tolerance (Paulsen 1994). If the stress is applied prior to anthesis, then heat tolerance may be associated with a high kernel number per spike, as found by Shpiler and Blum (1991) in an observation of 21 wheat cultivars growing under hot, irrigated conditions. Kernel number per spike was positively correlated with a longer duration from emergence to the double ridge stage.

Varietal differences in kernel weight under heat stress have been shown to be the result of differences in tolerance of both the rate and duration of grain filling to high temperature (Stone and Nicolas 1995b). Since it is the duration of grain growth that is influenced to a much greater extent by the environment, it has been suggested that the rate of grain growth is the more important selection criterion to improve kernel weight and grain yield (Whan *et al.* 1996). Cultivars with the ability to fill grain quickly have the opportunity to compensate for the reduction in the duration of grain growth in environments where high temperatures after anthesis are common. Genotypic differences in the responses of the rate

of grain growth to high temperature have been attributed to differing temperature sensitivities of the starch synthesising enzymes (Caley *et al*. 1990, Jenner 1991).

The designation of wheat cultivars as tolerant or sensitive to high temperature has also been found to depend largely on the method of high temperature application under controlled environment conditions. As discussed above, Stone and Nicolas (1995c) showed that a sudden increase in temperature reduced the kernel weight of a heat sensitive variety to a much greater extent than a gradual increase in temperature over the same range. There was no difference between the two treatments for a heat tolerant variety however.

Stone and Nicolas (1995a), in their study of 75 wheat cultivars subjected to short periods of heat shock conditions following anthesis, found genotypic variation in response to high temperature of the order of 20% for a number of yield and quality components, including individual kernel weight and protein composition. It is suggested that there is sufficient genetic variation in the response of wheat to brief periods of very high temperature to enable selection for a wide variety of responses in a number of yield and quality components. In addition, it was found that the responses to heat stress of a number of yield and quality components were largely independent. For example, kernel mass was found to be very sensitive to high temperature in some varieties, while the gliadin:glutenin ratio was little affected, and vice versa in other varieties. It is clear from these observations that it is difficult to make generalisations about the effects of heat stress on wheat yield and quality.

In addition to increased kernel growth rate, numerous other characteristics have been associated with the resistance of wheat to high temperature, and several of these have been suggested as useful selection criteria in breeding for heat tolerance. For example, Saadalla *et al*. (1990) were able to use the genetic variation in cellular membrane thermostability to select genotypes at the seedling stage that were tolerant to post anthesis temperature stress. Moreover, Al-Khatib and Paulsen (1990) found that genotypes that were the most tolerant of high temperature had either a stable rate and/or a long duration of photosynthetic activity, as measured in the flag leaf, indicating the importance of heat tolerance of the photosynthetic apparatus for sustained grain filling under heat stress.

Blumenthal *et al.* (1995b) observed a range of grain and dough quality responses to heat stress that vary between genotypes, and they identified several markers as potentially useful in breeding for heat tolerance, including the presence of glutenin subunits 5+10 at the *Glu-D1* locus, decreases in the gliadin:glutenin ratio and increases in the percentage of very large glutenin polymers. Accumulation of heat shock proteins (HSPs) has also been found to differ between genotypes, with thermotolerant wheat genotypes able to synthesise a greater number of unique HSPs in response to heat stress than the thermosensitive genotypes (Krishnan *et al.* 1989).

Panozzo *et al.* (1998) have summed up the current knowledge of genotypic variation in heat tolerance and suggest that it is the environment which produces the largest effect on the quality parameters of grain yield, kernel weight, protein content and gliadin synthesis, while genotype influences grain hardness, milling yield, water absorption and glutenin synthesis. The interaction of cultivar and environment is therefore an important consideration, since both will affect final grain quality and its breadmaking characteristics.

2.3 Zinc and crop productivity

The role of Zn in biological systems was first identified by the French scientist Raulin, in 1869, who found that bread mould (*Aspergillus niger*) would not grow in the absence of Zn. Following this discovery, Zn was identified as a ubiquitous component of both plant and animal tissue (Brown *et al*. 1993) and by the early part of the 20th century the agricultural significance of Zn had been recognised. In 1926, Sommer and Lipman demonstrated that Zn was generally essential for plant growth, and by the mid 1930's the first identification of Zn deficiency in field conditions was reported (Chandler 1937).

2.3.1 Extent and degree of zinc deficiency in soils

2.3.1.1 Worldwide

Zinc deficiency in soils is now recognised as one of the most common micronutrient deficiencies worldwide (Figure 2.2), and has become a significant constraint to crop production, particularly for cereal crops produced on the calcareous soils of the arid and

semi-arid regions (Cakmak *et al.* 1998a). The major reason for the widespread occurrence of Zn deficiency in soils is low availability of Zn to plant roots, rather than low Zn content in soils. High pH and high levels of $CaCO_3$, and low levels of organic matter and soil moisture, are the soil factors primarily responsible for low availability of Zn to plants (Marschner 1993). Altogether approximately 50% of the soils used for cereal production worldwide contain low levels of plant available Zn (Sillanpää and Vlek 1985), including 10 Mha of cropped land in Turkey and 8 Mha in Western Australia (White and Zasoski 1999). In India and Pakistan between 50 and 70% of crop-growing soils are affected by Zn deficiency (Alloway 2002), as well as approximately one third of the vast area of China (White and Zasoski 1999).

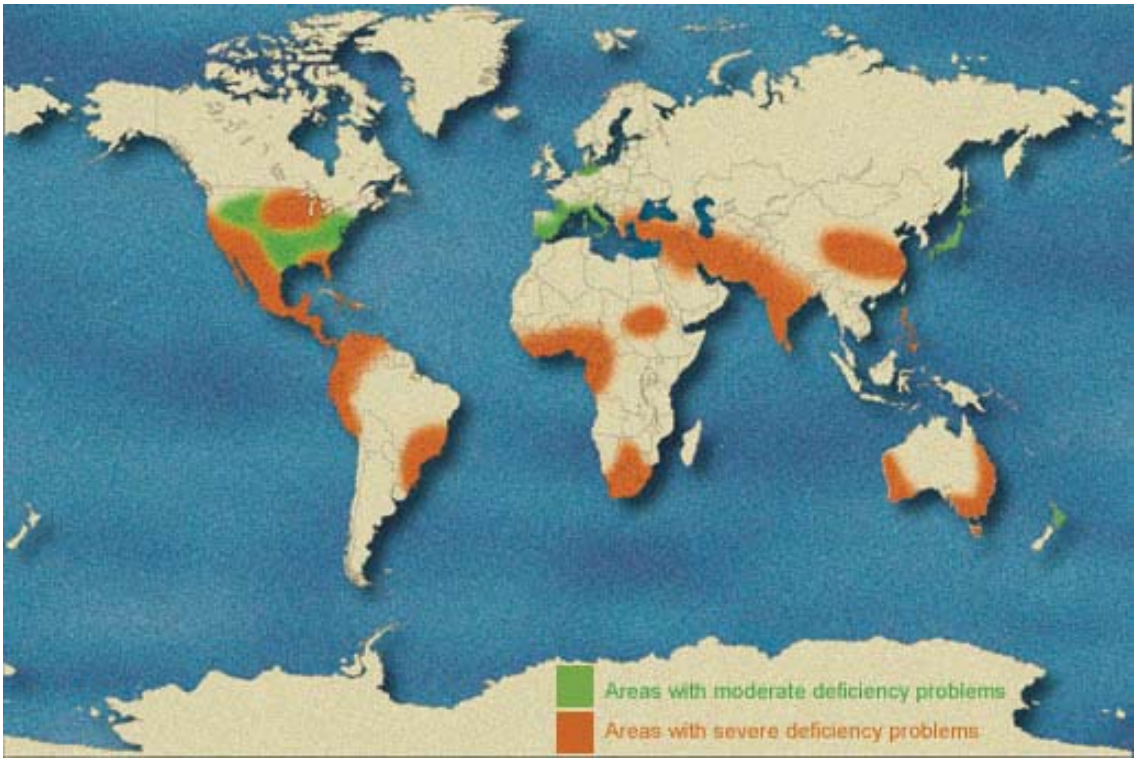


Figure 2.2 Worldwide distribution of Zn deficiency in soils (Alloway 2002).

2.3.1.2 Australia

Zinc deficiency in soils across Australia has been known for many years. The first field response to an application of Zn was in 1936, when zinc sulphate was applied as a foliar spray to citrus trees in Western Australia. A marked improvement in tree health was reported as a result (Pitman and Owen 1936). In 1948, severe Zn deficiency was reported

on the sandy soils of the Ninety Mile Plain, on the border of South Australia and Victoria (Riceman 1948), and in 1967 Zn deficiency in cereals grown on the Darling Downs, in south-eastern Queensland, was identified (Duncan 1967).

It is now acknowledged the Zn deficiency in soils can occur throughout the Australian wheatbelt, however it is most common on the alkaline soils of the Victorian Wimmera and South Australian Murray Mallee, as well as on the leached acid sands of Western Australia (Wilhelm *et al.* 1993). In a survey of the Zn concentration in barley (*Hordeum vulgare* L.) grains from each Hundred of South Australia, Spouncer *et al.* (1994) found that grain from the Murray Mallee, Eyre Peninsula and parts of Yorke Peninsula had the lowest concentration of grain Zn in the state, and in many cases these concentrations were in the critical deficiency concentration range of 10-15 mg kg^{-1} (Riley *et al.* 1992). Futhermore, modern management practices appear to have increased the extent and severity of Zn deficiency in Australia, as farmers change from using single superphosphate fertiliser on crops to high analysis fertiliser products (diammonium phosphate and triple superphosphate), which contain much lower quantities of Zn (Wilhelm *et al.* 1993).

2.3.2 Growth responses to zinc deficiency

Zinc deficiency in crop plants reduces both plant growth and ultimately crop yield, since Zn is involved in a large number of physiological and biochemical plant processes. A large decrease in wheat production due to Zn deficiency has been reported for several countries, including India (Takkar *et al.* 1975), Turkey (Cakmak *et al.* 1996a) and Australia (Graham *et al.* 1992).

2.3.2.1 Symptoms of zinc deficiency

In higher plants, typical visual symptoms of Zn deficiency include a shortening of the internodes, inhibition of leaf expansion, interveinal chlorosis and chlorotic bands with reddish-brown discolouration (Marschner 1995). In wheat, symptoms appear first on the lower half of the middle leaves, which originate halfway up the shoot. These symptoms commence as yellow chlorotic areas between the mid-vein and the leaf margin, and extend outwards towards the tip and base of the leaf (Grundon 1987). These chlorotic areas,

which eventually become necrotic, may remain discrete, producing many separate linear lesions, or may join together and involve the mid-vein. The latter expression of symptoms often results in the collapse of the leaf in the middle region, with the tip, base and margin of the leaf remaining green (Snowball and Robson 1983, Grundon 1987). When the deficiency is severe, these symptoms may also appear on the youngest leaves at the top and the oldest at the base of the shoot (Grundon 1987).

2.3.2.2 Diagnosis of zinc deficiency

Plant tissue analysis has become widely recognised as an effective tool for the diagnosis of the nutrient status of crop plants, and the information provided is used as a guide to nutrient management for optimal plant production. The critical deficiency concentration (CDC) for a particular nutrient has been defined as the nutrient concentration in the tissue where there is a 10% reduction in yield due to nutrient deficiency (Ulrich and Hills 1967). In practice, the CDC is not a single value, but rather a narrow range of nutrient concentrations, above which the crop is adequately supplied with nutrients, and below which the crop is deficient (Dow and Roberts 1982).

In wheat, the critical deficiency concentration has been reported to be 16-18 mg kg^{-1} dry weight, based on an analysis of the youngest fully emerged leaf blade (YEB) at both the seedling stage, and at anthesis (Riley *et al.* 1992, Wilhelm *et al.* 1993). However under some conditions this critical Zn deficiency concentration may be as low as 11 mg kg^{-1}, as found by Brennan (1992), using YEBs sampled at the sixth leaf stage. For diagnosis of past problems and planning for future action, the critical concentration of Zn in wheat grain has been found to be approximately 10-15 mg kg^{-1} (Rashid and Fox 1992, Riley *et al.* 1992).

2.3.3 Physiological effects of zinc deficiency

Zinc occurs in plants predominantly as a free ion, or as a complex with a variety of low molecular weight organic solutes. It may also be incorporated as a component of proteins, and although this may represent only a small amount of the total plant Zn content, it is these metalloproteins that represent the most important role of Zn in plant metabolism. As a component of proteins, Zn acts as a functional, structural or regulatory cofactor of a large number of enzymes, and many of the physiological effects resulting from Zn deficiency

are associated with the disruption of normal enzyme activity in certain tissues. The proportion of Zn involved in such metabolic reactions is termed the 'physiologically active' Zn, and has been used to distinguish between Zn efficient and inefficient genotypes (Cakmak *et* al. 1997). Reduced enzyme activity as a result of Zn deficiency can lead to impaired carbohydrate metabolism (a reduction in photosynthesis and decreased sucrose and starch formation), an increase in the permeability of membranes, accumulation of amino acids with a decrease in protein synthesis, reduced auxin levels, and a depression of male fertility (Brown *et al.* 1993).

2.3.3.1 Carbohydrate metabolism

Zinc deficiency appears to affect carbohydrate metabolism through its effect on both photosynthesis and sucrose and starch formation. Net photosynthesis can be reduced in Zn deficient plants by up to 70%, depending on species and the severity of the Zn deficiency (Brown *et al.* 1993). Several Zn dependent enzymes are involved in the various aspects of carbohydrate metabolism in plants, but although Zn deficiency may result in a decrease in enzymatic activity of most of these enzymes, only reduced activity in a certain few of these have been shown to result in a decrease in metabolic activity.

Carbonic anhydrase activity

Zinc is a component of the enzyme carbonic anhydrase (CA), which has been shown to decline in activity when plants encounter Zn stress (Randall and Bouma 1973, Ohki 1976, Gibson and Leece 1981). CA catalyses the conversion of CO_2 to HCO_3^- in the cytoplasm of the mesophyll cells of C_4 plants, thus providing substrate for phosphoenolpyruvate carboxylase. The function of CA in C_3 plants is less certain, however the enzyme is present within the chloroplasts, where it assists in converting HCO_3^- back to CO_2. This reaction will occur spontaneously, but is accelerated by CA. The enzyme is therefore usually present in excess of what is required for photosynthesis, resulting in a poor relationship between CA activity and the rate of photosynthesis in C_3 plants (Jacobson *et al.* 1975). The activity of CA is reduced in Zn deficient plants, but even when CA activity is low, maximum net photosynthesis can still occur (Randall and Bouma 1973). Sasaki *et al.* (1998) showed that CA functions to facilitate the supply of CO_2 from the stomatal cavity to the site of CO_2 fixation in rice leaves. The observation that Zn deficiency results

in impaired photosynthesis in all plant species, and yet the relative importance of CA to photosynthesis varies greatly between C_3 and C_4 plants, led Brown *et al*. (1993) to conclude that the primary role of Zn in photosynthesis could not be through its function in CA. Nevertheless, wheat is known to have a much lower CA activity than other species (Makino *et al*. 1992), and a decrease in CA activity in wheat under Zn deficiency may therefore result in a decrease in photosynthetic rate (Rengel 1995).

Activity of other enzymes

The activity of other Zn dependent enzymes of the photosynthetic process is also reduced in Zn deficient plants, including the activity of Rubisco, which catalyses the initial step of CO_2 fixation. This reduction in activity was first reported in navy bean (*Phaseolus vulgaris* L.) by Jyung *et al*. (1972), and later confirmed in rice and pearl millet (*Pennisetum americanum* L.) (Seethambaram and Das 1985).

Two other important enzymes of the Calvin cycle, fructose 1,6-bisphophatase and aldolase, are also Zn dependent enzymes, and the activities of both have been shown to be greatly reduced under Zn deficient conditions (O'Sullivan, 1970, Seethambaram and Das 1985). Fructose 1,6-bisphosphatase is a key enzyme in the partitioning of C_6 sugars in the chloroplasts and cytoplasm, while aldolase regulates the transfer of C_3 photosynthates from the chloroplasts into the cytoplasm (Marschner 1995). Depression of aldolase activity by Zn deficiency has reportedly resulted in a reduction in sucrose synthesis in some crops, including sugarbeet (*Beta vulgaris* L.) (Singh and Gangwar 1973) and maize (*Zea mays* L.) (Shrotri *et al*. 1980). Under Zn deficiency photosynthetic electron transport is reduced, and it is believed that the reduction in activity of these Calvin cycle enzymes may be due to lowered ratios of NADPH/NADP and ATP/ADP induced by this reduction in electron transport (Seethambaram and Das 1985).

There is some argument within the literature as to whether the Zn-induced changes in carbohydrate metabolism are responsible for the growth retardation and other visual symptoms associated with Zn deficiency. As discussed above, some authors have reported reduced sugar and starch formation under Zn deficiency, while others have found that sugars and starch accumulate in the leaves of Zn deficient plants, in crops such as cabbage (*Brassica olereacea* L.) (Sharma *et al*. 1982) and bean (Marschner and Cakmak 1989).

This may be due to impaired sucrose transport under Zn deficiency in some species, since any deficiency that slows shoot growth will decrease sink strength with feedback effects on sugar transport (Paul and Driscoll 1997, Pieters *et al*. 2001). In wheat, however, carbohydrates are preferentially partitioned to the roots to increase growth and therefore the surface area available for Zn uptake (Pearson and Rengel 1997).

Chlorophyll content and chloroplast structure

Reduced photosynthetic activity under Zn deficiency can also be a result of a decrease in chlorophyll content (Cakmak and Marschner 1993), and deleterious alterations to chloroplast structure (Brown *et al.* 1993). Electron microscopy has revealed that chloroplasts become swollen under Zn deficiency, and the number of thylakoids per granum stack is reduced (Thomson and Weier 1962, Shrotri *et al.* 1978, Gui-chang and Zhao-ming 1984). The orientation of the grana stacks in relation to the chloroplast envelope can also be altered (Thomson and Weier 1962), and in cases of severe Zn deficiency the chloroplast envelope ruptures (Wang *et al.* 1993). Again this damage to the chloroplast membranes would seem to be related to the role of Zn in maintaining the structure of biomembranes.

2.3.3.2 Membrane integrity

Several authors have demonstrated the role of Zn in maintaining the integrity and controlling the permeability of cellular membranes, including Bettger and O'Dell (1981), Welch *et al*. (1982) and Cakmak and Marschner (1988a). Welch *et al.* (1982) used root exudates as an indicator of root plasma membrane permeability in a number of plant species, including barley, sweet corn, subterranean clover (*Trifolium subterraneum* L.), tomato (*Lycopersicon esculentum* Mill.) and wheat, and found greater leakage of ^{32}P from roots of Zn deficient plants than from the roots of Zn adequate plants. Cakmak and Marschner (1998a) confirmed this result, and observed that Zn deficiency increased root exudation of K^+, amino acids, sugars and phenolics. Resupply of Zn to the deficient plants decreased the leakage of all solutes. This function of Zn is attributed to its stabilising and protective effect on membrane components, since Zn interacts with both membrane phospholipids (Von Glós and Boursnell 1981), and with the sulfhydryl groups of membrane proteins (Chvapil 1973).

Furthermore, Zn plays an important role in controlling both the generation and detoxification of ROS, which have the potential to damage membrane lipids and sulfhydryl groups, resulting in the increased leakage of solutes through increased membrane permeability (Römheld and Marschner 1991). This role of Zn in protecting cell components from oxidative stress is discussed at length below.

2.3.3.3 Protein metabolism

Both the rate of protein synthesis and the total protein content in the vegetative organs of plants have been found to be much reduced under Zn deficiency, while amino acids have been found to accumulate. Once Zn is resupplied to these plants however, protein synthesis resumes rapidly, which indicates a major role of Zn in protein synthesis (Cakmak *et al*. 1989). This inhibition of protein synthesis in Zn deficient plants is mainly caused by a sharp reduction in ribonucleic acid (RNA) concentration. It has been demonstrated that RNA declines under Zn deficiency for a number of reasons: (1) Zn deficiency causes a reduction in the activity of the Zn-containing RNA synthesising enzyme, RNA polymerase (Falchuk et al. 1978); (2) Zn deficiency is associated with higher rates of ribonuclease (RNase) activity and therefore RNA degradation is enhanced in Zn deficient plants (Dwivedi and Takkar 1974); and (3) the structural integrity of the ribosomes is reduced under Zn deficiency since Zn is a structural component of the ribosomes which have been found to disintegrate in the absence of the mineral (Prask and Plocke 1971).

A further effect of Zn deficiency on protein metabolism is via its involvement in DNA replication and transcription. Many nucleoproteins directly involved with replication and transcription of DNA are known to contain functionally important Zn atoms. The chromatin TFIIIA protein, essential for transcription, requires Zn in order to maintain the conformation of its DNA-binding domain (Hanas *et al.* 1983). Amino acid residues of the TFIIIA polypeptide chain form a tetrahedral complex with a Zn atom, thus generating a loop structure (zinc finger) containing the DNA binding domain of the protein. Similarly the $g^{32}P$ protein, which binds to and stabilises the conformation of single-stranded DNA so that it can serve as a substrate for replication and repair enzymes, also requires Zn to maintain the conformation of its DNA binding domain (Hanas *et al*. 1983, Giedroc *et al.* 1986, Vallee and Falchuk 1993). Zn is therefore directly involved in both the transcription

and translation steps of gene expression, and thus the total amount of protein is dramatically reduced in a Zn deficient plant (Brown *et al.* 1993, Marschner 1995).

2.3.3.4 Auxin metabolism

Another symptom of Zn deficiency is the lower concentrations of phytohormones in the shoots, particularly indoleacetic acid (IAA), which causes the symptoms of stunted growth and reduced leaf size that are typical of Zn deficient plants (Römheld and Marschner 1991). Lower concentrations of IAA are either due to enhanced oxidative degradation of IAA as a result of increased levels of oxygen radicals, or to decreased synthesis of IAA (Cakmak *et al.* 1989, Marschner 1995). High concentrations of tryptophan in the young leaves of Zn deficient plants may indicate an impaired conversion of tryptophan to IAA, but it is more likely that tryptophan accumulation is simply a result of inhibited protein synthesis under Zn deficiency (Cakmak *et al.* 1989), with lower IAA contents the result of increased oxidative degradation (Marschner 1995).

2.3.3.5 Reproduction

Flowering and seed production have been shown to be severely depressed in a number of crops under Zn deficiency, including wheat (Sharma *et al*. 1979). Small anthers with abnormal pollen grains were found to develop in wheat under Zn deficiency, while under severe Zn deficiency stress many anthers were empty and completely devoid of pollen grains (Sharma *et al*. 1979). It has been suggested that lower seed production under Zn deficiency is due either to enhanced formation of abscisic acid in the plant, causing premature abscission of both leaves and flower buds, or to disruption of the development and physiology of anthers and pollen grains (Brown *et al*. 1993). The growing tips of pollen tubes have been shown to have a particularly high requirement for Zn for protein synthesis (Ender *et al.* 1983), and this may further account for the reduction of seed set under Zn deficiency.

2.3.3.6 Oxidative stress

Many of the Zn deficiency-induced impairments in cellular function and integrity can be attributed to the enhanced levels of ROS that occur in Zn deficient plants. Zinc performs a

number of roles in protecting cells from damaging reactions caused by ROS, and under Zn deficient conditions there is both an increase in ROS generation and a decrease in ROS detoxification. The increase in oxidative stress that occurs in Zn deficient plants is responsible for the damage to membrane proteins, chlorophyll, nucleic acids, SH-containing enzymes and IAA that occurs under Zn deficiency, and therefore for much of the inhibition of plant growth (Cakmak 2000).

Generation of ROS

The generation of superoxide radicals has been found to occur at a higher rate under Zn deficiency as a result of increased activity of membrane-bound NADPH-oxidase. Zinc exerts a strong inhibitory effect on the generation of $O_2\cdot^-$ by this enzyme (Cakmak and Marschner 1988b) and this can be ascribed to one of two effects of Zn on enzyme activity. Either Zn can interfere with the enzymatic oxidation of NADPH by binding to it (Chvapil 1979a) or it can alter the redox properties of the NADPH-oxidising enzyme complex (Jeffery 1983). Toxic ROS produced by NADPH oxidases in Zn deficient plants destroy the double bonds of polyunsaturated fatty acids and phospholipids in the membranes, and increase the leakage of solutes (Cakmak and Marschner 1988a). Enhanced lipid peroxidation can lead to leaf chlorosis and necrosis, and inhibited shoot elongation, particularly under high light intensity (Marschner and Cakmak 1989).

Detoxification of ROS

Not only does an increased generation of $O_2\cdot^-$ occur in Zn deficient plants, but also a simultaneous impairment of the detoxification system of $O_2\cdot^-$, due to a decrease in the activity of Cu/Zn-SOD. As discussed above, Cu/Zn-SOD is the most abundant SOD in higher plants, while Mn-SOD and Fe-SOD form a smaller proportion of total SOD activity. Deficient supply of Zn has been found to decrease Cu/Zn-SOD activity in a number of plant species, including bean (Cakmak and Marschner 1993), wheat (Cakmak *et al.* 1997), cotton (*Gossypium hirsutum* L.) (Cakmak and Marschner 1988c), tobacco (Yu *et al.* 1998), lupin (*Lupinus angustifolius* L.) (Yu and Rengel 1999) and rice (Obata *et al.* 1999).

Under Mn deficiency, decreases in Mn-SOD activity have been found to be accompanied

by increases in Cu/Zn-SOD activity, but no such compensatory increase in Mn-SOD activity occurs in Zn deficient plants (Del Rio *et al.* 1978, Yu and Rengel 1999).

Cakmak *et al.* (1997) found that the activity of Cu/Zn-SOD in Zn deficient wheat leaves was closely related to the sensitivity of the various genotypes to Zn deficiency, and it is suggested that Cu/Zn-SOD activity can be used as a tool for assessing genotypic variation in sensitivity to Zn deficiency.

Reduced Cu/Zn-SOD activity accounts for a decrease in the detoxification of $O_2^{\cdot -}$ under Zn deficiency, but there is also a decrease in the detoxification of H_2O_2, since Zn is indirectly required for high activity of the enzymes involved in H_2O_2 detoxification, including CAT, APX and GR. Although Zn is not a required cofactor for CAT activity, the activity of the enzyme is reduced in Zn deficient plants. This is attributed to the increase in $O_2^{\cdot -}$, which inhibits the CAT enzyme (Cakmak and Marschner 1988c). Similarly, although APX and GR do not require Zn for their activity, the activities of both these enzymes are also reduced under Zn deficiency (Cakmak and Marschner 1993). This is due to impairment in the biosynthesis of these H_2O_2-scavenging enzymes, since Zn deficiency strongly reduces protein synthesis (Cakmak *et al.* 1989).

2.3.4 Genotypic variation in tolerance to zinc deficiency

Plants vary in their ability to grow in Zn deficient soils. This variation occurs not only between species, but also within a species. Tolerance to Zn deficient soils is usually termed "Zn efficiency", and is defined as the ability of a cultivar to grow and yield well in soils too deficient in Zn for a standard cultivar (Graham 1984).

Genotypic variation in Zn efficiency has been recognised and reported for a number of crops, including wheat (Shukla and Raj 1974, Graham 1988), maize (Clark 1978), oats (*Avena byzantina* K. Koch) (Brown and McDaniel 1978), tomato (Parker *et al*. 1992) and others. In the Zn deficient soils of southern Australia, Zn-efficient cultivars of wheat and barley have been found to yield up to three times that of Zn-inefficient cultivars (Graham 1988).

Several physiological mechanisms have been proposed to explain Zn efficiency in crop plants, including increased Zn uptake, increased Zn bioavailability in the rhizosphere due to the release of root exudates, and more efficient internal Zn use. However, these mechanisms of differential Zn efficiency among cultivars are still unclear, and it may be that more than one mechanism is responsible for the level of Zn efficiency in a particular cultivar (Graham and Rengel, 1993).

2.3.4.1 Zinc efficiency between species

Zinc uptake

Zinc efficient plant species have been found to possess higher rates of Zn uptake from the soil than Zn inefficient species, and this may account for the greater degree of Zn efficiency of these species. The higher Zn efficiency of rye (*Secale cereale*) in comparison to wheat is closely related to its greater Zn uptake capacity under Zn deficient conditions, while the greater capacity of bread wheats to take up Zn when compared to durum wheats accounts for the differences in Zn efficiency between these *Triticum* species (Rengel and Graham 1996, Erenoglu *et al.* 1999). However recent findings suggest that the variation in Zn efficiency that exists within a species (ie. between bread wheat cultivars) cannot be fully explained by cultivar differences in Zn uptake rate, since these vary little between cultivars (Erenoglu *et al.* 1999, Hacisalihoglu *et al.* 2001). The reason for a higher Zn uptake rate of some species is not well understood, but it has been shown that there is *de novo* synthesis of a 34 kDa polypeptide in the root-cell plasma membrane of a Zn efficient bread wheat genotype grown under Zn deficiency, which is not induced in a Zn inefficient durum genotype (Rengel and Hawkesford 1997, Rengel and Wheal, 1997). This polypeptide may be the main structural unit of a putative plasma membrane transporter for Zn (Erenoglu *et al.* 1999).

Phytosiderophore release

The enhanced ability of Zn efficient species to produce Zn mobilising phytosiderophores is another possible mechanism of Zn efficiency (Cakmak *et al*. 1994). Phytosiderophores are non-proteinogenic amino acids that are exuded by roots of graminaceous species to

mobilise Fe and other micronutrient cations from calcareous soils (Marschner 1995). Once released from the root phytosiderophores complex to Zn ions, leading to higher solubility and diffusion of Zn back to the root surface (Cakmak *et al*. 1996b). Once again this release of phytosiderophores can only be used to explain differences in Zn efficiency between species, as differences in the rate of phytosiderophore release do not correlate well with differential Zn efficiency within cereal species (Erenoglu *et al.* 1996, Cakmak *et al.* 1998a).

Carbonic anhydrase activity

Another characteristic of Zn efficient species is their ability to maintain greater CA activity under Zn deficiency, and so maintain their photosynthetic rate and dry matter production at a higher level (Rengel 1995). This characteristic may be especially important in C_4 plants (such as sugarcane (*Saccharum officinarum* L.) or maize) or in some C_3 species such as wheat, which has a lower CA activity compared with other species (Makino *et al*. 1992). Furthermore, Rengel (1995) found that CA activity in a Zn efficient bread wheat variety was two fold higher under Zn deficiency that in a Zn inefficient durum wheat variety.

2.3.4.2 Zinc efficiency within species

It has been suggested that mechanisms of Zn efficiency employed by Zn efficient cultivars within a species may include enhanced translocation of Zn from the roots to the shoots under reduced Zn supply (Cakmak *et al*. 1996b), differential compartmentalisation of Zn within the cell and/or differential utilisation of Zn within the cell (Graham and Rengel 1993).

Translocation and compartmentalisation

Cakmak *et al.* (1996b) found that those bread wheat cultivars with the greatest extent of Zn deficiency symptoms had the lowest root-to-shoot transport capacity of Zn, and therefore the lowest Zn content in the shoots. The reason for genotypic differences in Zn translocation to the shoot under Zn deficiency is not clear, but it is suggested that

phytosiderophores may form stable complexes with Zn in the xylem sap and thereby contribute to Zn translocation to the shoot (Welch 1995, Cakmak *et al.* 1996b). Furthermore, Hacisalinoglu *et al.* (2001) suggest that inefficient wheat cultivars may sequester a larger fraction of shoot Zn into the vacuole, where it may be unavailable for Zn-requiring physiological processes.

Zinc utilisation

Differences in the severity of Zn deficiency symptoms despite similar concentrations of Zn in leaves can be explained by differences in Zn utilisation efficiency. Zn inefficient genotypes seem to be less efficient in the use of Zn in metabolism and growth, causing less dry matter production per unit of Zn in the plant (Cakmak *et* al. 1997). Hacisalinoglu *et al.* (2001) have suggested that Zn-binding ligands may be involved in lowering the concentration of physiologically active Zn in the cytosol. As discussed above, Zn efficient bread wheat cultivars have been found to have a higher Cu/Zn-SOD activity than Zn inefficient cultivars, thus containing a higher amount of physiologically active Zn in their leaves (Cakmak *et al.* 1997). However, Erenoglu *et al.* (1999) suggest that the magnitude of these differences in Cu/Zn-SOD activity was not high enough to explain genotypic differences in Zn efficiency. Clearly further studies are required to elucidate the reasons for the differential expression of Zn efficiency among bread wheat cultivars.

2.4 Mineral nutrition and grain quality

It can be seen from the above discussion that the breadmaking quality of wheat is largely determined by protein concentration and protein composition. Both traits are dependent upon genotype (Johnson *et al*. 1985, Payne 1987, Stoddard and Marshall 1990), but environmental factors, including climate, soil moisture and soil fertility, have been found to substantially affect protein quantity and quality (Kramer 1979, Timms *et al.* 1981).

2.4.1 Grain protein concentration

Grain protein concentration depends on the total amount of N available to the crop and also on the rate and timing of the N application with respect to the different stages of the crop life cycle. N supplied after anthesis will prolong flag leaf duration and therefore has a

favourable impact on both starch and protein synthesis (Kosegarten and Mengel 1998). The later the N is applied however, the greater the effect on protein percentage and the less influence on yield (Pushman and Bingham 1976, Spiertz and De Vos 1983). Unless the supply of N to the grain is sustained during grain filling, the grain protein concentration of the crop may be low (Strong 1982, Lotfollahi *et al.* 1997). However, much higher levels of N are required to achieve optimal protein concentration than to maximise grain yield (Borghi *et al*. 1995).

Several studies have related grain mineral composition to grain protein content in wheat, and significant correlations have been found between protein content and the concentrations of Zn, Fe, phosphorus (P), calcium (Ca), potassium (K), copper (Cu) and magnesium (Mg) present in the grain (Lorenz and Loewe 1977, Dikeman *et al*. 1982). One of the few studies to have examined the effect of micronutrient supply on grain protein content is that of Hemantaranjan and Garg (1988), who showed that increasing supplies of Fe and Zn, up to an optimum concentration, significantly increased the starch and protein content of wheat grains, in addition to increasing grain yield and a number of yield components. In addition, Eltun (1996), in a study of the productivity and environmental side effects of six cropping systems, showed that reduced soil mineral availability – caused by variations in growing conditions - reduced grain protein content in addition to producing a smaller grain size.

2.4.2 Grain protein composition and baking quality

In addition to grain protein concentration, the seed storage protein composition is also an important factor in determining the breadmaking quality of wheat. The focus of research in this field, however, has concentrated mainly on the differences between genotypes, with little attention devoted to the differences within a specific genotype grown under nutrient deficient conditions. This is mainly because the seed storage protein fingerprint is primarily considered indicative of genotype (MacRitchie *et al.* 1990).

However, recent studies have suggested that this gluten protein pattern can also be altered, within a genotype, by environmental influences, including soil mineral content. Bonfil *et al*. (1997) examined the effects of soil type and deficiencies of nitrogen (N), P, K, Mg and sulphur (S) on seed storage protein composition in nine accessions of wild wheat (*Triticum*

turgidum var. *dicoccoides*) and three varieties (two *T. aestivum* and one *T. durum*). Although genotype was found to be the main factor in determining seed storage protein composition, this protein fingerprint was also found to vary in all but one genotype when grown under different mineral conditions. Bonfil *et al.* (1997) summarised this 'soil effect' in terms of three main quantitative changes in the seeds: (1) the relative amounts of HMW proteins, (2) the relative amounts of proteins in the range of 45 to 65 kD, and (3) the percentage distribution of HMW glutenins and other groups of seed storage proteins. The soils were also found to induce qualitative differences in the composition of seed storage proteins, mostly those of 45-65 kD. These differences were observed whenever a deficiency of N, P, K, Mg or S was identified, and it is suggested that these deficiencies may affect one of a number of seed storage protein processes, such as folding, assembly, transport or deposition.

The effects of N and S availability on seed storage protein composition have been studied a little more than that of the other nutrients, and while it is well accepted that an increase in N availability during grain filling will increase total grain protein content, some disagreement exists as to whether the gliadins or glutenins are responsible for the quality differences induced by N fertilisation. Several authors have observed an increase of gliadins but no change in the amount of glutenins following N fertilisation, with a consequent increase in the gliadin:glutenin ratio (Levy *et al*. 1985, Doekes and Wennekes 1982, Gupta *et al.* 1992). However other authors have suggested that it is a varietal-dependent increase in both gliadins and/or glutenins as a result of N fertilisation (Scheromm *et al*. 1992). Timms *et al*. (1981) found that the ratio of S to N in the grain fell as grain protein content increased with increased N fertilisation, and this correlated with a lower proportion of the S-containing amino acids cyst(e)ine and methionine. This led to an increase in the proportion of the S-poor ω-gliadins, at the expense of the S-rich α-, β- and γ–gliadins, with increased grain protein content.

Several studies have examined the effects of grain mineral content on dough properties and baking quality, but few have investigated the effects of nutrient supply on breadmaking characteristics. Douglas and Dyson (1985) investigated the relationship between the mineral composition of grain and its baking quality in 35 samples of wheat, and found positive relationships between N and S concentration and bake score (the sum of a volume

measurement score and a subjective texture score) but negative relationships between K, P, Mg and molybdenum (Mo) and bake score. Other elements, including Ca, Mn, Zn, Cu, Fe, sodium (Na), boron (B) and selenium (Se), were found to have no effect on bake score. A number of field experiments have confirmed the observation that S deficiency reduces the baking quality of wheat flour. Moss *et al.* (1983) and Kettlewell *et al.* (1998) showed that dough extensibility is dependent on flour S and N concentrations, and it is concluded that dough rheology is limited by the S status of flour from field grown wheat.

In one of few studies designed to assess the effects of nutrient supply on baking quality, Flynn and colleagues (1987) showed that flour from Cu deficient wheats produced doughs that were over-extensible and lacking in strength, resulting in collapsed loaves, despite having a higher grain protein content than the control plants. There was a slight improvement in dough and baking quality when Cu was applied at late tillering, but this was not as great as when a second application of Cu was made at the booting stage, indicating that it is essential for plants to have adequate Cu supply after pollen production to ensure balanced dough rheological properties. Supply at booting only, however, did not provide sufficient Cu to adequately overcome the deficiency. Although Cu is not directly involved in the biosynthesis of proteins, Flynn *et al.* (1987) suggest that the reduced activity of some of the Cu metalloenzymes may lead to impaired seed storage protein synthesis, resulting in weaker doughs and reduced breadmaking quality.

To summarise, the research to date would suggest that mineral nutrient supply does have some effect on the grain quality of wheat, largely by influencing protein quantity and quality. Increased supplies of Fe and Zn, as well as N, have been found to increase the protein content of wheat grains, while an increased supply of N, P, K, S and Mg seems to increase the relative amounts of HMW glutenins. This is generally correlated with an increase in breadmaking quality. Increased supply of S, Ca and Cu also has a positive influence on breadmaking quality. Some inconsistencies among the results have been found, however. For example, while P and K seem to increase the relative amounts of HMW glutenins, an increase in grain content of these nutrients is also correlated with weak doughs and poor breadmaking ability. This may be due to the type of HMW glutenin subunits produced under increased supply of these nutrients. Further work is necessary in order to clarify the effects of an increased supply of the various nutrients on grain quality.

2.5 Zinc nutrition and thermotolerance

Studies of genotypic variation in heat tolerance have revealed several criteria associated with thermotolerance, including the maintenance of photosynthetic activity under heat stress, improved membrane stability and maintenance of enzyme activity. Coincidently or otherwise, Zn is also involved in maintaining photosynthetic activity (including maintaining the activity of several themosensitive enzymes), preserving membrane integrity and sustaining protein synthesis. These independent effects of Zn deficiency and heat stress on plant growth provide circumstantial evidence that Zn nutrition may have a role to play in alleviating the detrimental effects of high temperature on crop plants. However few studies have examined any interaction between plant nutrition and thermotolerance, and very few reports are available in the literature.

2.5.1 Animal experiments

Chvapil (1979b, p. 169) was one of the first to recognise the possible role of Zn in providing organisms with stress tolerance when he proposed "…zinc ions have a vital role to play in a homeostatic mechanism, preserving the integrity of the cell membrane, especially when the cell is exposed to some noxious agent." Animal experiments conducted in this area of Zn nutrition and thermotolerance can be seen to support the hypothesis that Zn can ameliorate the effects of high temperature stress. Experiments with chickens, for example, have shown that hens fed a diet with supplementary Zn had better growth and significantly improved heat tolerance when exposed to 34°C, compared with hens with no supplementary Zn added to their diet (Männer and Wang 1991).

2.5.2 Heat shock protein synthesis

The expression of HSPs, which provide protection against heat stress in plants and animals, is induced by Zn ions independently of high temperature. This has been

demonstrated in animal and plant cell culture (Suzuki and Watanabe 1994, Wollgiehn and Neumann 1995, Liu *et al.* 1996) and *in vivo* in rats and pigs (Tons *et al.* 1997, Klosterhalfen *et al.* 1997 a,b). In tomato cell culture, treatment with various heavy metals, including Zn, resulted in the synthesis of HSPs, again independently of heat shock conditions (Wollgiehn and Neumann 1995).

Repka (1993) has provided additional evidence for the positive role of Zn nutrition in providing higher plants with tolerance to high temperature. Barley seedlings were pre-treated with various concentrations of Zn, Cu, Fe, Mg or Mn ions (from 0.025 mM up to 1 mM), for three days prior to a heat shock treatment of either 40ºC or 45ºC. All metals were found to have a thermoprotective effect against both temperature stresses, which were otherwise lethal to control seedlings with no metal ion pre-treatment. Both the shoot and the root of the pre-treated barley seedlings exhibited thermotolerance, even though the metal ions were applied only to the roots.

2.5.3 Defence against reactive oxygen species

As discussed above, Zn is involved in both the detoxification of ROS and the inhibition of ROS production, and is thus an important factor in an organism's defence against these destructive species. Superoxide dismutases, in particular, are known to play a critical role in the oxidative defence systems of all biological tissue (Bowler *et al.* 1992, Scandalios 1993), and it follows therefore that plants with reduced SOD activity under Zn deficiency should be more sensitive to oxidative stress factors, including high temperature stress (Cakmak 2000). Improvement of the Zn status of plants may therefore be of great importance for their survival under oxidative stress conditions.

This hypothesis is supported by results from experiments involving various causes of oxidative stress, although heat stress itself has not been addressed. Wenzel and Mehlhorn (1995) showed that Zn deficiency caused an enhanced sensitivity to ozone (O_3) toxicity in beans and further, that the higher O_3 sensitivity of Zn-deficient plants correlated well with a reduced activity of Cu/Zn-SOD. Similarly, overproduction of Cu/Zn-SOD in response to O_3 exposure protected transgenic tobacco plants from O_3 damage (Pitcher and Zilinskas

1996). Obata *et al.* (2001) showed that the activity of Cu/Zn-SOD in rice leaves was depressed under Zn deficient conditions, and this led to increased chlorosis under high oxidative stress levels induced by the herbicide paraquat. This increase in leaf chlorosis was not evident in plants supplied with adequate Zn.

Mittler and Zilinskas (1994) demonstrated that Cu/Zn-SOD activity increased during drought stress and following drought in peas, while field studies have shown that chilling stress in citrus and drought stress in wheat become more pronounced in plants suffering from Zn deficiency (Cakmak *et al.* 1995, Ekiz *et al.* 1998). Cakmak (2000) suggests that these results are due to the reduced activity of enzymes scavenging $O_2^{\cdot -}$ and H_2O_2 in Zn deficient tissues. Field observations of barley have indicated that adequate Zn nutrition can increase drought tolerance (King 1994), while decreases in grain yield due to drought stress in wheat were shown to be more marked when plants were Zn deficient (Ekiz *et al.* 1998).

Although there is little direct evidence to show that enhanced Zn nutrition will provide plants with protection against high temperature stress, the results from studies involving Zn and other oxidative stresses suggest that Zn may also enhance a plant's tolerance to high temperature. Bakardjieva and colleagues (2000) have shown that Zn ions will increase the activity and thermostability of extracted SOD isoforms *in vitro*, and they suggest that this is due to a stabilisation of the enzyme's subunit structure. However, to date there have been no studies that document the sensitivity of Zn deficient plants, with reduced SOD activity, to high temperature stress *in vivo.* Clearly further work is necessary to determine whether an enhanced Zn nutritional status is important for a plant's growth and vigour under high temperature conditions.

2.6 Conclusion

High temperature stress presents a significant environmental limitation to the quantity and quality of wheat production throughout many of the world's cereal growing areas. A number of changes to physiological processes occur when a wheat crop encounters heat

stress during the growing season, including a reduction in photosynthesis, damage to cellular membranes and inactivation of enzymes. In Australia and elsewhere, high temperature stress is also often accompanied by Zn deficiency, which further limits the productivity of crops growing in these areas. Zn is an essential micronutrient required for a number of plant growth processes, including carbohydrate metabolism, membrane stability, enzyme activity and protein synthesis.

It has been observed that several of the physiological processes within the plant cell that involve Zn are also associated with thermotolerance, including maintenance of photosynthetic activity, preservation of membrane integrity and continuation of enzyme activity. Furthermore, Zn is an important factor in a plant's defence against reactive oxygen species, which proliferate within plant tissues under stress conditions. This suggests a link between Zn deficiency and susceptibility to high temperature stress, an interaction that does not appear to have been studied before in plants to any depth.

The principal aim of this study was to determine the role of zinc nutrition in the provision of thermotolerance to wheat plants. A series of experiments were carried out with the following objectives:

(i) to evaluate the physiological responses of wheat to zinc supply and high temperature during vegetative growth,

(ii) to investigate some of the genotype by environment interaction responses of wheat to zinc fertilisation under field conditions,

(iii) to define the effects of zinc fertilisation and high temperature stress during grain filling on the physiology and grain yield of wheat,

(iv) to examine the effect of zinc supply and elevated temperatures on grain quality, and

(v) to gain insight into some of the mechanisms by which zinc may provide wheat seedlings with tolerance to high temperature stress.

CHAPTER 3

GENERAL MATERIALS AND METHODS

3.1 Introduction

This study was designed with the principal aim of determining the role that zinc (Zn) may play in the provision of thermotolerance to wheat plants. A series of pot experiments were conducted under controlled environment conditions in order to address this objective. A preliminary experiment was performed to evaluate the feasibility of the study, and to examine some of the effects of Zn supply and high temperature on the vegetative growth of wheat seedlings. Later experiments were carried out to further investigate some of the physiological responses of wheat to Zn deficiency and high temperature stress, and to assess the effects of these stresses during grain filling on wheat grain yield and quality. Six field experiments were also conducted on Zn deficient soils in South Australia and Victoria in 1998 and 1999. These field studies were designed primarily to evaluate some of the genotype by environment interaction responses of wheat to Zn fertilisation, with the secondary aim of verifying the results obtained from the pot experiments. This chapter presents the general materials and methods common to many of the experiments reported in this thesis. Further details and materials and methods specific to individual experiments are described in the appropriate chapters.

3.2 Selection of genotypes

A total of seven bread wheat (*Triticum aestivum* L.) cultivars and two durum wheat (*Triticum durum* Desf.) cultivars were used in this study. These were selected on the basis of previously reported differences in their levels of Zn efficiency or heat tolerance.

3.2.1 Cultivars with contrasting zinc efficiency

Zinc efficiency is defined as the ability of a cultivar to grow and yield better than a standard cultivar in a Zn deficient soil (Graham 1984). The bread wheat cultivars Excalibur and Trident have been established as Zn efficient varieties, based on screenings conducted in chelate-buffered nutrient solutions (Rengel and Graham 1995a, Rengel and Römheld 2000), in pot assays (Genc *et al*. 2002) and in field trials conducted on Zn deficient soils (Graham *et al.* 1992, McDonald *et al.* 2001). Frame appears to be a moderately Zn efficient variety, while Songlen, Goldmark and the durum wheat cultivar Daki-Cyn appear to be Zn inefficient genotypes, based on similar nutrient solution, pot assay and field trial data (Graham *et al.* 1992, Rengel and Römheld 2000, McDonald *et al.* 2001, Genc *et al*. 2002).

3.2.2 Cultivars with contrasting heat tolerance

The bread wheat cultivar Halberd has been shown to exhibit little change in grain protein content or dough strength with increasing temperature, and has thus been classified as a thermotolerant genotype (Blumenthal *et al.* 1991b, Wrigley *et al*. 1994). Likewise Kronos, a durum wheat cultivar, has been identified as thermotolerant, on the basis of its growth under high temperatures at the seedling stage (Coleman 1996). Conversely Meering, another bread wheat cultivar, has been classified as a heat sensitive on the basis of its reduced grain mass under high temperature conditions (Stone and Nicolas 1995a).

3.3 Soil preparation

A Zn-deficient siliceous sandy soil (Laffer sand; DTPA-extractable Zn = 0.07 mg kg^{-1}) was used in all the pot experiments conducted as a part of this study. This soil was collected from a naturally vegetated area near Tintinara, in the southeast of South Australia (Sparrow and Graham 1988). The topmost layer was removed to reduce the

amount of organic matter present, before the soil was passed through a 2 mm stainless steel sieve, washed three times with deionised water and air dried in a glasshouse. The soil was then weighed into polythene-lined pots, and calcium carbonate powder (0.3% w/w) was added to ensure moderate Zn deficiency in wheat plants when grown in a controlled environment (Dong *et al*. 1995). To ensure that Zn was the only nutrient limiting growth the following basal nutrients were applied separately to each pot in solution (in mg kg^{-1} dry soil): NH_4NO_3, 350; KH_2PO_4, 150; K_2SO_4, 120; $MgSO_4.7H_2O$, 90; $CuSO_4.5H_2O$, 5; $MnSO_4.4H_2O$, 7; H_3BO_3, 1; $CoSO_4.7H_2O$, 1; $FeSO_4.7H_2O$, 0.7; $H_2MoO_4.H_2O$, 0.5 and $NiSO_4.6H_20$, 0.15. Zn was applied as a solution of $ZnSO_4.7H_2O$ to correspond to the Zn treatments selected for each experiment. These nutrients were thoroughly mixed through the soil before nanopure water (18.2 Mohms cm^{-1} resistivity) was added to bring the soil moisture content to 12% (w/w), which corresponds to field capacity. The soil was allowed to equilibrate in the pots for 24 hours before the wheat seeds were sown.

3.4 Seed preparation and sowing

Wheat seeds that passed through a 3 mm mesh and were retained on a 2.5 mm mesh were surface sterilised by soaking in 70% ethanol (v/v) for 1 minute, followed by sodium hypochlorite (3% active chlorine, v/v) for 6 minutes. They were then rinsed three times thoroughly in nanopure water. Prior to sowing, seeds were germinated in sterile Petri dishes on two layers of Whatman® No. 42 ashless filter paper, pre-soaked with nanopure water, at 20˚C for 24 hours. Uniformly germinated seeds with their radicle emerged were then planted to a depth of approximately 15 mm, and a few drops of nanopure water was added to each pot to aid soil settling around the seeds. A layer of acid washed black polypropylene beads was placed over the soil surface of each pot to a depth of approximately 1 cm to act as mulch in order to reduce evaporation. Pots were watered to weight daily with nanopure water to maintain the 12% (w/w) soil water content.

3.5 Measurements of photosynthetic parameters

3.5.1 Chlorophyll fluorescence

Photosynthetic activity was estimated indirectly in this study using a Hansatech Plant Efficiency Analyser (PEA) (Hansatech Instruments Ltd., King's Lynn, UK) to measure chlorophyll fluorescence kinetics. The term "photosynthetic activity" is used throughout the study to refer to these chlorophyll fluorescence measurements, which examine the light reactions of photosynthesis. It is acknowledged, however, that photosynthesis is comprised of two components; the light reactions, in which captured light energy is used for the splitting of water and production of chemical energy; and the dark reactions, in which carbon dioxide is fixed into carbohydrates.

Chlorophyll fluorescence was measured by dark-adapting the adaxial surface of leaves for 30 minutes using a leaf clip situated approximately 2 cm from the leaf tip. The PEA sensor unit was then attached to the clip, the shutter was opened and the leaf was illuminated with saturating light of 3000 μmol m^{-2} s^{-1} at 650 nm for a period of 5 s. Key fluorescence parameters (Fo, Fm, Fv, Fv/Fm) were automatically calculated by the PEA. When a chloroplast has been dark adapted, the pools of oxidation or reduction intermediates for the electron transport pathway return to a common level. Upon exposure of a dark-adapted leaf to light, the initial chlorophyll fluorescence yield, Fo, is reached within nanoseconds, when all primary electron acceptors from photosystem II (Q_A) are in an oxidised state. This is followed by a rapid polyphasic rise to a peak level, and, if sufficient light is used, Q_A becomes fully reduced, and the maximum fluorescence level (Fm) is observed (Strasser *et al.* 1995). Chlorophyll variable fluorescence (Fv) is the difference between Fm and Fo, while the chlorophyll fluorescence ratio (Fv/Fm) represents the maximum quantum efficiency of photosystem II (PSII) primary photochemistry.

Only a portion of the light energy absorbed by a leaf at normal physiological temperatures is used in the photosynthetic apparatus; the balance is dissipated as heat or re-emitted as fluorescence (Papageorgiou 1975). Generally, fluorescence yield is highest when photochemistry and heat dissipation are lowest, and therefore changes in

fluorescence yield reflect changes in photosynthetic efficiency. Chlorophyll fluorescence is now recognised as a sensitive indicator of plant stresses that affect the photosynthetic process, especially those that directly affect the PSII antenna and reaction centres, such as high temperature stress (Krause and Santarius 1975, Schreiber and Berry 1977, Schreiber and Bilger 1993). PSII is located in the thylakoid membranes, and about 90% of chlorophyll fluorescence originates in this component of the photosynthetic system (Govindjee 1995). Damage to PSII by high temperature is often assessed by Fv/Fm, a measure of PSII quantum yield. This value is always close to 0.8 in healthy leaves, and a lower value may indicate that a proportion of the PSII reaction centres have been damaged (Schreiber and Bilger 1993, Araus *et al*. 1998, Kitao *et al*. 1998). Stability of photosynthetic activity is essential for crop productivity, particularly during vegetative growth (Al-Khatib and Paulsen 1990). Stability of Fv/Fm has thus been shown to be a sensitive indicator of plant growth, and even small decreases in this ratio have been shown to result in biologically meaningful decreases in photosynthesis and growth (Barbagallo *et al*. 2003).

When examining decreases in Fv/Fm, however, it is important to distinguish increases in Fo from decreases in Fv (Araus *et al*. 1998). Under high temperature conditions, Fv declines as a result of a decrease in Fm. This decrease in Fm is related to the denaturation of the chlorophyll-protein complexes during heat stress (Schreiber and Armond 1978, Yamane *et al.* 1997). Under more severe elevated temperatures, Fo may also increase, until the difference between Fo and Fm can no longer be distinguished (Smillie and Gibbons 1981, Smillie and Hetherington 1983). An increase in Fo is characteristic of the destruction of the PSII reaction centres and also of the separation of the chlorophyll *a/b* light harvesting complex of PSII (LHCII) from the PSII core complex (Armond *et al.* 1980, Sundby *et al.* 1986, Lu and Zhang 2000). Both Fo and Fv have been used to distinguish heat tolerance between and within species, including wheat (Smillie and Hetherington 1983, Moffatt *et al.* 1990), however Fo is better correlated with the grain yield of wheat under Mediterranean conditions than either Fm or Fv (Araus *et al*. 1998).

3.5.2 Chlorophyll content

Leaf chlorophyll content was estimated non-destructively by measuring leaf greenness using a portable SPAD (Soil Plant Analysis Development)-502® chlorophyll meter (Minolta Camera Co. Ltd., Japan). This meter operates by clamping the sensor head onto a leaf blade. A rubber boot seals out external light, and creates a closed chamber around the area to be measured. Two light emitting diodes are used to emit light through the leaf at two wavelengths, 650 nm (red) and 940 nm (infrared), when the chamber is closed. Light in the 650 nm range lies between the two primary wavelengths associated with chlorophyll activity (645 and 663 nm). Meter operation is based on the inverse relationship between absorbed radiation in the 650 nm region of the spectra, and that transmitted through the leaf. The 940 nm wavelength is not affected by leaf chlorophyll content and provides an internal meter calibration. A silicon photodiode receptor converts the transmitted light to analogue electrical signals, which are then converted into digital signals and used by the microprocessor to calculate the dimensionless SPAD unit value (Bavaresco 1995, Schepers *et al.* 1998).

Several authors have established a correlation between SPAD meter readings and total extractable chlorophyll content in a number of crops, including beans, peas, cotton and sorghum (Marquard and Tipton 1987), apple (Campbell *et al.* 1990), maize (Dwyer *et al.* 1991), rice, soybean and wheat (Monje and Bugbee 1992) and grapevine (Bavaresco 1995). Recent research has indicated that this is a quadratic relationship (Finnan *et al.* 1997, Azia and Stewart 2001), and although separate models are required for individual plant species and growth conditions (Marquard and Tipton 1987, Campbell *et al.* 1990), the SPAD meter is ideal for determining chlorophyll content within an experiment where relative rather than absolute values are required.

The sensor window of the SPAD meter only covers a small proportion of the total area of a leaf blade (6 mm^2). For this reason, three measurements were made on each leaf, at one quarter, one half, and three quarters of the way between the base and the tip. Where possible, the reading was taken midway between the mid-rib and leaf margin. Care was taken while recording the SPAD value to ensure that the sensor window fully covered the leaf lamina and that interference from the midrib was minimal.

3.6 Analysis of nutrient concentrations in plant tissues

Plant material was analysed for Zn and other nutrient concentrations using inductively coupled plasma (ICP) spectrometry (Zarcinas *et al.* 1987). In brief, wheat shoots were rinsed briskly in a mild strength detergent followed by three brisk rinses in nanopure water for approximately 5 s each time. This method of washing has been shown to remove dust, zinc and manganese residues from citrus leaves (Labanauskas 1966, Reuter *et al.* 1997b). After washing, excess water was drained from the sample which was then placed in a paper bag and dried at 80°C for 48 h before being chopped into small pieces using stainless steel scissors. Grain samples were simply dried at 80°C for 48 h prior to analysis. A homogenous sub-sample was weighed, pre-digested in nitric acid (or a mixture of 10:1 nitric:perchloric acid) overnight at room temperature and then heated to a temperature not exceeding 150°C (or 220°C for the nitric:perchloric digest) until complete digestion occurred. After cooling, digests were made up to volume with nanopure water, decanted and analysed by an ICP emission spectrometer (model ARL 3580, Analytical Research Laboratories, Switzerland). A set of standard plant materials of known concentration of Zn and other elements was digested and analysed in the same manner as the experimental samples to ensure the procedure was accurate.

3.7 Statistical analyses

A minimum of 3 replicates was used in all experiments, unless otherwise specified. Results were analysed by standard analysis of variance using routines of the Genstat 5 Release 4.1 statistical package (Lawes Agricultural Trust 1998). Data for some variables were transformed to logarithms where necessary, to overcome the problem of non-homogeneity of variance, before being analysed by analysis of variance. Treatment means were compared using least significant differences (LSD) (Steel and Torrie 1960) at the 5% level of probability (P=0.05).

CHAPTER 4

AN INVESTIGATION INTO THE RESPONSE OF WHEAT SEEDLINGS TO ZINC SUPPLY AND HEAT STRESS

4.1 Introduction

Wheat is a crop that is best adapted to cool growing conditions, and a decrease in productivity occurs as mean daily temperatures rise above 15°C (Wardlaw *et al.* 1989a, Reynolds *et al.* 2000). In Australia, heat stress (maximum daily temperatures above 35°C) is most likely to occur during the grain filling period (Blumenthal *et al.* 1991a), but in many of the world's arid, semi-arid and tropical regions wheat is grown in areas where such high temperatures may occur at any stage of the crop's lifecycle. Over 7 million ha of wheat are grown under continual heat stress in approximately 50 countries (Reynolds *et al.* 1997), including India (Hanchinal *et al.* 1997) and Sudan (Amani *et al.* 1997).

A number of the areas of the world that experience high temperatures during the cropping season are also highly likely to have soils that are deficient in zinc (Zn). Zinc deficiency in soils has been reported worldwide – approximately 50% of the soils used for crop production contain low levels of plant available Zn (Sillanpää and Vlek 1985) – however the problem is particularly widespread in the calcareous soils of the arid and semi-arid regions (Takkar and Walker 1993). Despite this incidence of high temperature and Zn deficient soils commonly occurring in combination, few studies have examined the interaction between these two stresses in plants.

While elevated temperatures are known to decrease photosynthetic activity in crop plants (Berry and Björkman 1980, Al-Khatib and Paulsen 1984), Zn deficiency has also been shown to reduce the net photosynthetic rate of plants, sometimes by up to 70% (Brown *et al.* 1993). Zinc is also involved in the preservation of membrane integrity and the maintenance of enzyme activity, both of which are key physiological processes associated with thermotolerance in plants. This suggests a possible link between Zn

deficiency and susceptibility to heat stress, however the role of Zn in the provision of thermotolerance to plants remains unknown. The main aim of this experiment was to examine the growth and photosynthetic responses of wheat seedlings to Zn deficiency and a period of heat stress during early vegetative growth, and thus to determine whether elevated supplies of Zn can play a role in alleviating some of the detrimental effects of high temperature on crop plants.

4.2 Materials and methods

Laffer sand was collected and prepared as described in Chapter 3 (Section 3.3). One hundred and fifty grams of this soil was packed into a 70 mm diameter x 45 mm high plastic pot with 4 small holes in the base, and calcium carbonate and basal nutrients were added as described in Chapter 3 (Section 3.3). Two Zn treatments were applied, 0 and 2 mg Zn kg^{-1} soil as $ZnSO_4.7H_20$, and designated as Zn_0 and Zn_2, respectively. A Zn fertilisation treatment of 2 mg kg^{-1} soil was chosen since this rate of Zn has been shown previously to provide sufficient Zn to wheat plants grown in Laffer sand to allow maximum growth, without producing Zn toxicity symptoms (Graham *et al.* 1992, Grewal *et al.* 1996). Nanopure water was added to bring the soil water content to field capacity (12% w/w), and the soil was allowed to equilibrate in the pots overnight before wheat seeds were sown.

Two wheat genotypes with contrasting Zn efficiency, Trident (Zn efficient) and Songlen (Zn inefficient), were chosen for this experiment (Chapter 3, Section 3.2.1). The seed of these varieties was prepared by removing two thirds of the seed (containing mostly endosperm) with a sharp razor blade leaving the embryo and a small amount of endosperm. This technique is believed to remove much of the seed nutrient reserves thus hastening the development of nutrient deficiency in young seedlings (Arun Aryan, pers. commun.). Seed pieces of a similar size were selected and eight seeds were sown into each pot to a uniform depth of 5 mm. A few drops of nanopure water were added to aid soil settling around the seeds. Plants were thinned to five plants per pot following emergence.

Plants were grown in a growth chamber at 25/15ºC day/night temperature, 12 h photoperiod and 335 µmol s^{-1} m^{-2} light intensity at plant height. Individual pots were placed in small trays, and for the first 5 days of the experiment plants were watered daily to weight with nanopure water to maintain soil water content at 12% (w/w). From 6 days after sowing (DAS) however, plants were watered daily to a soil moisture content of 25% (w/w). This allowed the water that drained through the pots to be collected in the trays beneath, and retaken up by capillary action, thus ensuring adequate soil moisture in the small volume pots.

Heat-treated plants were heated for 2 days starting from 15 DAS. Pots were moved during the night period to a growth chamber in which a night temperature of 25ºC was maintained for 12 h and a day temperature peak of 35ºC was maintained for 7 h. The rate of heating to and cooling from the maximum day temperature was 4ºC h^{-1}. Light intensity in this growth chamber was 410 µmol s^{-1} m^{-2} and the plants were watered to 25% (w/w) three times daily to ensure adequate soil moisture was available at all times. After 2 days plants were transferred back to the 25/15ºC growth chamber for a further 9 days.

Measurements of the length of the actively elongating (3^{rd}) leaf were taken prior to the heat stress treatment (15 DAS), at the end of the high temperature treatment (17 DAS) and again at the end of the experimental period (26 DAS). The relative leaf elongation rate (RLER) was calculated by the equation

$$RLER = (\ln L_2 - \ln L_1)/(t_2 - t_1)$$

where L_2 = the final leaf length; L_1 = the initial leaf length; t_2 = time of final measurement; and t_1 = time of initial measurement. Chlorophyll fluorescence measurements were taken at 15, 17, 19 and 26 DAS as described in Chapter 3 (Section 3.5.1). The shoots of two plants from each pot were harvested at 17 and 26 DAS and shoot fresh weight was obtained.

The experiment was set up in a completely randomised block design (2 genotypes x 2 Zn levels x 2 temperature treatments) with two replicates. Results were statistically

analysed as explained in Chapter 3 (Section 3.7). To overcome the problem of non-homogeneity of variances, the data for relative leaf elongation rate were log-transformed before being analysed by analysis of variance.

4.3 Results

4.3.1 Visual symptoms of Zn deficiency

Typical Zn deficiency symptoms of reduced growth and pale yellow, linear chlorotic regions on the oldest leaf appeared in the Zn_0 treatment of Songlen, the Zn inefficient variety, at 21 DAS and progressively worsened. No symptoms of Zn deficiency had appeared on the leaves of Trident, the Zn efficient variety, by the end of the experiment. With the exception of the Zn deficiency symptoms in Songlen, all plants appeared healthy, and no symptoms of any other nutrient deficiency were observed throughout the experimental period.

4.3.2 Leaf length

Zinc deficiency significantly reduced the length of the 3rd leaf of both wheat varieties at 15 DAS, but did not affect final leaf length (Figure 4.1). At 15 DAS the length of the 3rd leaf in Songlen was 26% shorter in plants supplied with no Zn than in plants supplied with 2 mg Zn kg^{-1} soil. Trident plants with no Zn fertilisation had leaves that were 16% shorter than plants supplied with supplementary Zn. By 26 DAS, however, these differences in leaf length between Zn treatments were no longer significant.

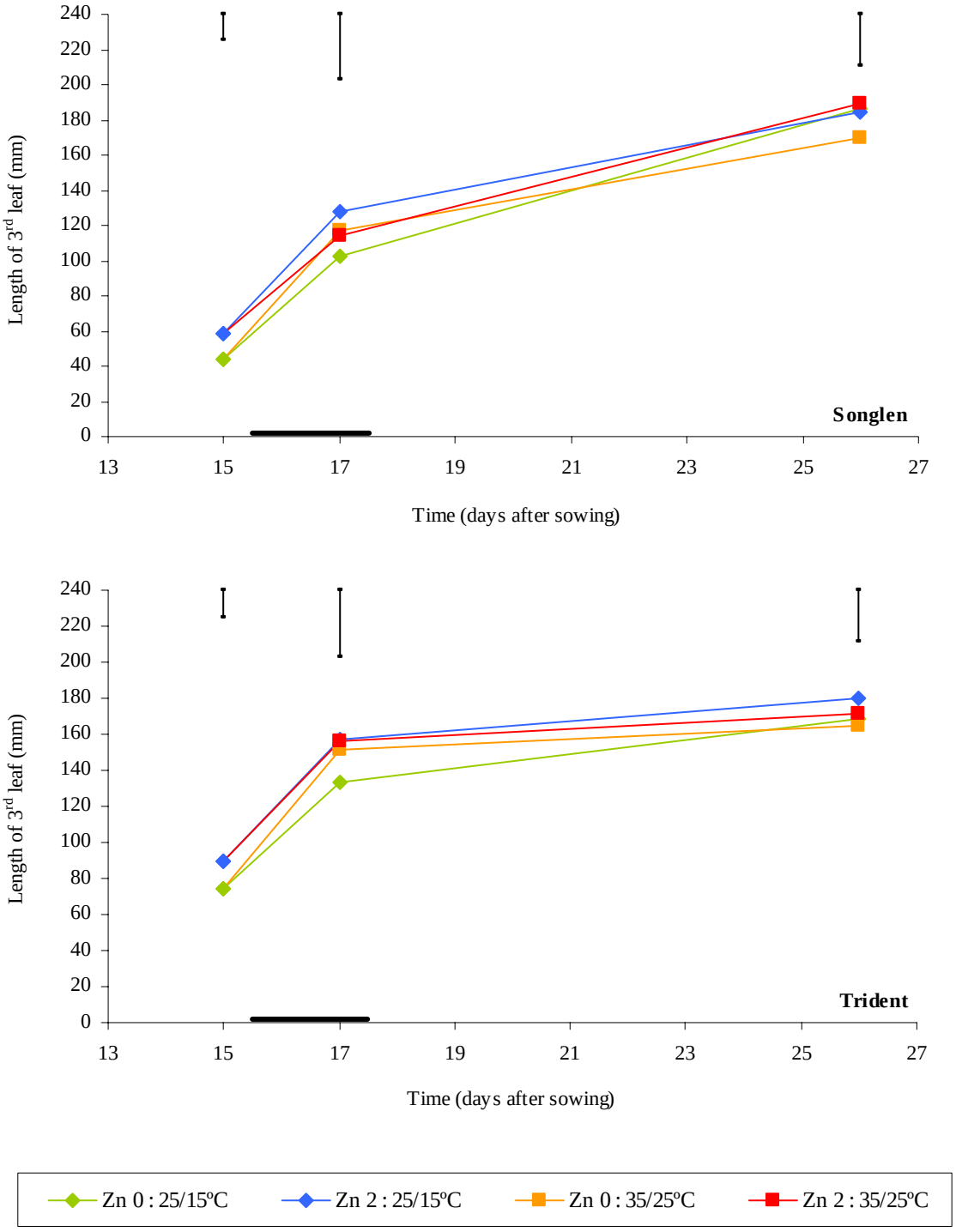


Figure 4.1. Effects of Zn supply (mg kg^{-1} soil) and temperature treatment on the 3rd leaf length (mm) of wheat genotypes Songlen and Trident. Vertical bars represent the $LSD_{0.05}$ for the interaction of Variety x Zinc x Temperature. Horizontal bars on x-axes represent the period of high temperature treatment.

The high temperature treatment of 35/25ºC for 2 days had no significant effect on the length of the 3rd leaf, the actively elongating leaf during the heat stress period, but did reduce the relative leaf elongation rate of these leaves (Table 4.1). Significant differences between varieties were also apparent. The relative leaf elongation rate of the 3rd leaf of Songlen was reduced only in the heat-treated plants with no supplementary Zn fertilisation. Conversely in Trident the elongation rate of the heat-treated plants was reduced only in those plants supplied with additional Zn.

Table 4.1. Effects of genotype, Zn fertilisation (mg kg^{-1} soil) and temperature treatment on the relative leaf elongation rates (mm/mm/day) of the 3rd leaf of wheat seedlings.

Temperature	**Songlen**		**Trident**		
	Zn 0	**Zn 2**	**Zn 0**	**Zn 2**	***Mean***
25/15ºC	0.220 (-0.67)[a]	0.168 (-0.82)	0.067 (-1.18)	0.086 (-1.07)	*0.135 (-0.94)*
35/25ºC	0.108 (-0.97)	0.112 (-0.99)	0.074 (-1.14)	0.041 (-1.41)	*0.084 (-1.13)*
Mean	*0.164 (-0.82)*	*0.140 (-0.91)*	*0.071 (-1.16)*	*0.063 (-1.24)*	
LSD$_{0.05}$ [b]					
Genotype		**(0.11)**			
Zinc		**n.s.**			
Temperature		**(0.11)**			
Genotype x Zinc		**n.s.**			
Genotype x Temperature		**n.s.**			
Zinc x Temperature		**n.s.**			
Genotype x Zinc x Temperature		**(0.22)**			
CV(%)		**(14.2)**			

4.3.3 Shoot fresh weight

The shoot fresh weight of seedlings was not affected by either Zn deficiency or high temperature at either harvest (Table 4.2). Neither Trident nor Songlen showed a significant increase in above ground biomass with the application of Zn up to almost 4 weeks after sowing, and the high temperature treatment of 35/25ºC for 2 days had no effect on biomass production either.

Table 4.2. Effects of genotype, Zn fertilisation (mg kg^{-1} soil) and temperature treatment on the shoot fresh weight (g) of wheat seedlings at 17 and 26 DAS.

Temperature	17 DAS				26 DAS			
	Songlen		Trident		Songlen		Trident	
	Zn 0	Zn 2	Zn 0	Zn 2	Zn 0	Zn 2	Zn 0	Zn 2
25/15°C	0.415	0.605	0.555	0.660	1.359	1.054	0.938	1.136
35/25°C	0.580	0.670	0.605	0.600	1.230	1.248	1.133	1.199
Mean	*0.497*	*0.637*	*0.580*	*0.630*	*1.294*	*1.151*	*1.036*	*1.167*
$LSD_{0.05}$								
Genotype	**n.s.**				**n.s.**			
Zinc	**n.s.**				**n.s.**			
Temperature	**n.s.**				**n.s.**			
Genotype x Zinc	**n.s.**				**n.s.**			
Genotype x Temperature	**n.s.**				**n.s.**			
Zinc x Temperature	**n.s.**				**n.s.**			
Genotype x Zinc x Temperature	**n.s.**				**n.s.**			
CV(%)	**15.8**				**12.9**			

n.s. = non-significant

4.3.4 Chlorophyll fluorescence

The chlorophyll fluorescence ratio (Fv/Fm) decreased significantly during the high temperature treatment and was lowest under both Zn deficiency and heat stress, although there were no significant differences between the two varieties (Figure 4.2). At 17 DAS plants not subjected to high temperature had an average Fv/Fm value of 0.841, compared to 0.805 for Zn adequate plants under heat stress and 0.781 for plants with no supplementary Zn fertilisation under heat stress.

Upon removal from the high temperature treatment, the Fv/Fm ratio was found to recover rapidly, but differed significantly between varieties. Thirty-six hours after removal from the high temperature (19 DAS) the Fv/Fm ratio of the heat-stressed plants of Trident had recovered to that of the control plants, while the Fv/Fm ratio of the heat-stressed plants of Songlen remained significantly lower than that of the control plants (Figure 4.3). Furthermore one week later (26 DAS) this Fv/Fm ratio remained significantly lower than the plants that had not been subjected to high temperature stress. There was no significant interaction between Zn fertilisation and temperature treatment at 19 or 26 DAS.

The decrease in the Fv/Fm ratio at 17 DAS under high temperature and low Zn was due mainly to an increase in chlorophyll initial fluorescence (Fo) during the high temperature treatment (Figure 4.4), rather than any effect on chlorophyll maximum fluorescence (Fm). While there was a significant decrease in Fm during the high temperature treatment, there was no interaction with Zn fertilisation and the Fm of both Zn treatments was lowered equally during this period (Figure 4.5a). The Fo, however, increased significantly during the high temperature treatment in the nil Zn plants only, to 611, up from an average of 565 for the Zn adequate plants and those not subjected to heat stress. This chlorophyll fluorescence parameter was found to recover rapidly in both varieties, and was no different to that of the control plants by 36 h following the high temperature treatment.

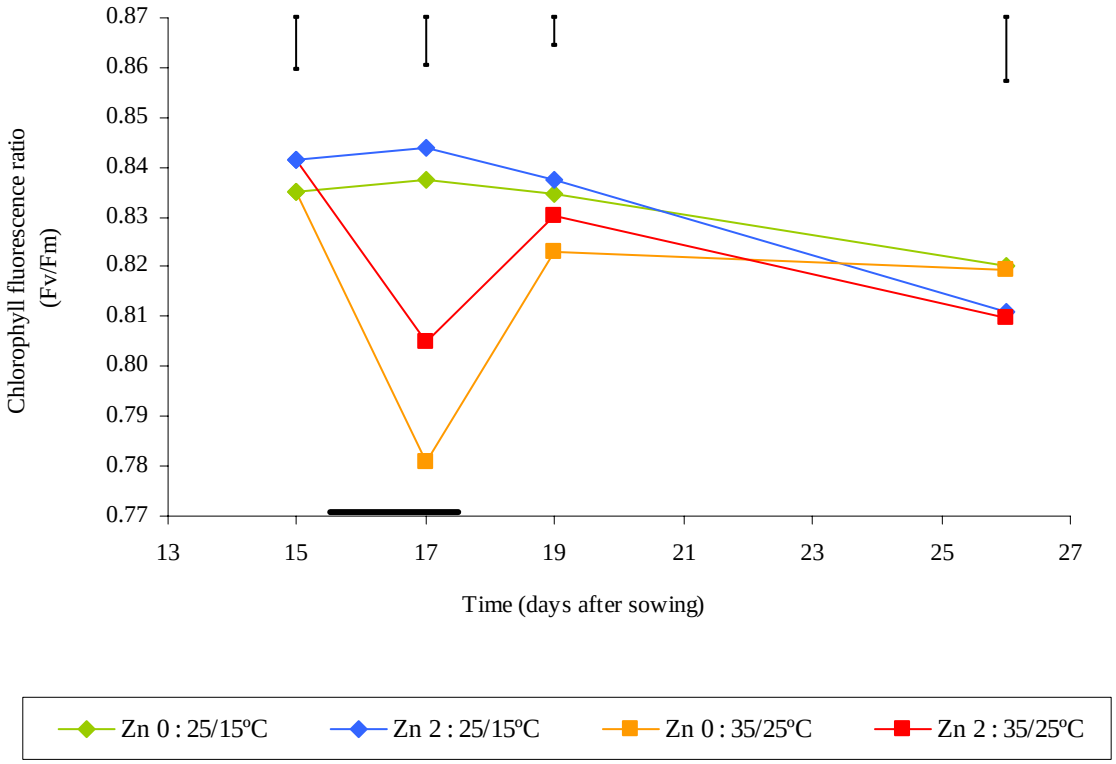


Figure 4.2. Effects of Zn supply (mg kg^{-1} soil) and temperature treatment on the chlorophyll fluorescence ratio, Fv/Fm, of wheat seedlings. Data are the mean of both genotypes; vertical bars represent the $LSD_{0.05}$ for the interaction of Zinc x Temperature. The horizontal bar on the x-axis represents the period of high temperature treatment.

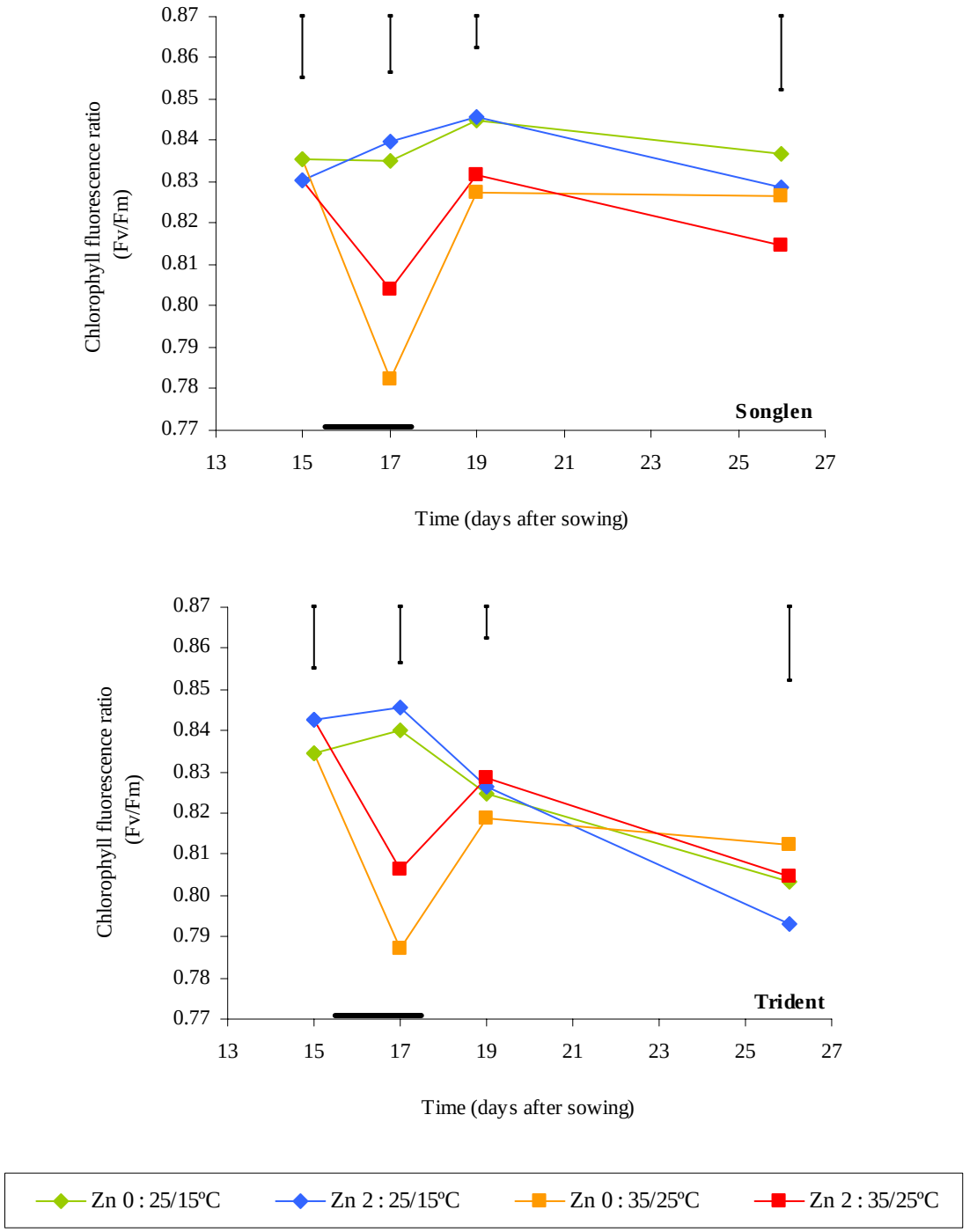


Figure 4.3. Effects of Zn supply (mg kg^{-1} soil) and temperature treatment on the chlorophyll fluorescence ratio, Fv/Fm, of wheat genotypes Songlen and Trident. Vertical bars represent the $LSD_{0.05}$ for the interaction of Genotype x Zinc x Temperature. Horizontal bars on x-axes represent the period of high temperature treatment.

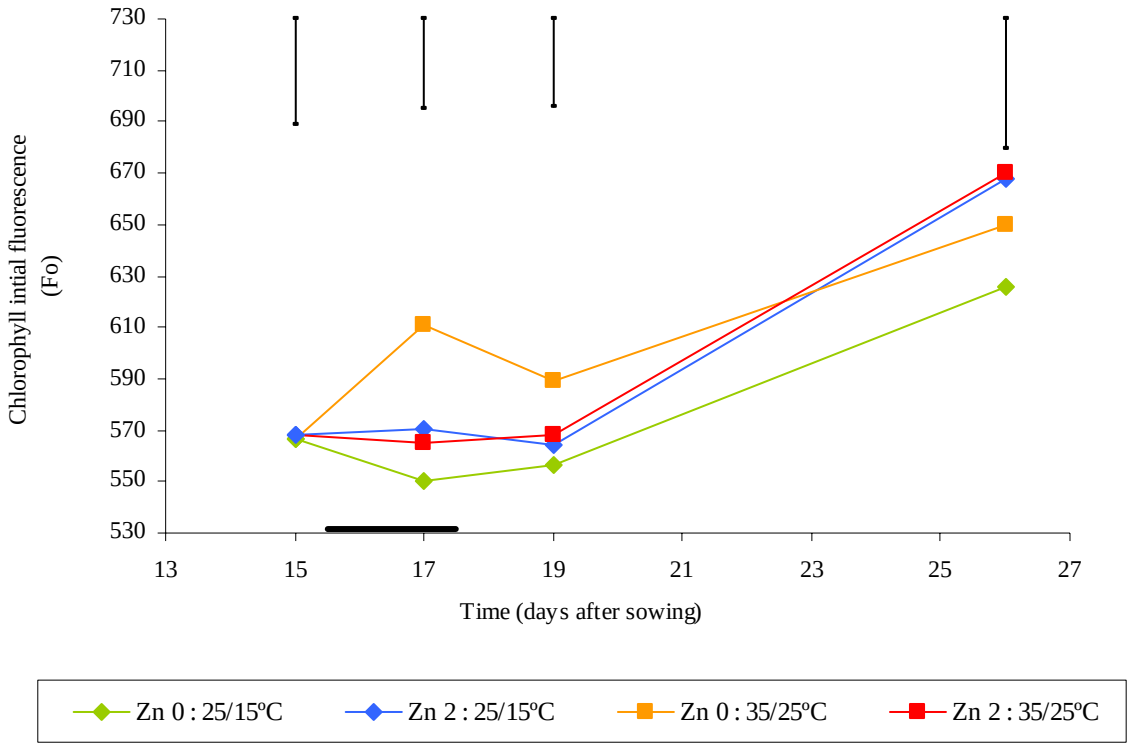


Figure 4.4. Effects of Zn supply (mg kg^{-1} soil) and temperature treatment on the chlorophyll initial fluorescence, Fo, of wheat seedlings. Data are the mean of both genotypes; vertical bars represent the $LSD_{0.05}$ for the interaction of Zinc x Temperature. The horizontal bar on the x-axis represents the period of high temperature treatment.

While the interaction between Zn fertilisation and temperature treatment was significant for Fo but not for Fm during the high temperature treatment, the main effects of Zn and temperature were both found to have a significant effect on Fm during the heat stress period. Zn deficiency lowered Fm by 6% and high temperature reduced this parameter by 19%. The observed values for chlorophyll variable fluorescence (Fv) showed a similar trend to those observed for Fm (Figures 4.5b).

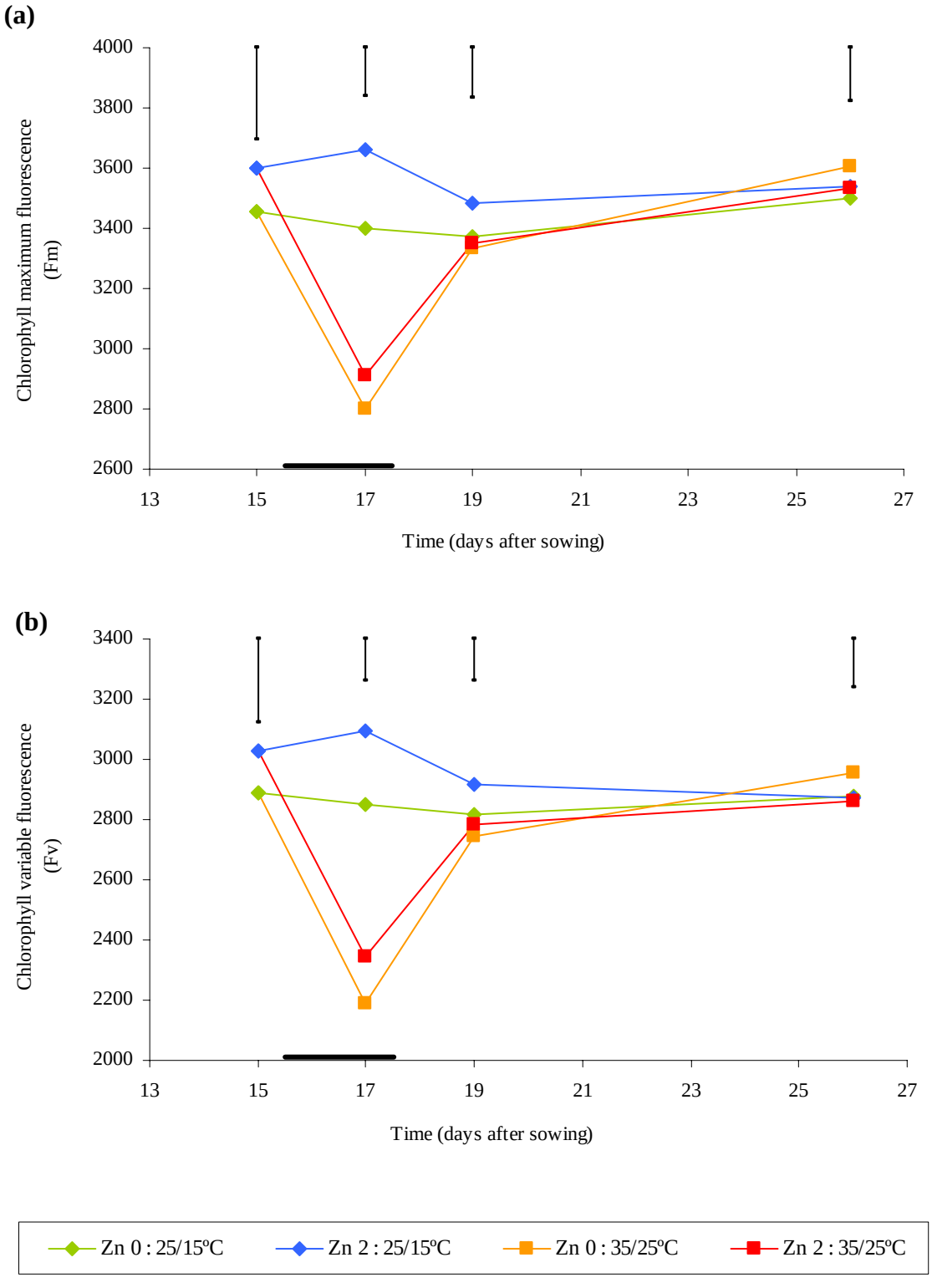


Figure 4.5. Effects of Zn supply (mg kg^{-1} soil) and temperature treatment on chlorophyll maximum fluorescence (a) and chlorophyll variable fluorescence (b) of wheat seedlings. Data are the mean of both varieties; vertical bars represent the $LSD_{0.05}$ for the interaction of Zinc x Temperature. Horizontal bars on x-axes represent the period of high temperature treatment.

4.4 Discussion

The twin stresses of Zn deficiency and high temperature imposed on wheat seedlings during early growth in this experiment were not severe enough to reduce plant growth. However both stresses did significantly affect photosynthetic activity, as assessed by chlorophyll fluorescence, and furthermore, a significant interaction was observed between Zn fertilisation and temperature treatment on the chlorophyll fluorescence ratio, Fv/Fm. This suggests that Zn may be able to provide wheat seedlings with some level of physiological tolerance to high temperature stress. High temperature stress significantly reduced Fv/Fm, however under adequate Zn fertilisation this reduction was considerably less than under Zn deficiency, indicating that Zn may be important in maintaining photosynthetic activity during a period of heat stress.

Photosynthesis is one of the most heat sensitive processes in plant cells, with thylakoid membrane activities being more vulnerable than the chloroplast envelope or the water soluble enzymes of the photosynthetic pathways located in the stroma (Santarius 1975, Monson *et al.* 1982, Sayed *et al.* 1989). Within the thylakoid membrane, PSII is considered to be most heat sensitive, whereas PSI function appears to be relatively tolerant to heat injury (Berry and Björkman 1980, Al-Khatib and Paulsen 1989, Havaux *et al.* 1991). The chlorophyll fluorescence ratio is a measure of PSII quantum yield, and is linearly related to PSII electron transport capacity (Al-Khatib and Paulsen 1989, Krause *et al.* 1990). In the present study, Fv/Fm was significantly lowered in both varieties when measured on the youngest emerged leaf blade during exposure to the high temperature treatment of 35/25ºC.

Genotypic differences in the recovery of Fv/Fm following the high temperature period were also observed in the present experiment. While the Fv/Fm of the heat-treated plants of the Zn efficient variety, Trident, had recovered to that of the control (unheated) plants by 36 h following removal from the heat treatment, the Fv/Fm of the Zn inefficient variety, Songlen remained lower than that of the control plants at the end of the experimental period. Chlorophyll fluorescence parameters have been used previously to distinguish high temperature tolerance among wheat genotypes (Al-Khatib and Paulsen 1990, Moffatt *et al.* 1990). Although the thermotolerance of the two

genotypes used in the present experiment is unknown at this time, these results suggest that Trident is a more thermotolerant cultivar than Songlen, at least with respect to photosynthetic activity.

The decrease in Fv/Fm during the high temperature period in the present experiment was largely due to a reduction in the Fm component of chlorophyll fluorescence, rather than any effect on Fo. It has been found previously that a rise in Fo fluorescence occurs at severely elevated temperatures (above 37ºC) rather than at moderately high temperatures (30-37ºC) (Smillie and Hetherington 1983, Nash *et al*. 1985, Lu and Zhang 2000). A decrease in Fm under heat stress is a reflection of the physical dissociation of the chlorophyll *a/b* light-harvesting complex of PSII (LHCII) from its core reaction centre complex (Schreiber and Armond 1978, Armond *et al.* 1980, Sundby *et al.* 1986). This dissociation has been attributed to an increase in thylakoid membrane permeability in the 35-45ºC temperature range; a result of changes in the lipid-protein interactions that are associated with increasing lipid fluidity of the thylakoid membranes at elevated temperatures (Berry and Björkman 1980, Rekika *et al.* 1997). It has been suggested that the strength of the hydrophilic interactions which link the light-harvesting antennae with the PSII complexes decreases with increasing temperature, while that of the hydrophobic interactions increases, so that the pigment-protein complexes tend to associate more with the lipids than with each other, resulting in their dissociation (Berry and Björkman 1980).

Zinc deficiency also reduced Fm in the present experiment, with no interaction with the high temperature treatment. Godde and Hefer (1994) also reported a decrease in the Fm component of chlorophyll fluorescence in leaves under nutrient deficiency stress. Photosynthetic electron transport was reduced in spinach (*Spinacia oleracea*) leaves suffering from Mg deficiency alone or in combination with S deficiency, and resulted from an accumulation of inactivated PSII centres under the stress conditions. The authors attributed this inhibition of PSII to an inhibition of assimilate transport, as indicated by a substantial accumulation of starch. Starch accumulation is known to lead to an increase in the NADPH/NADP ratio (Stitt 1986) which slows down the reoxidation of plastoquinone in the light, and possibly its reduction in the dark, thus

leading to a reduction in electron flow at the acceptor side of PSII (Godde and Hefer 1994, Dannehl *et al.* 1996).

The high temperature treatment of the present experiment reduced Fv/Fm and this was due to a reduction in Fm. However, supplying Zn to the seedlings improved the Fv/Fm of both varieties during the period of high temperature in comparison to those plants that had received no Zn, and this was due to a stabilisation of Fo, rather than any effect on Fm. The increase in Fo by heat stress has been attributed to both the separation of LHCII from the PSII core complexes, and to a partly reversible inactivation of the reaction centre of PSII (Yamane *et al.* 1997). Physical dissociation of the LHCII from the PSII core complex, and its subsequent migration from the appressed to the non-appressed thylakoid regions, results from changes in lipid-protein interactions that are associated with increased lipid fluidity of the thylakoid membranes at elevated temperatures (Armond *et al.* 1980, Berry and Björkman 1980, Gounaris *et al.* 1984, Sundby *et al.* 1986). While neither heat stress nor Zn deficiency alone had any significant effect on Fo, in combination the two stresses produced a significant increase in this chlorophyll fluorescence parameter. It is feasible that adequate Zn fertilisation may be able to reduce the increase in Fo during heat stress through its role in the preservation of membrane integrity. Zn has been found to interact with both membrane phospholipids (Von Glós and Boursnell 1981) and with the sulfhydryl groups of membrane proteins (Chvapil 1973), thus providing a stabilising and protective effect on membrane components. Furthermore, Zn plays an important role in controlling both the generation and detoxification of reactive oxygen species, which also have the potential to increase membrane permeability (Cakmak and Marschner 1988c).

The level of Zn deficiency stress in the present experiment was not severe enough to significantly suppress plant growth. Leaf elongation was retarded as a result of Zn deficiency, and small genotypic differences in tolerance to Zn deficiency between the two wheat varieties studied were apparent. However final leaf length and shoot fresh weight were unaffected by the Zn treatment.

The high temperature treatment of two days of temperatures above 35ºC for 7 hours per

day in the present experiment was also not severe enough to reduce leaf length or shoot fresh weight, but did reduce the elongation rate of the actively elongating leaf. Leaf elongation rate has been established as one of the most important visual traits for monitoring plant growth potential, and is highly controlled by meristem temperature (Watts 1972, Salah and Tardieu 1996). The results of the present study are in agreement with the findings of Kemp and Blacklow (1982), who also reported a reduction in the rate of wheat leaf elongation at high temperature. Using copper coils to control the temperature of the leaf elongation zone, Kemp and Blacklow (1982) generated a response curve for leaf elongation over a range of temperatures from 4ºC to 38ºC, and found an optimum temperature of 28.4ºC for the rate of leaf extension. Changes in the physical and metabolic processes of expanding cells due to high temperature include the disruption of photosynthesis (Berry and Björkman 1980), increased membrane permeability (Raison *et al.* 1980) and reduced enzyme activity (Burke *et al.* 1988).

The leaf elongation rate of Songlen, the Zn inefficient variety, was suppressed in the heat-treated plants of the nil Zn treatment only, while the elongation rate of plants supplied with adequate Zn fertilisation did not differ from that of the unheated control plants. This suggests that supplementary Zn fertilisation may prevent the decline in the rate of leaf elongation of Zn inefficient wheat genotypes under high temperature. However, an unexpected result from the present experiment was the observation that in Trident, the Zn efficient variety, high temperature reduced the relative leaf elongation rate in the adequate Zn plants only, not in those plants grown without Zn fertiliser. This anomaly may be due to a number of factors, such as a nutrient imbalance leading to other nutrient deficiencies or toxicities in the Zn_2 plants, although this is not supported by the final leaf length or shoot fresh weight data. Another possibility is that it is a developmental response to high temperature by the Zn_0 plants of this Zn efficient variety, which is transient. Further investigation is clearly required in order to clarify the response of leaf elongation to the twin stresses of Zn deficiency and high temperature stress.

4.5 Conclusion

The primary aim of this experiment was to determine whether wheat plants supplied with adequate Zn are better able to cope with a short period of heat stress during vegetative growth than plants grown under conditions of Zn deficiency. Wheat seedlings were subjected to both Zn deficiency and 2 days of high temperature stress during the early stages of growth. Although neither stress was severe enough in this experiment to significantly affect plant growth, the results of the study demonstrate that elevated supplies of Zn can reduce the decline of the chlorophyll fluorescence ratio, and therefore also the decline of PSII quantum yield, that occurs during heat stress. Stability of photosynthesis is essential for wheat productivity during vegetative growth (Al-Khatib and Paulsen 1990), and from the results reported here, it appears that adequate Zn fertilisation, on Zn deficient soils at least, may offer a potential stabilising effect on photosynthetic activity under heat stress conditions. Additional research is required to determine whether these beneficial effects of Zn on photosynthesis will result in better plant growth under high temperature; to determine whether the advantageous effects of Zn on photosynthetic activity will persist during the reproductive phase; and also to further elucidate the methods by which Zn offers stability to the photosynthetic apparatus under heat stress.

CHAPTER 5

SOME RESPONSES OF WHEAT GENOTYPES TO ZINC FERTILISATION UNDER FIELD CONDITIONS

5.1 Introduction

Micronutrient deficiencies occur in crops on a range of soils across southern Australia, and are common on the highly alkaline calcareous soils of the Victorian Wimmera and South Australian Murray Mallee (Wilhelm *et al.* 1993). Zinc (Zn), along with manganese (Mn), is acknowledged as one of the most frequently diagnosed micronutrient disorders of cereals in these regions (McDonald *et al.* 2001). In a survey of the Zn concentration in barley grains from each Hundred of South Australia, Spouncer *et al.* (1994) found that grain from the Murray Mallee, Eyre Peninsula and parts of Yorke Peninsula had the lowest concentration of grain Zn in the state, and in many cases these concentrations were in the critical deficiency concentration range of 10-15 mg kg^{-1} (Riley *et al.* 1992).

Zinc deficiency is particularly common in the arid and semi-arid regions of the world (Cakmak 2000) and these areas of Australia are no exception. Cereals growing in these regions are therefore also likely to experience other environmental stresses, including drought and high temperature stress, during their growing season, and especially during grain filling. It has been hypothesised that improvement in the Zn nutritional status of plants may be important for their survival under such stress conditions (Cakmak 2000). Zn plays an important role in controlling both the generation and detoxification of toxic reactive oxygen species (ROS), the by-products of normal cell metabolism that are over-produced under various environmental stresses, including heat and water stress (Bowler *et al.* 1992, Scandalios 1993). These ROS are responsible for damage to a number of cellular components, including membrane proteins, phospholipids, chlorophyll and nucleic acids (Mead 1976, Brawn and Fridovich 1981, Davies 1987, Foyer and Harbinson 1994). Zn not only has an inhibitory effect on the generation of ROS by NADPH-oxidase, but is also involved in the detoxification of ROS as a

component of the copper/zinc superoxide dismutase (Cu/Zn-SOD) enzyme (Chvapil 1979a, Fridovich 1986, Cakmak and Marschner 1988b, Cakmak *et al.* 1997, 1998a).

There is limited evidence for the involvement of Zn in stress tolerance. In experiments with animals, supplementary Zn improved growth and heat tolerance when exposed to high temperature stress (Männer and Wang 1991). Wenzel and Mehlhorn (1995) found that Zn deficiency caused a higher sensitivity to O_3 toxicity in bush bean (*Phaseolus vulgaris* L.), which correlated well with a reduced activity of Cu/Zn-SOD. Chilling stress in citrus and drought stress in wheat have been found to be more pronounced in plants suffering from Zn deficiency (Cakmak *et al.* 1995, Ekiz *et al.* 1998). In a controlled environment experiment with lucerne (*Medicago sativa* L.), Grewal and Williams (2000) showed that plants supplied with adequate Zn nutrition were better able to cope with both water stress and excessive soil moisture during the early vegetative stage. However the response of plants to Zn fertilisation under different temperature conditions in the field, and the genetic variation for yield performance that may exist under such situations, has been largely overlooked.

A series of field trials was conducted at six sites throughout South Australia and Victoria with the primary purpose of evaluating some of the genotype by environment interaction responses of wheat to Zn fertilisation. The growth and yield responses of seven genotypes of wheat, known to differ in either their Zn efficiency or high temperature tolerance, were compared at various levels of Zn nutrition, and sites were chosen where different levels of Zn deficiency and high temperature stress during grain filling could be expected. A secondary objective of the study was to produce grain that had developed where different levels of high temperature and Zn deficiency had been encountered, for the subsequent assessment of grain protein content and protein composition (Chapter 7). A further aim of the study was to determine whether elevated supplies of Zn could alleviate the adverse effects of heat stress on photosynthetic activity in wheat under field conditions, thus supporting some of the results obtained from the pot experiments (Chapters 4 and 6).

5.2 Materials and methods

5.2.1 Sites

Six field experiments were sown throughout South Australia and Victoria in 1998 and 1999 (Table 5.1). These locations were chosen because different levels of high temperature stress could be expected to occur during grain filling. On-site or near site weather stations were used to collect daily maximum and minimum air temperatures during grain fill, from anthesis until physiological maturity, for each trial location.

Table 5.1. Long-term average monthly maximum temperatures, annual rainfall and growing season rainfall, calculated from a minimum of 35 years of climatological data (Bureau of Meteorology 2002), for sites chosen for field experiments in 1998 and 1999.

Location	Latitude & Longitude	Long-term average maximum temperature (°C)			Average annual rainfall (mm)	Average April – October rainfall (mm)**
		Oct	*Nov*	*Dec*		
Minnipa	32.84°S 135.15°E	24.2	27.3	29.4	327	242
Waite Institute	34.97°S 138.63°E	20.2	23.2	25.8	622	484
Lameroo	35.33°S 140.52°E	22.5	26.0	28.9	387	278
Tintinara*	35.53°S 140.04°E	21.5	24.6	27.5	466	350
Birchip	35.98°S 142.92°E	21.9	25.8	29.2	347	256
Horsham	36.65°S 142.10°E	20.9	24.7	27.6	449	317

***** Since there is no weather station at Tintinara, the data provided are from the nearest weather station, at Keith (36.10°S, 140.35°E), approximately 36 kilometres away.

****** The growing season rainfall (April-October) is useful to predict potential yields under South Australian conditions (French and Schultz 1984).

5.2.2 Genotypes

Six bread wheat cultivars and one durum wheat cultivar were chosen for these field studies on the basis of previously reported differences in their Zn efficiency or thermotolerance (Chapter 3). The bread wheat genotypes used in 1998 were Excalibur and Trident (Zn efficient), Frame (moderately Zn efficient) and Goldmark (Zn inefficient). Also used was Halberd (heat tolerant) and Meering (heat sensitive); while the durum wheat genotype used was Kronos (heat tolerant). In 1999 a second durum wheat cultivar was included in the experiments, Daki-Cyn (Zn inefficient). The Zn efficiency of Halberd, Meering and Kronos was unknown at the time of planting, while the thermotolerance of Excalibur, Trident, Frame, Goldmark and Daki-Cyn had also not been reported.

5.2.3 Lameroo and Tintinara, 1998

These experiments were conducted during the growing season of 1998 in the Mallee region of South Australia. The soil at Lameroo was a calcareous sandy earth, while that at Tintinara was a sandy alkaline yellow bleached duplex soil (Stace *et* al. 1968). DTPA-extractable soil Zn was 0.27 mg kg^{-1} at the Lameroo site and 0.30 mg kg^{-1} at Tintinara. The trials were sown in a randomised block, split-plot design with 5 replications. Each replicate was split into the seven wheat varieties as the main plots, and the four Zn treatments were randomised within these as the subplots. Each plot was approximately 4.5 m^2 and consisted of eight rows, 15 cm apart and 5 m long. Seed was planted with a cone seeder at a depth of approximately 25-30 mm and a rate of 180 – 190 seeds m^{-2}. Sowing took place on 28 May 1998 at Lameroo and on 12 June 1998 at Tintinara. Basal fertiliser (Hi-Fert Pty Ltd, Gillman, South Australia) was applied at sowing at a rate of 190 kg ha^{-1} and consisted of 15:17:0 coated with Cu, Co, Mn and Mo (Table 5.2). No Zn was added in the basal fertiliser.

Table 5.2. Basal fertiliser used for field trials at Lameroo, Tintinara, Horsham and Birchip (Hi-Fert Pty Ltd).

Element	%	Application rate (kg ha^{-1})
N	14.7	28.90
P	17.2	32.70
S	1.25	2.40
Cu	1.15	2.20
Co	0.14	0.27
Mn	2.9	5.50
Mo	0.14	0.27

Zn treatments consisted of 0, 7.5 and 22.5 kg Zn ha^{-1}, delivered directly with the seed as granules of zinc oxysulphate, and a fourth treatment of 7.5 kg Zn ha^{-1} applied at sowing and supplemented with 2 foliar sprays of Zincsol® (Incitec Pivot Limited, Southbank, Victoria), which delivered 334 g Zn ha^{-1} at each spray. These sprays were applied at tillering and at stem elongation, 8 and 14 weeks after sowing, respectively.

A single harvest was conducted at anthesis at Lameroo, 19 weeks after sowing. Quadrats of 0.5 m by 4 rows (2 adjacent rows from each end of the plot) were harvested from the 0 and 22 kg Zn ha^{-1} treatments of all five replicates. Samples were oven-dried at 70ºC for a minimum of 48 h and weighed to determine dry weight. Plots were reduced to a length of 4.76 m before grain yield was reaped by a Wintersteiger® small plot harvester from the inner six rows of each plot on 17 December 1998 at Lameroo and 21 December 1998 at Tintinara. Clean sub-samples of grain from each plot were counted and weighed to establish kernel weight and number of grains per m^2. Grain was analysed for Zn and other nutrient concentrations by ICP spectrometry (Chapter 3, Section 3.6).

5.2.4 Minnipa, 1998

This experiment was also conducted during the growing season of 1998, at the Minnipa Agricultural Centre on the Upper Eyre Peninsula of South Australia. The soil at this site was a red-brown calcareous sandy earth (Stace *et al*. 1968). The DTPA-extractable Zn content of the soil at this site was not measured, but it is accepted that the soil at this research centre would be higher in Zn content than that at Lameroo or Tintinara. This trial was also planted in a randomised block, split-plot design with 5 replications. Again the varieties formed the main plots and the Zn treatments the subplots. Each plot was 10.1 m^2 and consisted of eight rows, 17.8 cm apart and 7 m long. Sowing took place on 17 June 1998 at a rate of 200 seeds m^{-2}. Basal fertiliser (MAP) was applied at sowing at a rate of 50 kg ha^{-1} and consisted of 10:22:0, with 2% S. Four Zn treatments, 0, 2.5, 7.5 and 22.5 kg Zn ha^{-1}, were applied at sowing as granules of zinc oxysulphate.

Dry matter samples were taken at maturity, from 10 plants sampled at random from the inner two rows of each plot of three replicates. Fertile and infertile tillers were counted and dry weight was measured. Plots were reduced to a length of 5 m before grain yield was reaped from the inner six rows of each plot using a small plot harvester on 26 November 1998. Clean sub-samples of grain from each plot were counted and weighed to establish kernel weight and number of grains per m^2, and grain was analysed for nutrient concentrations by ICP spectrometry.

5.2.5 Waite Institute, 1998

This experiment was conducted in a bird proof enclosure at the Waite Institute, Urrbrae, South Australia. The soil at this site is a hard alkaline red duplex soil (Stace *et al*. 1968). The DTPA-extractable soil Zn concentration was not established for this site, but since the enclosure is primarily used for seed multiplication of breeding lines, it can be assumed that the site is not deficient in Zn. Since the aim of this experiment was to examine the effects of an elevated post-anthesis supply of Zn on plants growing under heat stress, later sowing dates than usual were used. The trial was planted on two

sowing dates, 25 July 1998 (SD 1) and 11 September 1998 (SD 2). Both of these sowing dates are later than the optimum sowing time of late April – June for this environment (Cawood *et al.* 1996), but were chosen purposefully to induce high temperature stress during grain filling.

The experimental design was a split-split-plot design with three replicates. The two sowing dates were the main plots, the two Zn treatments were randomised within the subplots and the seven wheat varieties were further randomised within these as the sub-subplots. Each plot consisted of three rows 2 m in length, with 25 cm between rows. Sixty-seven seeds were sown per row, a seeding rate of approximately 200 plants m^{-2}. Yates Complete Manure 'D'® fertiliser was applied at stem elongation at the rate of 1 t ha^{-1}. This delivered 80 kg ha^{-1} of N, 34 kg ha^{-1} of P and 95 kg ha^{-1} of K; plus S and Ca. Additionally, urea was applied to the soil at anthesis at 40 kg ha^{-1}, which delivered 19 kg N ha^{-1}. Plots were frequently sprinkler irrigated to avoid any drought stress, and weeds were controlled by hand weeding. All plots were sprayed twice for rust with Bayleton® at 1 kg ha^{-1} on 30 October 1998 and 29 November 1998.

Zinc was applied to the +Zn subplots at the beginning of anthesis as a foliar spray of $ZnSO_4$, at a rate of 570 g Zn ha^{-1}. Control plots (-Zn) were sprayed with water only. In order to assess the effectiveness of this treatment, three plants per plot of the variety Kronos were sampled 12 days after the application of the Zn treatment. The main stems of each plant were separated into the following plant parts: ear, flag leaf, other leaves and stem, and then washed prior to analysis for Zn concentration by ICP spectrometry (Chapter 3, Section 3.6). After washing, excess water was drained from the sample, which was then placed in a paper bag and dried at 80ºC for 48 h prior to analysis.

Each main stem ear was labelled with the date of first anther exsertion, and the date of all subsequent operations was related to this. Only plants that had flowered on the same day were sampled at each harvest. For SD 1, plot harvests were performed at 5-day intervals, beginning at 12 days after anthesis (DAA) and concluding at 37 DAA. Final harvests took place at 57 DAA. For SD 2 plots, harvests were performed at 5-day intervals beginning at 12 DAA and concluding at 27 DAA, for Excalibur, Goldmark,

Frame and Meering. For Kronos, Halberd and Trident, harvests were performed at 12 and 27 DAA only. Final harvests occurred at 45 DAA for all varieties except Kronos, which was performed at 52 DAA. At each harvest, a total of 12 grains per plot were removed from the *a* and *b* florets of the two central spikelets on each side of the main stem ear. Following fresh weight determination, grains were oven-dried at 70°C for a minimum of 48 hours, and their dry weights were recorded. At maturity, ten main stem ears were harvested and hand-threshed. Grains were counted to determine grain number and weighed to establish grain yield and individual kernel weight.

Chlorophyll fluorescence measurements were made on the flag leaves of two varieties, Frame and Goldmark, at SD 2, as described in Chapter 3 (Section 3.5.1). Six measurements were made between 5 and 20 DAA, prior to any leaf senescence. Measurements were taken on 3 plants per plot, during the hours of 11.30 am and 3.00 pm. The air temperature during each chlorophyll fluorescence measurement was determined using a thermometer placed in a ventilated box, similar to a Stevenson Screen, and this was correlated with the current temperature recorded by the on-site weather station, located approximately 500 m away from the plots.

5.2.6 Birchip and Horsham, 1999

These experiments took place during the growing season of 1999 in the Mallee and Wimmera regions of Victoria. The soil at the Birchip site was a hard alkaline red-brown bleached duplex soil; while at Horsham the soil was a grey cracking clay with self-mulching surface (Stace *et al*. 1968). DTPA-extractable Zn was 0.30 mg kg^{-1} at Birchip and 0.52 mg kg^{-1} at the Horsham site. At Horsham, two trials were conducted at the one site and were based on different sowing dates; one sown at the 'normal' sowing time, after the opening rains, and the other sown 'late', some five weeks later. Each trial was again sown in a split-plot design set up as completely randomised blocks, with 5 replications. Each replicate was split into the eight wheat varieties as the main plots, and the four Zn treatments were randomised within these as the subplots. Plot

size and seeding details were identical to those used at Lameroo and Tintinara in 1998. Sowing took place on 9 June 1999 at Birchip, and on 8 June 1999 and 15 July 1999 at Horsham for sowing dates 1 and 2, respectively. Basal fertiliser was applied as at Lameroo and Tintinara the previous year.

The four Zn treatments consisted of the following: (1) nil Zn, hereafter designated as Zn_0, (2) 7.5 kg Zn ha^{-1} applied at sowing as zinc oxysulphate, designated as Zn_1, (3) 7.5 kg Zn ha^{-1} applied at sowing plus a single foliar spray of Zincsol® at anthesis, designated as Zn_2, and (4) 7.5 kg Zn ha^{-1} applied at sowing plus 2 foliar sprays of Zincsol® applied at tillering and at stem elongation, designated as Zn_3.

A single harvest was conducted during the vegetative stage at Birchip, 11 weeks after sowing. Quadrats of 0.5 m by 4 rows (2 adjacent rows from each end of the plot) were harvested from the Zn_0 and Zn_3 treatments of 3 replicates of all eight varieties. Fifteen youngest emerged leaf blades (YEBs) were sub sampled from each plot and analysed for Zn by ICP spectrometry (Chapter 3, Section 3.6). Samples were oven-dried at 70ºC for a minimum of 48 h before being weighed to determine dry weight. Canopy temperature depression (CTD) readings were taken during grain filling at both sites. These readings were taken between the hours of noon and 3 pm, on sunny days, from each end of the plot using a hand-held infrared thermometer. Three replications of each variety from the Zn_0 and Zn_3 treatments were evaluated. Measurements were taken 1m from the edge of each plot; approximately 50 cm above the canopy and the view angle was around 40º to the horizontal line above the canopy so as to avoid the confounding effect of soil temperature (Amani *et al.* 1997). Air temperature was simultaneously measured and CTD was calculated as $CTD = T_a - T_c$, where T_a was the air temperature and T_c the temperature of the canopy (Royo *et al.* 2002).

Plots at each site were reduced to a length of 4.76 m before grain yield was reaped using a Wintersteiger® small plot harvester from the inner six rows of each plot on 2 December 1999 at Birchip and on 11 December 1999 at Horsham (both sowing dates). Clean sub-samples of grain from each plot were counted and weighed to establish kernel weight and number of grains per m^2. Grain from two varieties, Frame and

Goldmark, was analysed for Zn and other nutrient concentrations by ICP spectrometry (Chapter 3, Section 3.6).

5.2.7 Statistical analyses

Each location was analysed separately as a split plot design or split/split plot (Waite Institute) using Genstat 5 (Chapter 3, Section 3.7). The data were subjected to an analysis of variance to separate the main effects of genotype and Zn treatment, and their interaction (genotype x Zn treatment). Data from the Waite Institute and Horsham included the main effect of sowing date and its interaction effects. For a description of the effect of Zn on grain yield stability across sites a Finlay-Wilkinson regression model (Finlay and Wilkinson 1963) was used.

Grain filling data from the Waite Institute was fitted with the ordinary logistic growth model (France and Thornley 1984), which has been found to adequately describe grain growth in wheat (Loss *et al.* 1989, Zahedi 2001). Grain dry weight was used as the response variable and time (days after anthesis) was used as the independent variable. The ordinary logistic growth equation is of the form

$$y = A + C/[1 + \exp(-B(t-M))]$$

where A is the initial size of the grain; C is the final dry weight, B is the rate of growth (a slope parameter) and M is the time at which the inflexion point occurs. These parameters were estimated using the Genstat statistical program, with A set to 0. This function is symmetrical about its inflexion point, and the maximum growth rate was calculated as BC/4 (Loss *et al.* 1989) and the duration of grain filling as (BM + 2.944)/B (Zahedi 2001).

5.3 Results

5.3.1 Meteorological data

Maximum temperatures were near average during the grain filling period in both 1998 and 1999 (Table 5.3).

Table 5.3. A summary of maximum temperatures during grain filling and growing season rainfall for the six sites used for the field experiments in 1998 and 1999.

Site	Mean maximum temperature (ºC)			Number of days > 30ºC		Number of days >35ºC		Mean maximum temperature during grain filling (ºC)	Mean April – October rainfall (mm)
	Oct	*Nov*	*Dec*	*Oct*	*Nov*	*Oct*	*Nov*		
1998									
Minnipa	24.7	28.3	31.2	4	11	2	3	26.6	232
Waite Institute	19.6	23.4	26.1	1	4	0	0	SD 1 : 23.8 SD 2 : 26.7	Irrigated
Lameroo	22.5	26.1	30.3	4	8	2	1	25.6	289
Tintinara*	20.1	24.8	28.1	1	5	1	1	23.9	355
1999									
Birchip**	21.9	23.4	26.6	0	2	0	0	23.0	216
Horsham	22.0	23.4	27.2	1	3	0	0	SD 1 : 23.9 SD 2 : 24.0	264

* Since there is no weather station at Tintinara, the data provided are from the nearest weather station, at Keith, S.A., approximately 36 kilometres away.

** Since there is no weather station at Birchip, the data provided are from the nearest weather station, at Donald, Victoria, approximately 44 kilometres away.

Minnipa was the warmest site, with an average maximum temperature of 26.6ºC during grain filling. During October-November Minnipa experienced 15 days above 30ºC and 5 days above 35ºC. In contrast, Birchip was the coolest site, with an average

temperature of 23.0ºC during grain filling and only 2 days above 30ºC during October-November. The growing season rainfall (April–October) was close to average at Lameroo and Tintinara in 1998, but below average at Minnipa in 1998 (40 mm below the mean) and also below average in 1999 at Birchip (40 mm below the mean) and Horsham (53 mm below the mean).

5.3.2 Lameroo, Tintinara and Minnipa, 1998

5.3.2.1 Shoot dry matter production

The mid-season dry matter harvest conducted at Lameroo on 9 October 1998 showed significant differences between varieties, but not between Zn treatments (Table 5.4). At this stage of growth, Meering had produced the least amount of shoot dry matter (775 g m^{-2}), while Excalibur had produced the most (896 g m^{-2}).

Table 5.4. Shoot dry matter production at anthesis (19 weeks after sowing) of the 7 wheat genotypes grown at Lameroo in 1998.

Genotype	Shoot dry weight (g m^{-2})		
	0 kg Zn ha^{-1}	**22.5 kg Zn ha^{-1}**	***Mean***
Excalibur	863	929	*896*
Frame	857	888	*872*
Goldmark	781	771	*776*
Halberd	780	856	*818*
Kronos	886	829	*857*
Meering	793	757	*775*
Trident	815	867	*841*
Mean	*825*	*842*	
$LSD_{0.05}$			
Genotype	**69**		
Zinc	**n.s.**		
Genotype x Zinc	**n.s.**		
CV (%)	**8.0**		

n.s. = non-significant

There was no significant effect of Zn on dry matter production at maturity at Minnipa, however there were significant differences between varieties in tiller production (Table 5.5). Kronos, the durum wheat, produced significantly fewer tillers than any of the bread wheat varieties. Meering produced significantly more fertile tillers than the other varieties, although the total number of tillers was not significantly different.

Table 5.5. Fertile tillers, total tillers and shoot dry weight at maturity (g m^{-2}) of the 7 wheat genotypes grown at Minnipa in 1998.

Genotype	Number of fertile tillers per plant	Total number of tillers per plant	Shoot dry weight (g m^{-2})
Excalibur	1.7	2.8	650
Frame	1.8	2.8	574
Goldmark	1.8	2.7	602
Halberd	1.8	2.9	612
Kronos	1.3	2.0	544
Meering	2.1	2.8	589
Trident	1.8	2.6	590
$LSD_{0.05}$	**0.2**	**0.3**	**n.s.**

n.s. = non-significant

5.3.2.2 Grain yield and yield components

The main effects of variety and Zn treatment were both highly significant ($P < 0.001$) for grain yield at Lameroo, as was their interaction ($P = 0.022$). Five varieties, Excalibur, Frame, Goldmark, Kronos and Trident, showed a significant increase in yield with the application of Zn, while Halberd and Meering were unresponsive to Zn (Table 5.6). The response to the different Zn treatments varied between genotypes, however. Excalibur, Kronos and Trident showed an average increase of 8% with the 7.5 kg Zn ha^{-1} treatment, Goldmark showed an 8% increase with the Zn foliar spray, while Frame only showed an increase in yield with the application of 22.5 kg of Zn ha^{-1} (6%). These increases in grain yield at Lameroo were due solely to an increase in grain number

(Table 5.6), as none of the Zn treatments caused an increase in grain weight. The foliar spray of Zn increased the number of kernels per m^2 by an average 14% in Excalibur, Goldmark, Kronos, Meering and Trident but this was offset by an average 9% decrease in the kernel weight of these same varieties, with the exception of Trident. The highest yielding varieties at this site were Excalibur (352 g m^{-2}), Trident (347 g m^{-2}) and Frame (335 g m^{-2}), while the lowest was Halberd (277 g m^{-2}).

The application of Zn also increased grain yield at Tintinara, but there was no interaction between genotype and Zn treatment at this site (Table 5.7). The 7.5 kg ha^{-1} and 22.5 kg ha^{-1} Zn treatments both increased yield by 6%, whereas the yield of the 7.5 kg ha^{-1} plus foliar spray treatment did not differ from that of the nil Zn treatment. Grain yield increases due to supplementary Zn were again a result of an increase in grain number (Table 5.7), with the 7.5 kg ha^{-1} and 22.5 kg ha^{-1} Zn treatments increasing the number of grains per m^2 by 5% and 6%, respectively. Grain number was also increased by the 7.5 kg ha^{-1} plus foliar spray treatment, but this was offset by a 6% decrease in grain weight. This is a similar result to that observed at Lameroo. The highest yielding varieties at this site were again Trident, Frame and Excalibur (426, 422 and 407 g m^{-2}, respectively), while Halberd was again the lowest yielding variety (301 g m^{-2}).

Grain yield at Minnipa was not significantly affected by Zn fertilisation, but there were significant differences between varieties (Table 5.8). Once again Trident (255 g m^{-2}) and Excalibur (254 g m^{-2}) were the highest yielding cultivars. However, at this site Meering, an early maturing variety that is reported to be heat sensitive with respect to kernel weight (Stone and Nicolas 1995a, Cawood *et al.* 1996), produced the lowest grain yield (188 g m^{-2}). These differences in grain yield between the bread wheat varieties were associated with differences in grain set, since all bread wheat genotypes produced grain of a similar kernel weight. Meering produced the lowest number of grains per m^2, and this was reflected in the significantly lower grain yield. This was despite producing significantly more fertile tillers, indicating that the number of grains per tiller of Meering was considerably lower than that of the other varieties. Kronos, the durum wheat, produced kernels of a significantly higher weight than the bread wheats, but significantly fewer tillers and fewer grains per m^2.

Table 5.6. Effects of genotype and zinc fertilisation (kg ha^{-1}) on the grain yield (g m^{-2}), kernel weight (mg) and kernel number (grains m^{-2}) of wheat plants grown at Lameroo in 1998.

Genotype	Grain Yield (g m^{-2})					Kernel Weight (mg)					Kernel number (grains m^{-2})				
	0	**7.5**	**7.5 + spray**	**22.5**	***Mean***	**0**	**7.5**	**7.5 + spray**	**22.5**	***Mean***	**0**	**7.5**	**7.5 + spray**	**22.5**	***Mean***
Excalibur	335	372	350	350	*352*	38.2	37.6	36.1	37.8	*37.4*	8795	9905	9742	9263	*9426*
Frame	325	334	334	346	*335*	37.7	36.4	36.5	38.1	*37.2*	8618	9187	9146	9063	*9003*
Goldmark	263	281	285	295	*281*	30.1	29.6	28.4	30.7	*29.7*	8789	9518	10028	9589	*9481*
Halberd	271	277	278	284	*277*	34.8	35.3	35.0	34.9	*35.0*	7730	7856	7938	8152	*7919*
Kronos	291	310	307	300	*302*	44.8	45.2	39.4	42.9	*43.1*	6490	6896	7794	7005	*7093*
Meering	311	301	325	305	*310*	27.6	26.8	25.2	26.8	*26.6*	11299	11299	12901	11405	*11726*
Trident	332	357	365	336	*347*	35.1	35.3	34.0	34.7	*34.8*	9451	10104	10714	9689	*9989*
Mean	*304*	*319*	*320*	*317*		*35.5*	*35.2*	*33.5*	*35.1*		*8739*	*9248*	*9752*	*9166*	
LSD$_{0.05}$															
Genotype			**25**					**1.7**					**745**		
Zinc			**7**					**0.6**					**258**		
Genotype x Zinc															
(a)			**19**					**1.5**					**681**		
(b)			**29**					**2.1**					**935**		
CV (%)			**4.8**					**3.4**					**5.9**		

(a) For comparisons within the same genotype

(b) For other comparisons

n.s. = non-significant

Table 5.7. Effects of genotype and zinc fertilisation (kg ha^{-1}) on the grain yield (g m^{-2}), kernel weight (mg) and kernel number (grains m^{-2}) of wheat plants grown at Tintinara in 1998.

Genotype	Grain Yield (g m^{-2})					Kernel Weight (mg)					Kernel number (grains m^{-2})				
	0	**7.5**	**7.5 + spray**	**22.5**	***Mean***	**0**	**7.5**	**7.5 + spray**	**22.5**	***Mean***	**0**	**7.5**	**7.5 + spray**	**22.5**	***Mean***
Excalibur	397	396	402	430	*407*	39.8	39.1	37.0	38.1	*38.5*	9994	10141	10921	11295	*10588*
Frame	427	435	404	424	*422*	39.1	37.8	35.0	37.9	*37.5*	10931	11512	11520	11230	*11298*
Goldmark	321	343	353	359	*344*	33.8	34.6	33.2	35.9	*34.4*	9509	9993	10691	10015	*10052*
Halberd	287	304	280	334	*301*	38.8	38.9	36.5	39.5	*38.4*	7363	7819	7665	8482	*7832*
Kronos	308	381	364	364	*354*	50.3	51.6	49.2	51.9	*50.8*	6129	7389	7389	7013	*6980*
Meering	399	407	364	394	*391*	29.3	30.8	26.5	29.7	*29.1*	13667	13232	13766	13326	*13498*
Trident	412	443	428	421	*426*	37.9	36.8	35.2	37.6	*36.9*	11012	12042	12173	11289	*11629*
Mean	*364*	*387*	*371*	*389*		*38.4*	*38.5*	*36.1*	*38.6*		*9801*	*10304*	*10589*	*10376*	
$LSD_{0.05}$															
Genotype			**31**					**2.2**					**857**		
Zinc			**15**					**0.9**					**416**		
Genotype x Zinc			**n.s.**					**n.s.**					**n.s.**		
CV (%)			**8.2**					**4.9**					**8.5**		

n.s. = non-significant

Table 5.8. Effects of genotype and zinc fertilisation (kg ha^{-1}) on the grain yield (g m^{-2}), kernel weight (mg) and kernel number (grains m^{-2}) of wheat plants grown at Minnipa in 1998.

Genotype	Grain Yield (g m^{-2})					Kernel Weight (mg)					Kernel number (grains m^{-2})				
	0	**2.5**	**7.5**	**22.5**	***Mean***	**0**	**2.5**	**7.5**	**22.5**	***Mean***	**0**	**2.5**	**7.5**	**22.5**	***Mean***
Excalibur	259	251	256	251	*254*	31.3	31.1	32.1	32.0	*31.6*	8279	8083	7959	7832	*8038*
Frame	239	241	241	242	*241*	33.6	32.7	32.6	33.4	*33.1*	7121	7374	7403	7264	*7291*
Goldmark	235	238	230	235	*234*	31.8	31.7	31.1	31.5	*31.6*	7383	7494	7403	7454	*7434*
Halberd	230	229	242	235	*234*	30.6	32.0	31.7	32.2	*31.6*	7503	7154	7624	7296	*7394*
Kronos	222	219	209	217	*217*	39.0	39.5	38.7	38.1	*38.8*	5710	5544	5398	5718	*5592*
Meering	179	188	201	183	*188*	30.6	31.1	31.2	31.4	*31.1*	5912	6050	6487	5847	*6074*
Trident	257	251	256	256	*255*	31.0	31.0	31.0	30.0	*30.7*	8280	8110	8273	8533	*8299*
Mean	*231*	*231*	*233*	*231*		*32.6*	*32.7*	*32.6*	*32.7*		*7170*	*7116*	*7221*	*7135*	
LSD$_{0.05}$															
Genotype			**19**					**1.3**					**630**		
Zinc			**n.s.**					**n.s.**					**n.s.**		
Genotype x Zinc			**n.s.**					**n.s.**					**n.s.**		
CV (%)			**5.8**					**3.1**					**5.8**		

n.s. = non-significant

5.3.2.3 Grain zinc concentration and content

Supplementary Zn fertilisation enhanced Zn concentration in the grain at Lameroo and Tintinara, but not at Minnipa (Table 5.9). No increase in grain Zn concentration was observed with the 7.5 kg Zn ha^{-1} treatment at either site, but the 22.5 kg Zn ha^{-1} treatment boosted grain Zn by an average of 23% at Tintinara, and by 29% at Lameroo. The Zn concentration was further increased in all varieties by the Zn foliar spray at both sites. At Tintinara this was an increase of 83%, from 13 mg kg^{-1} to 23 mg kg^{-1}, while at Lameroo the increase was 139%, from 11 mg kg^{-1} to 27 mg kg^{-1}. These grain Zn concentrations indicate that plants grown with no supplementary Zn fertilisation at Lameroo, and to a lesser extent at Tintinara, were grown under Zn deficiency stress. The critical level of Zn in wheat grain is approximately 10 mg Zn kg^{-1} (Riley *et al.* 1992), although a range of up to 13 mg Zn kg^{-1} has also been found in Zn deficient wheat plants (Graham *et al.* 1992).

Grain Zn content increased with Zn fertilisation at all three sites in 1998 (Table 5.10), including Minnipa, despite there being no differences in kernel weight with Zn supplementation at this site. Both the 7.5 kg Zn ha^{-1} and 22.5 kg Zn ha^{-1} treatments increased the grain Zn content at Minnipa, by 12% and 10% respectively, relative to the nil Zn control. Goldmark and Meering were found to accumulate significantly less Zn in the grain than the other varieties (585 and 554 ng Zn per seed, compared with 944 and 975 ng Zn per seed for Frame and Halberd, respectively, in the foliar treatment at Lameroo) and this was a reflection of both a lower grain weight and lower grain Zn concentration in these two varieties.

Table 5.9. Effects of genotype and zinc fertilisation (kg ha^{-1}) on zinc concentration (mg kg^{-1}) in the grain produced at Lameroo, Tintinara and Minnipa in 1998.

Genotype	Lameroo				Tintinara				Minnipa			
	0	7.5	7.5 + spray	22.5	0	7.5	7.5 + spray	22.5	0	2.5	7.5	22.5
Frame	11.1	12.8	28.3	15.6	12.2	13.9	25.7	16.2	14.8	15.3	17.9	16.4
Goldmark	11.6	11.9	23.7	13.3	12.2	14.0	21.0	15.1	13.4	13.1	13.6	14.5
Halberd	11.5	13.6	31.0	15.1	13.3	14.3	25.0	15.6	16.3	16.0	17.1	15.6
Meering	10.7	12.3	24.3	13.7	12.8	13.9	20.7	14.8	14.6	15.7	16.5	15.6
Mean	*11.2*	*12.7*	*26.8*	*14.5*	*12.6*	*14.0*	*23.1*	*15.5*	*14.8*	*15.0*	*16.3*	*15.5*
Excalibur*	10.2	9.5	32.0	12.0	10.9	12.2	22.0	13.6	13.7	11.2	12.3	13.3
Kronos*	8.8	10.8	27.0	13.4	12.1	12.5	24.0	15.0	11.8	12.0	12.9	13.6
Trident*	9.6	11.5	30.0	14.6	10.8	14.2	24.0	14.7	16.2	16.0	16.4	16.7
LSD$_{0.05}$												
Genotype	**n.s.**				**n.s.**				**n.s.**			
Zinc	**1.1**				**1.5**				**n.s.**			
Genotype x Zinc					**n.s.**				**n.s.**			
(a)	**2.3**											
(b)	**2.8**											
CV (%)	**8.3**				**11.1**				**8.9**			

* Only one replicate of these varieties analysed for elemental composition; the data were not included in the ANOVA

(a) For comparisons within the same genotype

(b) For other comparisons

n.s. = non-significant

Table 5.10. Effects of genotype and zinc fertilisation (kg ha^{-1}) on the zinc content (ng seed^{-1}) of grain produced at Lameroo, Tintinara and Minnipa in 1998.

Genotype	Lameroo				Tintinara				Minnipa			
	0	7.5	7.5 + spray	22.5	0	7.5	7.5 + spray	22.5	0	2.5	7.5	22.5
Frame	382	417	944	549	429	477	806	526	454	458	537	517
Goldmark	303	308	585	359	379	428	624	484	378	380	386	413
Halberd	363	425	975	464	471	505	826	556	457	468	488	474
Meering	260	289	554	326	344	399	534	417	392	441	466	436
Mean	*327*	*360*	*765*	*424*	*406*	*452*	*697*	*496*	*420*	*437*	*469*	*460*
Excalibur*	351	320	986	416	409	400	686	477	380	309	350	390
Kronos*	366	438	900	509	533	574	1047	683	390	378	422	444
Trident*	301	356	836	434	373	453	717	414	465	434	480	467
LSD$_{0.05}$												
Genotype		**55**				**51**				**n.s.**		
Zinc		**42**				**49**				**37**		
Genotype x Zinc						**n.s.**				**n.s.**		
(a)		**83**										
(b)		**85**										
CV (%)		**10.6**				**11.3**				**9.8**		

* Only one replicate of these varieties analysed for elemental composition; the data were not included in the ANOVA

(a) For comparisons within the same genotype

(b) For other comparisons

n.s. = non-significant

5.3.3 Waite Institute, 1998

5.3.3.1 Shoot zinc concentration

The Zn concentration within the flag leaves of the durum wheat variety, Kronos, harvested at 12 DAA, showed that the –Zn plants of SD 1 were deficient in Zn (Table 5.11). The average Zn concentration of these flag leaves was 12 mg kg^{-1}, which is well below the critical deficiency level of 16-17 mg Zn kg^{-1} for flag leaves of wheat (Sharma and Katyal 1986, Rashid and Fox 1992). This is in contrast to that in the flag leaves of the –Zn, SD 2, plants, which, with a Zn concentration of 20 mg kg^{-1}, were well within the adequate Zn range for this stage of plant growth. With the addition of the Zn foliar spray at anthesis, the Zn concentration in the flag leaves had increased markedly at both sowing dates, to 44 mg kg^{-1} for the SD 1 plants, and to 103 mg kg^{-1} for the SD 2 plants.

Zinc concentration in the various parts of the main stem revealed that 12 days after the application of the Zn treatment most of the Zn taken up by the plants had remained in the leaves (Table 5.11). This can be attributed to the fact that the leaves of wheat act as temporary storage reserves for Zn during the early stages of grain development, and it is later, during grain maturity, when the Zn is remobilised from the leaves to the grain (Pearson and Rengel 1994). The concentration of Zn in the flag leaves and other leaves increased with Zn fertilisation by an average of 353% and 224%, respectively, whereas there was no significant difference in Zn concentration of the stem and ear between the two Zn treatments.

Table 5.11. Zinc concentration within the various parts of the main stem of Kronos wheat plants grown at the Waite Institute in 1998, 12 days after the application of a zinc foliar spray.

Plant part	- Zinc			+ Zinc		
	SD #1	**SD #2**	***Mean***	**SD #1**	**SD #2**	***Mean***
Flag leaf	12.0	20.3	*16.1*	43.5	102.5	*73.0*
Other leaves	13.3	32.0	*22.6*	39.0	107.5	*73.2*
Stem	31.5	39.5	*35.5*	36.5	48.0	*42.2*
Ear	49.5	52.5	*51.0*	50.5	68.5	*59.5*
Mean	*26.6*	*36.1*		*42.4*	*81.6*	

$LSD_{0.05}$	
Sowing Date	**n.s.**
Zinc	**9.9**
Plant Part	**n.s.**
Sowing Date x Zinc	
(a)	**14.0**
(b)	**12.6**
Sowing Date x Plant Part	
(a)	**19.8**
(b)	**18.3**
Zinc x Plant Part	**19.8**
Sowing Date x Zinc x Plant Part	**n.s.**
CV (%)	**28.0**

(a) For comparisons within the same sowing date

(b) For other comparisons

n.s. = non-significant

5.3.3.2 Temperatures during grain filling

Mean daily maximum temperatures for SD 1 plants from anthesis until physiological maturity ranged from 23.7°C, for Kronos, to 24.6ºC, for Halberd and Trident (Table 5.12). For SD 2 plants, mean daily maximum temperatures ranged from 26.5°C, for Kronos, to 27.6ºC, for Excalibur, which was 0.5ºC above the other five bread wheat varieties. SD 1 plants experienced 3-4 days of temperatures above 35ºC during grain filling, and these occurred between 42 and 55 DAA, depending on variety (Appendix 5.1). SD 2 plants experienced 9 days of temperatures greater than 35ºC during grain filling, and these occurred between 16 and 52 DAA, depending on variety.

Table 5.12. A summary of grain filling duration and post-anthesis temperature conditions for each of the seven wheat varieties grown at the Waite Institute in 1998.

Variety	Duration of grain filling (days)		Mean maximum temperature during grain filling (ºC)		Number of days > 30ºC		Number of days >35ºC	
	SD 1	*SD 2*	*SD 1*	*SD 2*	*SD 1*	*SD 2*	*SD 1*	*SD 2*
Excalibur	57	45	24.2	27.6	11	19	4	9
Frame	57	45	24.0	27.1	11	18	4	9
Goldmark	57	45	23.9	27.1	11	18	4	9
Halberd	57	45	24.6	27.1	13	18	4	9
Kronos	57	52	23.7	26.5	11	18	3	9
Meering	57	45	24.3	27.1	12	18	4	9
Trident	57	45	24.6	27.1	13	18	4	9

5.3.3.3 Chlorophyll fluorescence

Chlorophyll fluorescence measurements were taken on six occasions during grain filling, between 5 and 20 DAA. The time interval between each measurement varied between 1 and 5 days as the temperature fluctuated, but all measurements were performed prior to any flag leaf senescence. The chlorophyll fluorescence ratio (Fv/Fm) decreased significantly in both varieties examined, Frame and Goldmark, as the ambient temperature rose above 37ºC (Figure 5.1). At 37ºC there was no decline in the Fv/Fm ratio of either Zn treatment in the Zn efficient variety, Frame, but at an ambient temperature of 40ºC this ratio was reduced considerably, from 0.774 to 0.668, with no difference between Zn treatments. In the Zn inefficient variety Goldmark, however, a significant decline in the Fv/Fm ratio was observed at 37ºC, in the –Zn plants only. At this temperature, the Fv/Fm ratio was 0.754 in the plants that had received a foliar spray of Zn, but declined to 0.698 in the plants that had received no supplementary Zn fertilisation. When the temperature rose to 40ºC however, the Fv/Fm was further reduced, to 0.671, with no significant difference between Zn treatments.

This decrease in the Fv/Fm ratio of the –Zn plants of Goldmark at 37ºC was the result

of an increase in chlorophyll initial fluorescence (Fo) (Figure 5.2) rather than any decrease in chlorophyll maximum fluorescence (Fm) (Figure 5.3a and b). The Fo of the –Zn plants of Goldmark was significantly higher than their +Zn counterparts at 37ºC, but that of the +Zn plants rose at 40ºC so that at this temperature there was no significant difference between Zn treatments.

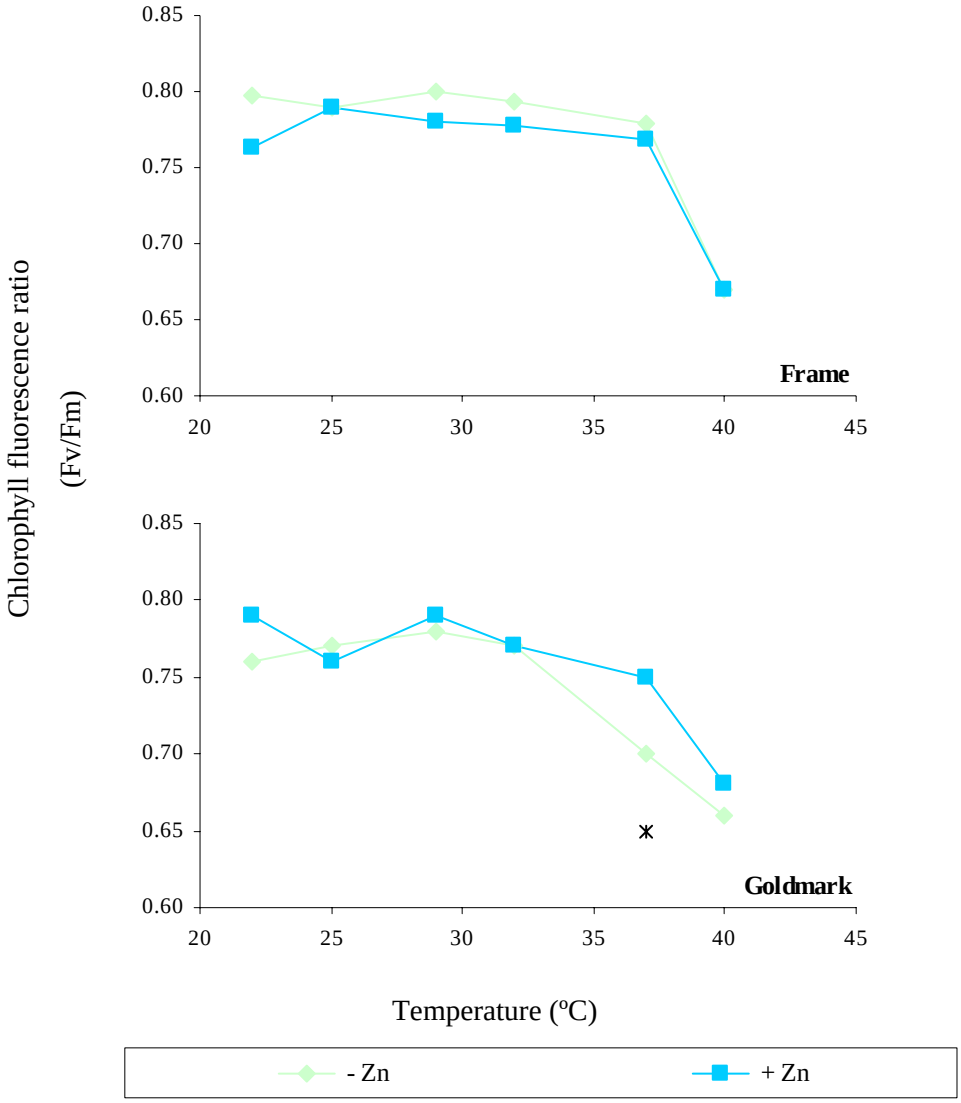


Figure 5.1. Effects of genotype, zinc fertilisation and temperature on the chlorophyll fluorescence ratio, Fv/Fm, of wheat flag leaves of sowing date 2 plants, grown at the Waite Institute in 1998. Measurements were taken intermittently between 5 and 20 days after anthesis, and were not sequential, but have been rearranged to show the effect of temperature. Asterisk indicates a significant difference; P < 0.05.

While the Fm of both varieties declined considerably as the ambient temperature increased (Figure 5.3a and b), there was no difference in Fm between Zn treatments at any temperature in either variety. Similarly the chlorophyll variable fluorescence (Fv), which is the difference between Fm and Fo, did not differ significantly between Zn treatments at any temperature in either variety (Figure 5.3c and d).

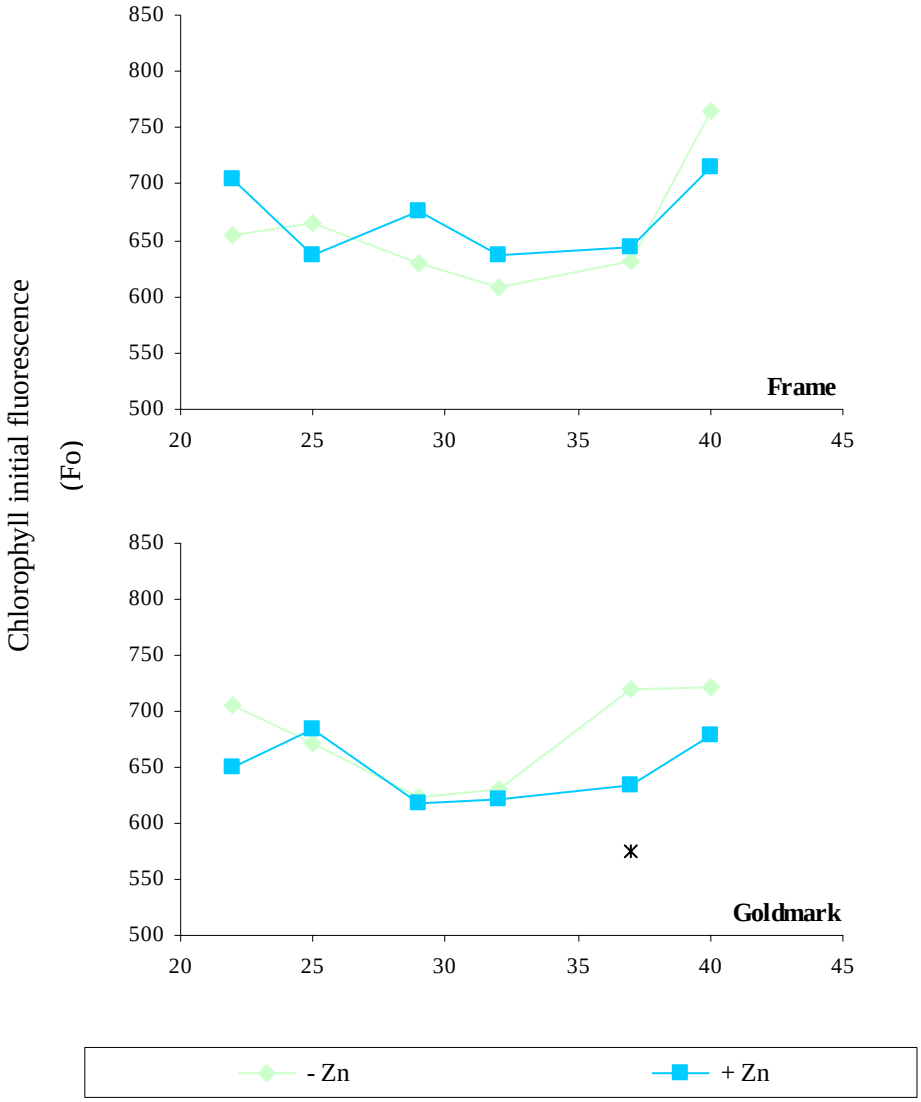


Figure 5.2. Effects of genotype, zinc fertilisation and temperature on chlorophyll initial fluorescence of wheat flag leaves of sowing date 2 plants, grown at the Waite Institute in 1998. Measurements were taken intermittently between 5 and 20 days after anthesis, and were not sequential, but have been rearranged to show the effect of temperature. Asterisk indicates a significant difference; P < 0.05.

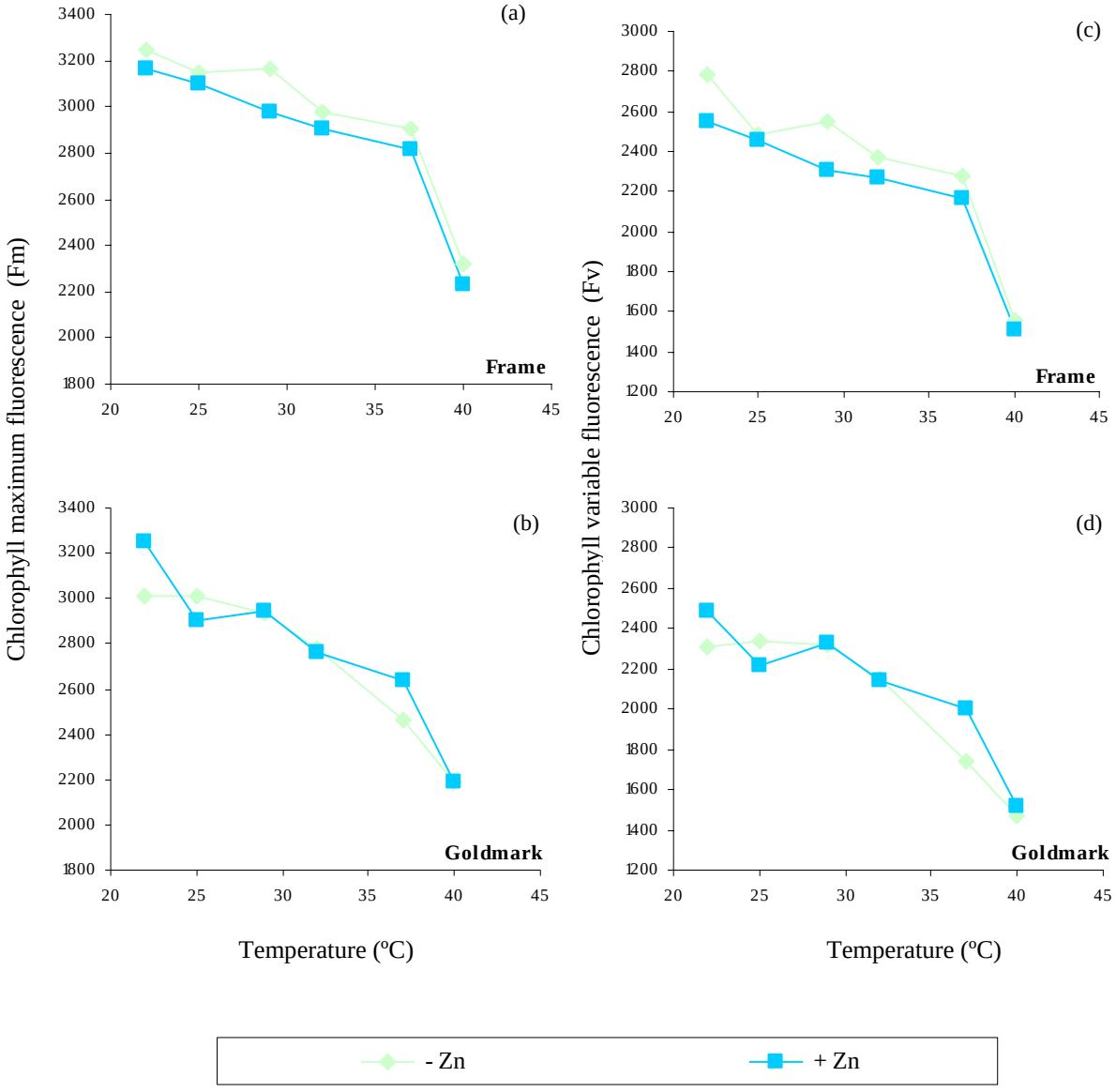


Figure 5.3. Effects of zinc fertilisation and temperature on the chlorophyll fluorescence parameters (a-b, chlorophyll maximum fluorescence; c-d, chlorophyll variable fluorescence) of wheat flag leaves of sowing date 2 plants, grown at the Waite Institute in 1998.
Measurements were taken intermittently between 5 and 20 days after anthesis, and were not sequential, but have been rearranged to show the effect of temperature

5.3.3.4 Parameters of grain filling

The estimated values of grain filling parameters for cultivars Excalibur, Frame, Goldmark and Meering are shown in Tables 5.13 and 5.14. These parameters were all estimated by the ordinary logistic growth model, as described above.

Maximum rate of grain filling

The maximum rate of grain filling is the rate of dry matter accumulation at the inflexion point, and represents the maximum achievable rate of grain filling under the prevailing conditions. There was a significant effect of Zn on the maximum rate of grain filling (Table 5.13), which decreased with Zn fertilisation. The average maximum rate of grain filling over the two sowing dates was 1.96 mg day^{-1} in the –Zn plants, compared to 1.73 mg day^{-1} in the +Zn plants.

There was also a significant interaction between sowing date and genotype on the maximum rate of grain filling, with Excalibur, Frame and Goldmark having a significantly higher rate in the SD 2 plants that those planted 7 weeks earlier (Figure 5.4). There was no difference in the maximum rate of grain filling between sowing dates in Meering, however. Furthermore, while there was little difference between genotypes in the maximum rate of grain filling at SD 1, at SD 2 Meering and Frame (1.55 and 1.84 mg day^{-1} respectively) were significantly lower than Goldmark (2.27 mg day^{-1}), which was significantly lower than Excalibur (2.74 mg day^{-1}).

Time to inflexion point

The inflexion point represents the instant in time when dry matter is accumulated in the grain at its maximum rate. The time to inflexion point (Table 5.13) was 28% lower at SD 2, from 19 days at SD 1 down to 14 days at SD 2 ($P < 0.05$). The percentage reduction in time to inflexion point was highest in Excalibur (35%), followed by Frame (31%), Goldmark (24%) and Meering (20%). There was no effect of Zn on the number of days to the inflexion point.

Table 5.13. Effects of sowing date, zinc fertilisation and genotype on the maximum rate of grain filling (mg day^{-1}) and the time to inflexion point (days), estimated by the ordinary logistic model, of wheat plants grown at the Waite Institute in 1998.

Genotype	Maximum rate of grain filling (mg day^{-1})						Time to inflexion point (days)					
	Sowing Date #1			Sowing Date #2			Sowing Date #1			Sowing Date #2		
	- Zn	+ Zn	*Mean*	- Zn	+ Zn	*Mean*	- Zn	+ Zn	*Mean*	- Zn	+ Zn	*Mean*
Excalibur	1.67	1.66	*1.67*	3.11	2.37	*2.74*	20.5	21.1	*20.8*	12.7	14.4	*13.5*
Frame	1.39	1.35	*1.37*	1.80	1.87	*1.84*	19.9	19.7	*19.8*	13.2	13.9	*13.6*
Goldmark	1.84	1.72	*1.78*	2.58	1.95	*2.27*	19.2	18.7	*18.9*	14.0	14.8	*14.4*
Meering	1.67	1.46	*1.56*	1.65	1.46	*1.55*	20.6	18.9	*19.7*	16.0	15.5	*15.8*
Mean	*1.64*	*1.55*		*2.29*	*1.91*		*20.0*	*19.6*		*14.0*	*14.7*	
LSD$_{0.05}$												
Sowing Date			**n.s.**						**1.6**			
Zinc			**0.18**						**n.s.**			
Genotype			**0.25**						**n.s.**			
Sowing Date x Zinc			**n.s.**						**n.s.**			
Sowing Date x Genotype												
(a)			**0.36**						**1.6**			
(b)			**0.47**						**1.6**			
Zinc x Genotype			**n.s.**						**n.s.**			
Sowing Date x Zinc x Genotype			**n.s.**						**n.s.**			
CV (%)			**16.4**						**8.1**			

(a) For comparisons within the same sowing date

(b) For other comparisons

n.s. = non-significant

Duration of grain filling

The duration of grain filling was reduced in plants from the second sowing date (Table 5.14). There was a significant interaction between sowing date and genotype however, with only a 10% (non-significant) reduction in grain filling duration at SD 2 in Meering. This is in contrast to a significant reduction in duration of 41% at SD 2 in Excalibur, 27% in Frame and 18% in Goldmark (Figure 5.4).

Final grain dry weight

Final grain dry weight, as estimated by the ordinary logistic model, was affected by genotype, sowing date and Zn treatment (Table 5.14). There was a significant interaction between genotype and sowing date on kernel weight, which showed that only Excalibur had a significant reduction in grain dry weight at the second sowing date (14%). There was no significant difference in the dry weights of Frame, Goldmark or Meering between SD 1 and SD 2. This indicates that the increase in the rate of grain filling in Excalibur could not compensate for the shortened duration at SD 2, resulting in a reduction in grain weight. In Frame and Goldmark however, the higher rate of grain filling at SD 2 could make up for the reduced duration, resulting in no difference in dry weight between sowing dates. The grain growth characteristics of Meering did not differ between sowing dates (with the exception of time to inflexion point), and no difference in final grain weight between sowing dates was observed in this variety.

There was also a significant interaction between sowing date and Zn treatment on final grain weight (Table 5.14). Zinc had no effect on grain weight at SD 1, but caused a significant increase in grain weight at SD 2. Furthermore, plants that had received no supplementary Zn at SD 1 had significantly larger grains (11%) than plants that received no supplementary Zn at SD 2 (Figure 5.5).

Table 5.14. Effects of sowing date, zinc fertilisation and genotype on the duration of grain filling (days) and the final grain dry weight (mg), estimated by the ordinary logistic model, of wheat plants grown at the Waite Institute in 1998.

Genotype	Duration of grain filling (days)						Final grain dry weight (mg)					
	Sowing Date #1			Sowing Date #2			Sowing Date #1			Sowing Date #2		
	- Zn	+ Zn	*Mean*	- Zn	+ Zn	*Mean*	- Zn	+ Zn	*Mean*	- Zn	+ Zn	*Mean*
Excalibur	37.4	39.4	*38.4*	19.3	26.3	*22.8*	38.3	40.5	*39.4*	30.1	37.5	*33.8*
Frame	37.9	37.6	*37.8*	26.8	28.3	*27.6*	32.8	32.8	*32.8*	31.2	35.0	*33.1*
Goldmark	34.2	34.1	*34.1*	24.4	31.2	*27.8*	36.7	36.1	*36.4*	36.2	42.8	*39.5*
Meering	36.7	34.9	*35.8*	31.8	32.5	*32.1*	36.3	31.7	*34.0*	32.1	33.1	*32.6*
Mean	*36.5*	*36.5*		*25.6*	*29.6*		*36.0*	*35.3*		*32.4*	*37.1*	
***LSD*$_{0.05}$**												
Sowing Date			**7.4**						**n.s.**			
Zinc			**n.s.**						**n.s.**			
Genotype			**n.s.**						**2.9**			
Sowing Date x Zinc			**n.s.**									
(a)									**2.9**			
(b)									**3.2**			
Sowing Date x Genotype												
(a)			**5.3**						**4.1**			
(b)			**6.1**						**4.1**			
Zinc x Genotype			**n.s.**						**n.s.**			
Sowing Date x Zinc x Genotype			**n.s.**						**n.s.**			
CV (%)			**14.1**						**9.9**			

(a) For comparisons within the same sowing date

(b) For other comparisons

n.s. = non-significant

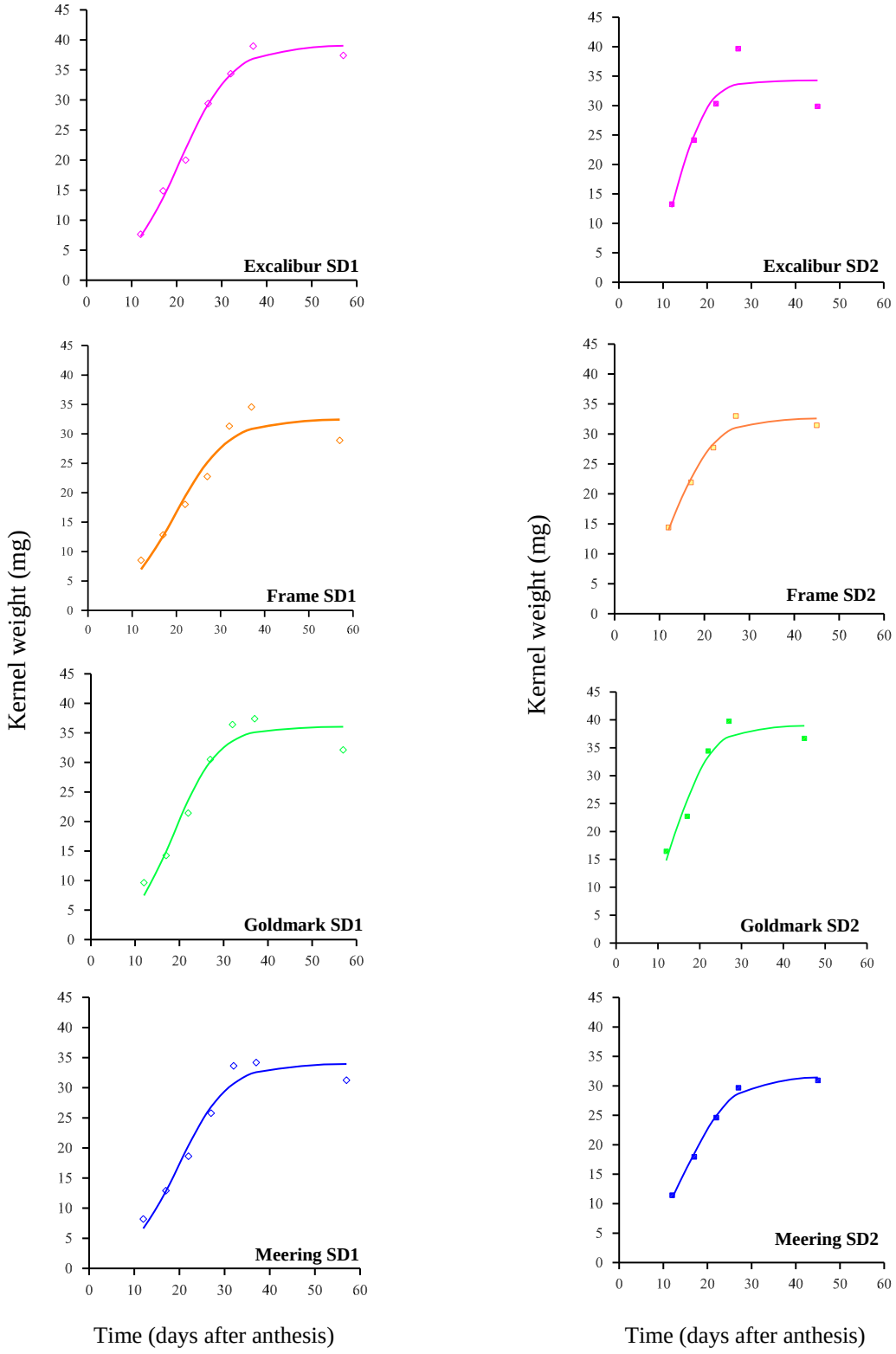


Figure 5.4. Dry matter accumulation in grains of the central spikelets of the main culm ear of wheat cultivars Excalibur, Frame Goldmark and Meering, grown at 2 sowing dates(SD) at the Waite Institute in 1998. Data are fitted to the ordinary logistic growth model, and are the mean of both Zn treatments.

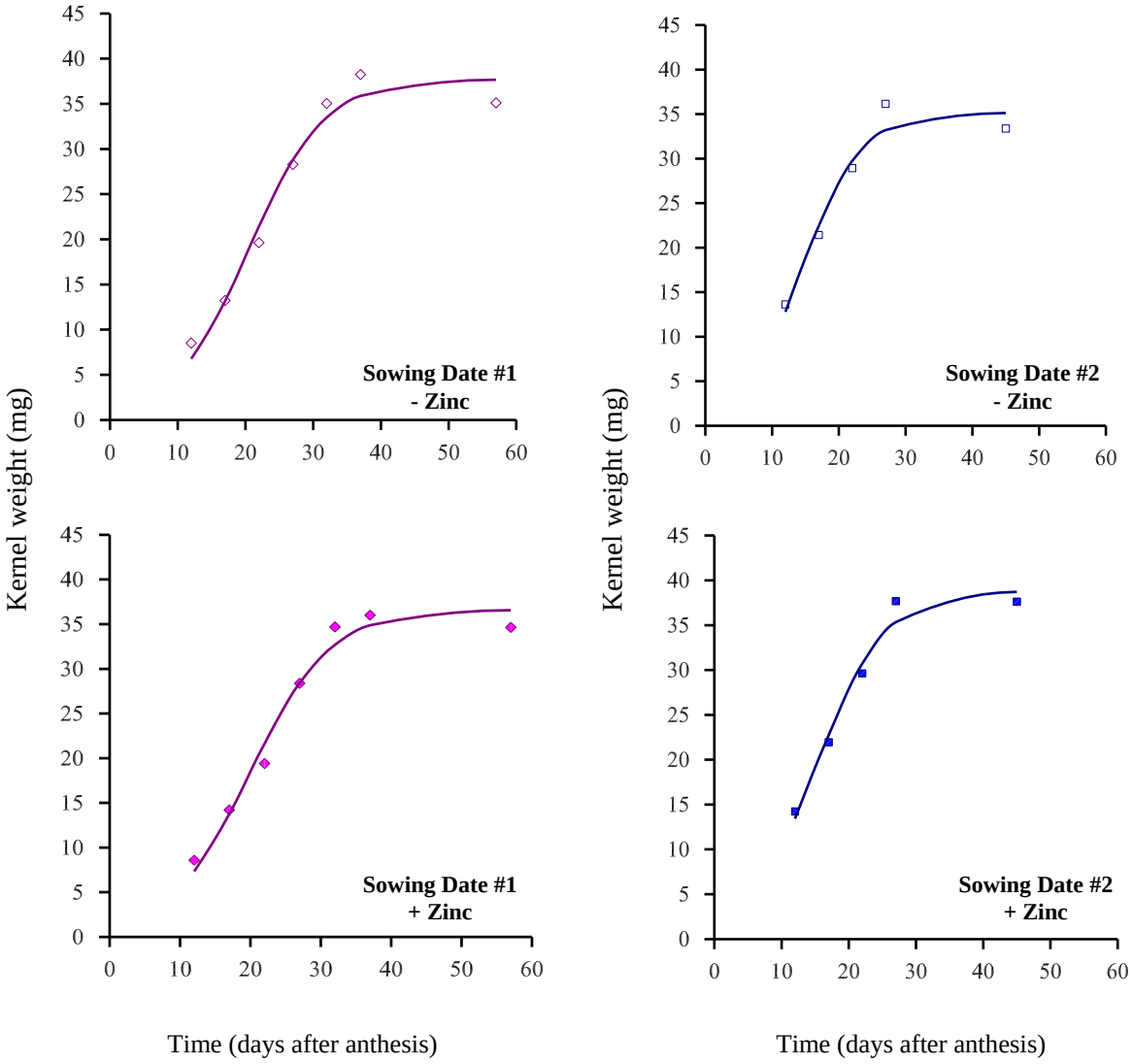


Figure 5.5. Effects of sowing date and zinc fertilisation on the growth of grains of the central spikelets of the main culm ear of wheat plants grown at the Waite Institute in 1998. Data are fitted to the ordinary logistic growth model, and are the mean of all genotypes.

5.3.3.5 Grain yield and yield components

Neither sowing date nor Zn fertilisation had any effect on grain yield or the number of grains per main stem ear (Tables 5.15 and 5.16). There was a significant interaction between sowing date and genotype however. Of the bread wheat varieties, Goldmark was found to have the highest grain yield at SD 1 (1.42 g plant^{-1}), while Halberd had the

lowest (0.96 g plant^{-1}). The high grain yield of Goldmark was due to both a high kernel weight and a high number of grains per plant, whereas the low yield of Halberd was due mainly to a low number of grains per plant. At SD 2, Trident had the highest yield of all varieties (1.25 g plant^{-1}), while Excalibur had the lowest grain yield (1.04 g plant^{-1}). Again the higher yield of Trident was due both to a high kernel weight and grain number, while the low yield of Excalibur was also due to both of these factors.

It is interesting to note that the bread wheat varieties that showed an increase in grain yield at SD 2 had been previously classified as either heat tolerant (Halberd, 28%) or Zn efficient (Trident, 11%). Varieties that showed a reduced grain yield at SD 2 had been previously found to be heat sensitive (Meering, -19%) or Zn inefficient (Goldmark -17%). Frame, previously classified as moderately Zn efficient, showed no change in grain yield between sowing dates. Only Excalibur, previously classified as Zn efficient, did not fit the predicted outcome, with a reduced grain yield of 18% at SD 2.

As estimated by the ordinary logistic growth model, above, Excalibur was the only variety to have a significantly lower kernel weight at SD 2. The average weight of kernels from the main stem of Excalibur was 36 mg in the SD 1 plants and 31 mg in the SD 2 plants, a reduction of 14%, as was estimated by the ordinary logistic model.

5.3.3.6 Grain zinc concentration and content

Supplementary Zn fertilisation increased the Zn concentration in the grain of wheat grown at the Waite Institute in 1998 by an average of 9% (Table 5.17). Plants that did not have Zn applied as a foliar spray at anthesis had an average grain Zn concentration of 55 mg kg^{-1}, and this increased to 60 mg kg^{-1} for those that did receive the Zn foliar spray. Both of these values are somewhat higher than the adequate levels of 20-35 mg Zn kg^{-1} commonly reported in wheat grain (Bansal *et al.* 1990, Graham *et al.* 1992), but are below toxicity levels (Rashid and Fox 1992, Marschner 1995).

Table 5.15. Effects of sowing date, zinc fertilisation and genotype on the grain yield (mg plant^{-1}) of the main stem of wheat plants grown at the Waite Institute in 1998.

Genotype	Grain Yield (mg plant^{-1})					
	Sowing Date #1			Sowing Date #2		
	- Zn	+ Zn	*Mean*	- Zn	+ Zn	*Mean*
Excalibur	1231	1308	*1270*	1018	1058	*1038*
Frame	1079	1056	*1067*	1002	1132	*1067*
Goldmark	1434	1412	*1423*	1128	1225	*1176*
Halberd	965	950	*957*	1196	1251	*1224*
Kronos	1425	1500	*1463*	1064	*1268*	*1166*
Meering	1358	1251	*1304*	1086	1038	*1062*
Trident	1090	1179	*1134*	1200	1307	*1253*
Mean	*1226*	*1237*		*1099*	*1183*	
LSD$_{0.05}$						
Sowing Date			**n.s.**			
Zinc			**n.s.**			
Genotype			**137**			
Sowing Date x Zinc			**n.s.**			
Sowing Date x Genotype						
(a)			**194**			
(b)			**445**			
Zinc x Genotype			**n.s.**			
Sowing Date x Zinc x Genotype			**n.s.**			
CV (%)			**14.1**			

(a) For comparisons within the same sowing date

(b) For other comparisons

n.s. = non-significant

Table 5.16. Effects of sowing date, zinc fertilisation and genotype on the kernel weight (mg) and kernel number of the main stem of wheat plants grown at the Waite Institute in 1998.

Genotype	Kernel Weight (mg plant^{-1})						Kernel number (per plant)					
	Sowing Date #1			Sowing Date #2			Sowing Date #1			Sowing Date #2		
	- Zn	+ Zn	*Mean*	- Zn	+ Zn	*Mean*	- Zn	+ Zn	*Mean*	- Zn	+ Zn	*Mean*
Excalibur	35.2	36.8	*36.0*	29.5	32.2	*30.9*	35.1	35.6	*35.4*	34.8	33.0	*33.9*
Frame	28.0	29.2	*28.6*	28.4	31.5	*29.9*	38.5	36.2	*37.4*	35.4	36.2	*35.8*
Goldmark	34.4	34.2	*34.3*	32.9	34.5	*33.7*	41.2	41.2	*41.2*	34.1	35.9	*35.0*
Halberd	31.4	32.0	*31.7*	31.6	33.5	*32.5*	30.7	29.6	*30.2*	37.9	37.1	*37.5*
Kronos	49.7	46.7	*48.2*	42.0	45.1	*43.6*	28.7	32.1	*30.4*	25.2	27.6	*26.4*
Meering	31.3	29.1	*30.2*	28.0	27.6	*27.8*	43.5	43.0	*43.3*	37.9	37.3	*37.6*
Trident	30.9	32.4	*31.6*	33.4	33.9	*33.6*	35.2	36.4	*35.8*	36.1	38.4	*37.2*
Mean	*34.4*	*34.3*		*32.2*	*34.0*		*36.1*	*36.3*		*34.5*	*35.1*	
LSD$_{0.05}$												
Sowing Date			**n.s.**						**n.s.**			
Zinc			**n.s.**						**n.s.**			
Genotype			**2.0**						**2.9**			
Sowing Date x Zinc			**n.s.**						**n.s.**			
Sowing Date x Genotype												
(a)			**2.8**						**4.1**			
(b)			**5.0**						**10.8**			
Zinc x Genotype			**n.s.**						**n.s.**			
Sowing Date x Zinc x Genotype			**n.s.**						**n.s.**			
CV (%)			**7.1**						**10.1**			

(a) For comparisons within the same sowing date

(b) For other comparisons

n.s. = non-significant

Table 5.17. Effects of zinc fertilisation, sowing date and genotype on the zinc concentration (mg kg^{-1}) in the grain of wheat plants grown at the Waite Institute in 1998.

Genotype	- Zinc			+ Zinc		
	SD #1	**SD #2**	***Mean***	**SD #1**	**SD #2**	***Mean***
Excalibur	47.0	57.0	*52.0*	49.3	65.0	*57.2*
Frame	47.3	67.0	*57.2*	54.3	69.3	*61.8*
Goldmark	40.7	60.0	*50.3*	47.3	59.0	*53.2*
Halberd	47.7	63.0	*55.3*	46.7	72.0	*59.3*
Kronos	58.0	76.7	*67.3*	58.0	87.3	*72.7*
Meering	38.7	59.0	*48.8*	46.7	64.0	*55.3*
Trident	46.0	63.0	*54.5*	48.0	72.7	*60.3*
Mean	*46.5*	*63.7*	***55.1***	*50.1*	*69.9*	***60.0***

$LSD_{0.05}$	
Sowing Date	**4.1**
Zinc	**3.8**
Genotype	**4.7**
Sowing Date x Zinc	**n.s.**
Sowing Date x Genotype	**n.s.**
Zinc x Genotype	**n.s.**
Sowing Date x Zinc x Genotype	**n.s.**
CV (%)	**4.1**

n.s. = non-significant

Significant differences in grain Zn concentration were observed between sowing dates at the Waite Institute, with the grain of the SD 1 plants having an average Zn concentration of 48 mg kg^{-1}, 18% lower than the grain of the SD 2 plants, which averaged 67 mg kg^{-1}. The main effect of genotype was also significant for grain Zn concentration, with the durum variety Kronos loading more Zn into the grain than the bread wheat varieties.

Grain Zn content increased by 12% with supplementary Zn fertilisation and by 38% at SD 2 compared with SD 1 (Table 5.18). This was a reflection of the higher grain Zn concentration with Zn fertilisation and at SD 2, rather than any effect on kernel weight.

Kronos was found to have a significantly higher grain Zn content than the bread wheat varieties (2890 ng Zn per seed, compared with an average of 1600 ng Zn per seed for Excalibur, Frame, Halberd and Trident) and this was due to both a higher grain weight and a higher grain Zn concentration in the durum variety. Meering had a significantly lower grain Zn content than the other varieties (1330 ng Zn per seed) and this was a reflection of both the smaller grains and generally lower grain Zn concentration of this variety.

Table 5.18. Effects of zinc fertilisation, sowing date and genotype on the zinc content (ng seed^{-1}) in the grain of wheat plants grown at the Waite Institute in 1998.

Genotype	- Zinc			+ Zinc		
	SD #1	**SD #2**	***Mean***	**SD #1**	**SD #2**	***Mean***
Excalibur	1409	1407	*1408*	1539	1826	*1683*
Frame	1143	1679	*1411*	1414	1975	*1695*
Goldmark	1183	1790	*1486*	1348	1874	*1611*
Halberd	1338	1844	*1591*	1361	2199	*1780*
Kronos	2600	2978	*2789*	2370	3622	*2996*
Meering	1065	1500	*1282*	1147	1617	*1382*
Trident	1274	1839	*1556*	1343	2189	*1766*
Mean	*1430*	*1862*	***1646***	*1503*	*2186*	***1845***

$LSD_{0.05}$	
Sowing Date	**494**
Zinc	**162**
Genotype	**204**
Sowing Date x Zinc	**n.s.**
Sowing Date x Genotype	**n.s.**
Zinc x Genotype	**n.s.**
Sowing Date x Zinc x Genotype	**n.s.**
CV (%)	**4.1**

n.s. = non-significant

5.3.4 Birchip and Horsham, 1999

5.3.4.1 Shoot dry matter production and zinc concentration

Shoot dry matter harvested at Birchip during stem elongation (11 weeks after sowing) showed significant effects of Zn fertilisation and genotype, but no interaction between the two (Table 5.19). The Zn fertilisation treatment of 7.5 kg^{-1} Zn ha^{-1} plus 1 foliar spray of Zn at tillering had increased shoot dry matter by an average of 30%, and had increased the Zn concentration in the shoot by an average of 51% (Table 5.19). The average Zn concentration in plants grown with no additional Zn was 14 mg kg^{-1}, whereas that in the Zn fertilised plants was 22 mg kg^{-1}, with no difference between varieties. At this stage of growth Excalibur had produced the highest amount of shoot dry matter (193 g m^{-2}), and, of the bread wheat varieties, Goldmark had produced the least (127 g m^{-2}).

Table 5.19. Effect of zinc fertilisation (7.5 kg Zn ha^{-1} + 1 foliar spray at tillering) on shoot dry matter production at stem elongation and zinc concentration in the YEBs of wheat genotypes grown at Birchip in 1999.

Genotype	Shoot dry weight (g m^{-2})			Zinc concentration (mg kg^{-1})		
	0	**7.5 + spray**	***Mean***	**0**	**7.5 + spray**	***Mean***
Daki-Cyn	96	136	*116*	11.9	23.7	*17.8*
Excalibur	167	220	*193*	12.9	24.7	*18.8*
Frame	144	185	*164*	14.2	21.2	*17.7*
Goldmark	118	137	*127*	13.1	18.7	*15.9*
Halberd	139	179	*159*	15.8	20.3	*18.0*
Kronos	115	166	*141*	15.3	24.3	*19.8*
Meering	117	148	*133*	14.7	16.3	*15.5*
Trident	138	172	*155*	16.4	23.3	*19.9*
Mean	*129*	*168*		*14.3*	*21.6*	
$LSD_{0.05}$						
Genotype		**36**			**n.s.**	
Zinc		**10**			**1.9**	
Genotype x Zinc		**n.s.**			**n.s**	
CV (%)		**11.1**			**17.0**	

n.s. = non-significant

5.3.4.2 Canopy temperature depression

Supplementary Zn, in the form of 7.5 kg Zn ha^{-1} at sowing plus two foliar sprays, significantly increased the canopy temperature depression (CTD) of some varieties during grain filling at Birchip (Figure 5.6). The CTD of the Zn-inefficient varieties, Goldmark and Daki-Cyn, was increased with Zn fertilisation by 24% and 18% respectively, as was the CTD of the other durum wheat variety, Kronos (28%). Supplementary Zn also increased the CTD of Excalibur, by 26%, a variety previously classified as Zn efficient. Conversely the CTD of the Zn-efficient cultivars, Trident and Frame, and thermotolerant Halberd, was unaffected by Zn fertilisation.

Figure 5.6. Effect of zinc fertilisation (7.5 kg Zn ha^{-1} plus 2 foliar sprays) on the canopy temperature depression during grain filling of wheat plants grown at Birchip in 1999. The vertical bar represents the $LSD_{0.05}$ for the Genotype x Zinc interaction, for comparisons within the same genotype.

Canopy temperature depression was also measured at Horsham during grain filling, but there were no differences between genotypes and Zn treatments at this site.

5.3.4.3 Grain yield

The main effects of variety and Zn treatment were both highly significant ($P < 0.001$) for grain yield at Birchip, but there was no significant interaction between the two (Table 5.20). The two Zn treatments of 7.5 kg Zn ha^{-1} only and 7.5 kg Zn ha^{-1} plus one foliar spray of Zn at anthesis increased yield by 10% and 8% respectively, with no significant difference between these two treatments. The third Zn application of 7.5 kg Zn ha^{-1} plus two foliar sprays, at tillering and at stem elongation, further increased yield, by 15% relative to the control plots with no applied Zn. These increases in yield were due to an increase in the number of grains per m^2; there was no effect of Zn fertilisation on grain weight (Table 5.20). Excalibur was again the highest yielding variety at this site (379 g m^{-2}), followed by Trident (353 g m^{-2}) and Frame (334 g m^{-2}), while Halberd was again the lowest yielding variety (264 g m^{-2}).

There was little effect of sowing date or Zn fertilisation on grain yield at Horsham, with the exception of the Zn treatment involving 7.5 kg Zn ha^{-1} plus two foliar sprays, which increased yield by 3% at SD 1 (Table 5.21). Again Excalibur (253 g m^{-2}), Trident (240 g m^{-2}) and Frame (238 g m^{-2}) were the highest yielding varieties at this low yielding site, with little difference among the other five genotypes.

In a similar result to that at Birchip, there was no effect of Zn fertilisation on grain weight at Horsham (Table 5.22). There was a significant interaction between sowing date and genotype however, with four varieties, Daki-Cyn, Excalibur, Goldmark and Kronos, having a higher kernel weight at SD 2. Interestingly these are the same four varieties that showed an increase in CTD with Zn fertilisation at Birchip, as discussed above.

The increase in yield due to Zn fertilisation (7.5 kg Zn ha^{-1} plus 2 foliar sprays) in the SD 1 plots at Horsham was due to a significant increase of 4% in the number of grains per m^2 for this Zn treatment (Table 5.23). The interaction between sowing date and genotype was significant, with Daki-Cyn having 11% fewer grains per m^2 at the second sowing date, and Meering having 9% more grains per m^2 at the second sowing date.

Table 5.20. Effects of genotype and zinc fertilisation (kg ha^{-1}) on the grain yield (g m^{-2}), kernel weight (mg) and kernel number (grains m^{-2}) of wheat plants grown at Birchip in 1999.

Genotype	Grain Yield (g m^{-2})					Kernel Weight (mg)					Kernel number (grains m^{-2})				
	0	7.5	7.5 + 1 spray	7.5 + 2 sprays	*Mean*	0	7.5	7.5 + 1 spray	7.5 + 2 sprays	*Mean*	0	7.5	7.5 + 1 spray	7.5 + 2 sprays	*Mean*
Daki-Cyn	286	329	298	340	*313*	43.6	44.0	43.7	44.3	*43.9*	6573	7482	6826	7664	*7136*
Excalibur	364	380	383	390	*379*	36.6	38.3	38.4	37.1	*37.6*	9980	9912	9989	10524	*10101*
Frame	298	343	341	353	*334*	39.1	40.0	40.5	38.8	*39.6*	7634	8604	8450	9093	*8445*
Goldmark	249	281	292	294	*279*	33.3	35.0	33.5	33.1	*33.7*	7526	8036	8708	9053	*8331*
Halberd	252	269	261	276	*264*	35.7	36.7	35.2	35.0	*35.6*	7045	7333	7423	7871	*7418*
Kronos	251	272	275	302	*275*	47.5	47.5	46.7	47.3	*47.2*	5302	5732	5902	6408	*5836*
Meering	270	310	293	326	*300*	29.8	28.1	29.3	28.3	*28.9*	9134	11013	9970	11590	*10427*
Trident	337	360	349	367	*353*	37.6	38.2	37.9	37.6	*37.8*	8930	9415	9159	9762	*9316*
Mean	*288*	*318*	*311*	*331*		*37.9*	*38.5*	*38.1*	*37.7*		*7766*	*8441*	*8303*	*8996*	
LSD$_{0.05}$															
Genotype			**51**					**1.5**					**1382**		
Zinc			**10**					**n.s.**					**338**		
Genotype x Zinc			**n.s.**					**n.s.**					**n.s.**		
CV (%)			**7.4**					**3.8**					**9.1**		

n.s. = non-significant

Table 5.21. Effects of sowing date, genotype and zinc fertilisation (kg ha^{-1}) on the grain yield (g m^{-2}) of wheat plants grown at Horsham in 1999.

Genotype	Grain Yield (g m^{-2})									
	Sowing Date #1					Sowing Date #2				
	0	7.5	7.5 + 1 spray	7.5 + 2 sprays	*Mean*	0	7.5	7.5 + 1 spray	7.5 + 2 sprays	*Mean*
Daki-Cyn	216	211	225	228	*220*	230	231	219	222	*226*
Excalibur	245	240	256	259	*250*	262	246	260	254	*255*
Frame	230	234	228	248	*235*	240	251	243	228	*240*
Goldmark	207	221	206	210	*211*	203	214	198	199	*203*
Halberd	219	209	201	211	*210*	211	218	222	214	*216*
Kronos	203	211	207	204	*206*	211	219	226	210	*217*
Meering	201	203	210	210	*206*	226	225	229	226	*227*
Trident	238	238	240	248	*241*	237	245	235	239	*239*
Mean	*220*	*221*	*222*	*227*		*227*	*231*	*229*	*224*	

$LSD_{0.05}$	
Sowing Date	**n.s.**
Genotype	**10.9**
Zinc	**n.s.**
Sowing Date x Genotype	**n.s.**
Sowing Date x Zinc	
(a)	**5.3**
(b)	**13.1**
Genotype x Zinc	**n.s.**
Sowing Date x Genotype x Zinc	**n.s.**
CV (%)	**5.4**

(a) For comparisons within the same sowing date

(b) For other comparisons

n.s. = non-significant

Table 5.22. Effects of sowing date, genotype and zinc fertilisation (kg ha^{-1}) on the kernel weight (mg) of wheat plants grown at Horsham in 1999.

Genotype	Kernel Weight (mg)									
	Sowing Date #1					Sowing Date #2				
	0	7.5	7.5 + 1 spray	7.5 + 2 sprays	*Mean*	0	7.5	7.5 + 1 spray	7.5 + 2 sprays	*Mean*
Daki-Cyn	44.5	44.8	44.0	44.0	*44.3*	50.5	51.1	51.8	50.6	*51.0*
Excalibur	40.5	41.3	39.8	40.7	*40.6*	43.9	43.4	43.3	42.8	*43.4*
Frame	43.2	42.7	44.0	43.9	*43.4*	42.8	43.5	42.3	42.0	*42.7*
Goldmark	41.1	40.5	41.4	39.9	*40.7*	42.0	41.9	41.8	42.3	*42.0*
Halberd	38.9	38.6	39.1	38.6	*38.8*	37.8	38.4	38.9	39.2	*38.6*
Kronos	47.5	48.1	48.3	48.1	*48.0*	51.7	52.6	53.1	52.0	*52.3*
Meering	34.9	33.9	35.2	33.0	*34.3*	34.3	35.0	34.6	34.1	*34.5*
Trident	40.8	40.7	40.3	40.5	*40.6*	41.7	41.9	41.8	42.1	*41.9*
Mean	*41.4*	*41.3*	*41.5*	*41.1*		*43.1*	*43.5*	*43.4*	*43.2*	

$LSD_{0.05}$	
Sowing Date	**0.6**
Genotype	**0.7**
Zinc	**n.s.**
Sowing Date x Genotype	
(a)	**1.0**
(b)	**1.1**
Sowing Date x Zinc	**n.s.**
Genotype x Zinc	**n.s.**
Sowing Date x Genotype x Zinc	**n.s.**
CV (%)	**2.7**

(a) For comparisons within the same sowing date

(b) For other comparisons

n.s. = non-significant

Table 5.23. Effects of sowing date, genotype and zinc fertilisation (kg ha^{-1}) on the kernel number (grains m^{-2}) of wheat plants grown at Horsham in 1999.

Genotype	Kernel number (grains m^{-2})									
	Sowing Date #1					Sowing Date #2				
	0	7.5	7.5 + 1 spray	7.5 + 2 sprays	*Mean*	0	7.5	7.5 + 1 spray	7.5 + 2 sprays	*Mean*
Daki-Cyn	4854	4698	5120	5191	*4966*	4553	4535	4231	4384	*4425*
Excalibur	6064	5804	6437	6346	*6163*	5962	5659	6005	5933	*5890*
Frame	5322	5482	5184	5656	*5411*	5615	5767	5753	5424	*5640*
Goldmark	5040	5477	4981	5251	*5187*	4826	5106	4738	4715	*4846*
Halberd	5630	5394	5134	5471	*5407*	5578	5668	5708	5465	*5605*
Kronos	4261	4393	4297	4247	*4300*	4076	4167	4258	4041	*4136*
Meering	5761	5996	5975	6359	*6023*	6596	6427	6622	6641	*6572*
Trident	5831	5859	5946	6105	*5935*	5684	5845	5621	5669	*5705*
Mean	*5345*	*5388*	*5384*	*5578*		*5361*	*5397*	*5376*	*5284*	

LSD$_{0.05}$	
Sowing Date	**n.s.**
Genotype	**279**
Zinc	**n.s.**
Sowing Date x Genotype	
(a)	**394**
(b)	**433**
Sowing Date x Zinc	
(a)	**139**
(b)	**276**
Genotype x Zinc	
(c)	**278**
(d)	**366**
Sowing Date x Genotype x Zinc	**n.s.**
CV (%)	**5.9**

(a) For comparisons within the same sowing date

(b) For other comparisons

(c) For comparisons within the same genotype

(d) For other comparisons

n.s. = non-significant

5.3.4.4 Grain zinc concentration and content

Supplementary Zn fertilisation in the form of a foliar spray increased grain Zn concentration at both sites, and, at Horsham, at both sowing dates (Table 5.24). At neither site did the grain Zn concentration of the soil only Zn treatment (7.5 kg Zn ha^{-1} at sowing) differ from that of the nil Zn treatment, but the application of the foliar sprays increased grain Zn concentration at both sites. At Birchip, the Zn treatment involving a single foliar spray at anthesis increased the concentration of Zn in the grain by 86% relative to the nil Zn plots, and this did not differ significantly from the treatment involving the two foliar sprays at tillering and stem elongation. At Horsham, however, the single foliar spray of Zn increased the concentration of Zn in the grain by 31% (SD 1) and 23% (SD 2), while the treatment involving two foliar sprays further increased grain Zn concentration, by 48% (SD 1) and 67% (SD 2), relative to the nil Zn treatments.

Grain Zn concentration was the lowest at Birchip, 11 mg kg^{-1} for the nil Zn treatment, boosted to 21 mg kg^{-1} for the treatments involving the Zn foliar spray. At Horsham, grain Zn concentration was 20 mg kg^{-1} in the nil Zn control plots, boosted to 26 mg kg^{-1} in the single spray treatment and 32 mg kg^{-1} in the two spray treatment, with little difference between the two sowing dates.

Zinc content in the grain increased with all Zn treatments at Birchip relative to the nil Zn control (Table 5.24). With the application of 7.5 kg Zn ha^{-1} at sowing only, grain Zn content increased by 20%, from 353 ng per seed to 425 ng per seed. With additional Zn supplementation in the form of either one or two foliar sprays, grain Zn content increased by a further 61%, to 687 and 684 ng per seed for the one and two foliar spray treatments, respectively. At Horsham however, grain Zn content did not significantly increase with the soil only application of Zn, but with the foliar Zn treatments only. At SD 1, this was an increase of 32%, from 782 ng per seed to 1031 ng per seed, for the one Zn spray treatment, and 45%, to 1133 ng per seed, for the two spray treatment. At SD 2 similar increases of 22% (one spray) and 65% (two sprays) were observed.

Table 5.24. Effect of zinc fertilisation (kg ha^{-1}) on the grain zinc concentration (mg kg^{-1}) and grain zinc content (ng seed^{-1}) of Frame and Goldmark grown at Birchip and Horsham in 1999.

Genotype	Birchip				Horsham (sowing date #1)				Horsham (sowing date #2)			
	0	**7.5**	**7.5 + 1 spray**	**7.5 + 2 sprays**	**0**	**7.5**	**7.5 + 1 spray**	**7.5 + 2 sprays**	**0**	**7.5**	**7.5 + 1 spray**	**7.5 + 2 sprays**
	Zinc concentration (mg kg^{-1})				*Zinc concentration (mg kg^{-1})*				*Zinc concentration (mg kg^{-1})*			
Frame	11.0	11.2	19.6	19.8	20.7	21.1	27.0	28.1	19.0	19.1	22.8	31.6
Goldmark	10.8	13.4	21.0	22.2	20.4	22.4	26.6	32.7	20.9	21.2	26.2	35.1
Mean	*10.9*	*12.3*	*20.3*	*21.0*	*20.5*	*21.8*	*26.8*	*30.3*	*19.9*	*20.1*	*24.5*	*33.3*
LSD$_{0.05}$												
Genotype		**n.s.**				**n.s.**				**n.s.**		
Zinc		**2.3**				**2.8**				**1.2**		
Genotype x Zinc		**n.s.**				**n.s.**				**n.s.**		
CV (%)		**11.4**				**9.0**				**4.0**		
	Zinc content (ng seed^{-1})				*Zinc content (ng seed^{-1})*				*Zinc content (ng seed^{-1})*			
Frame	384	417	730	708	813	824	1078	1102	740	762	883	1189
Goldmark	321	433	644	659	750	807	983	1164	789	813	991	1136
Mean	*353*	*425*	*687*	*684*	*782*	*815*	*1031*	*1133*	*765*	*787*	*937*	*1263*
LSD$_{0.05}$												
Genotype		**n.s.**				**n.s.**				**70**		
Zinc		**60**				**96**				**59**		
Genotype x Zinc		**n.s.**				**n.s.**				**n.s**		
CV (%)		**8.8**				**8.1**				**5.0**		

n.s. = non-significant

5.3.5 Grain yield stability across sites

The population mean and regression lines for the six bread wheat varieties are shown in Figure 5.7 to illustrate the varietal response across the six field sites in 1998 and 1999. The individual variety yields are plotted against the mean of all the variety yields, giving the population mean a regression coefficient of 1.0.

Varieties characterised by regression coefficients of the order of 1.0 have average stability over all environments (Finlay and Wilkinson 1963). Three varieties in the present experiments, Excalibur, Frame and Trident, showed average yield stability, with linear regression coefficients (*b*) of 1.00, 1.10 and 1.10, respectively (Table 5.25). These varieties produced above average yields at all sites, indicating that they have general adaptability (Finlay and Wilkinson 1963). In contrast, Goldmark also had a regression coefficient that was not significantly different to 1.0 (b = 0.89), but consistently produced below average yields. This indicates that Goldmark is poorly adapted to all environments.

Table 5.25. Intercepts (*a*) and regression coefficients (*b*) derived from individual variety yields plotted against the mean of all the variety yields for the average of all Zn treatments, under adequate Zn fertilisation, and under nil Zn fertilisation for the six wheat genotypes grown at six sites in 1998 and 1999.

Genotype	Average of all Zn treatments		Adequate Zn fertilisation 7.5 kg Zn ha^{-1}		Nil Zn fertilisation 0 kg Zn ha^{-1}	
	a	***b***	***a***	***b***	***a***	***b***
Excalibur	0.049	1.001	0.001	1.017	0.115	0.976
Frame	-0.211	1.098	-0.160	1.078	-0.424	1.186
Goldmark	0.243	0.886	0.489	0.787	0.434	0.804
Halberd	0.834*	0.640*	0.899*	0.614*	0.963*	0.586*
Meering	-0.605*	1.239*	-0.543	1.213	-1.001*	1.403*
Trident	-0.210	1.103	-0.250	1.120	-0.231	1.113

* indicates a significant difference from the population mean regression ($P < 0.05$).

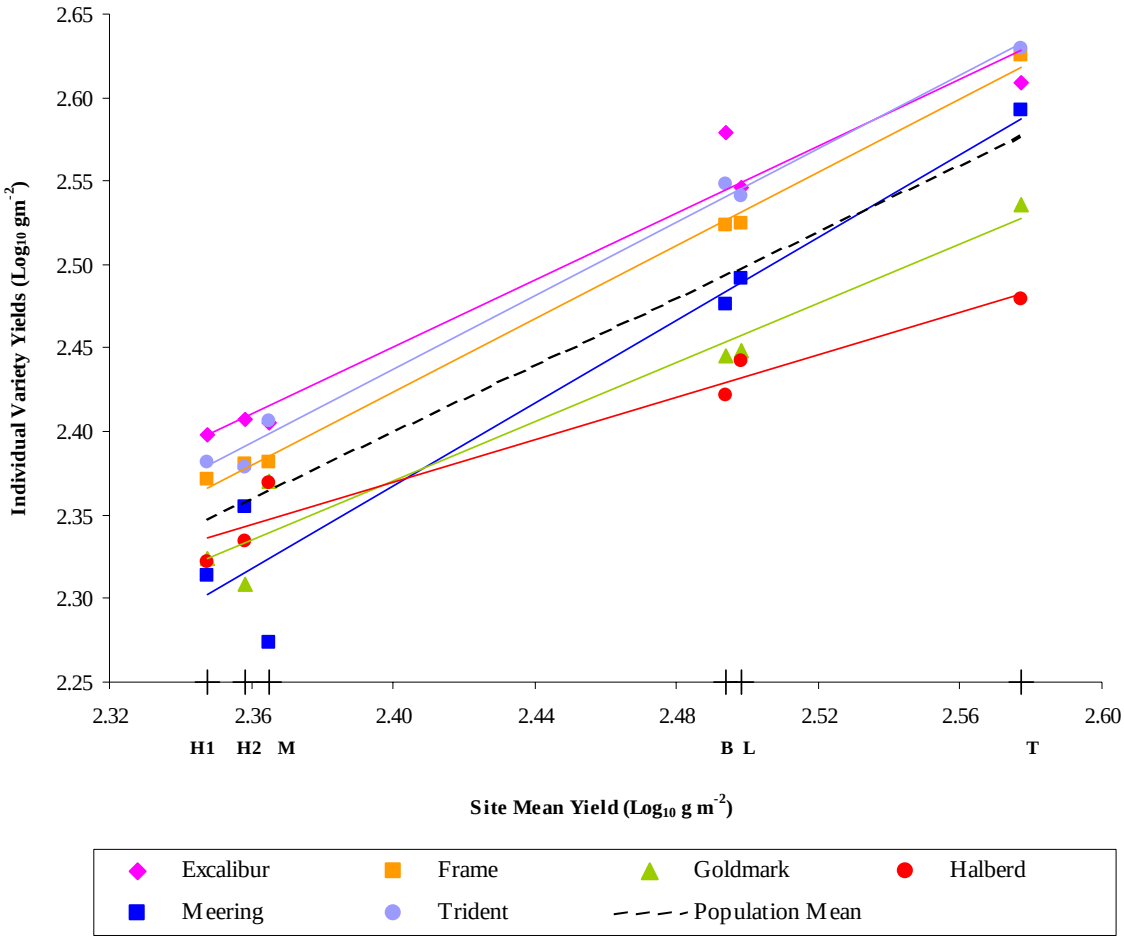


Figure 5.7. Regression lines showing the relationship of individual yields of six bread wheat varieties and population mean of wheat grown at different sites and seasons.
Each data point is the mean of all zinc treatments.

H1 = Horsham 1999 - Sowing Date 1, H2 = Horsham 1999 - Sowing Date 2,
M = Minnipa 1998, B = Birchip 1999, L = Lameroo 1998, T = Tintinara 1998

Meering is typical of varieties that are sensitive to changes in the environment and therefore have below average yield stability; small changes in the environment produce large changes in yield (Finlay and Wilkinson 1963). Previously classified as a thermosensitive variety (Stone and Nicolas 1995a), Meering produces low yields in low-yielding environments, but as the environment improves, thus favouring higher yields, the yield of Meering increases at a rate that is above average for the group. Meering is thus characterised by a regression coefficient that is significantly greater than 1.0 (b = 1.24).

Halberd exhibits the opposite type of adaptation to Meering, with very little change in yield despite large changes in the environment. Halberd therefore has above average yield stability, but consistently below average yields. Previously classified as thermotolerant (Wrigley *et al.* 1994) the present results also show that Halberd is relatively insensitive to environmental change, and therefore has a regression coefficient significantly less than 1.0 (b = 0.64).

When the individual variety yields under adequate Zn fertilisation were regressed against the site mean yields (Figure 5.8), few differences was observed between these regression coefficients and those of the mean variety yields of all Zn treatments, described above (Table 5.25). One important difference however, was the regression coefficient of Meering, which was not significantly different to 1.0 when supplied with adequate Zn (b = 1.21). This indicates that Meering has average yield stability in all environments when grown with supplementary Zn fertilisation. Under Zn deficient conditions however, Meering exhibited a regression coefficient that was significantly greater than 1.0 (b = 1.40), indicating below average yield stability. This variety therefore produces low yields in low yielding environments when deprived of Zn, but becomes one of the highest-yielding varieties with above average yields when grown in more favourable conditions without Zn fertilisation (Figure 5.8).

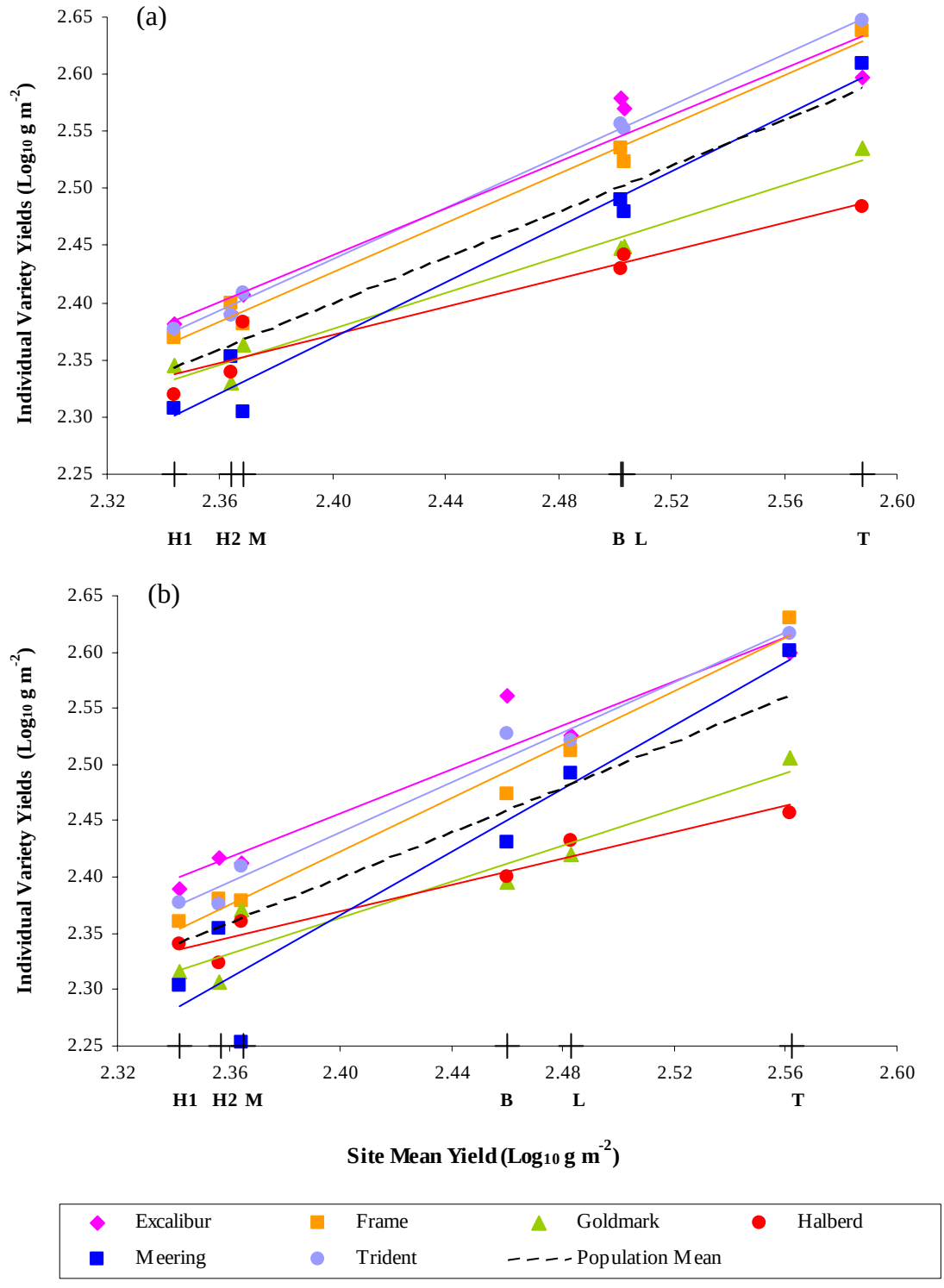


Figure 5.8. Regression lines showing the relationship of individual yields of six bread wheat varieties and population mean (a, supplied with adequate Zn fertilisation (7.5 kg Zn ha^{-1}); b, supplied with nil Zn fertilisation) of wheat grown at different sites and seasons.

H1 = Horsham 1999 - Sowing Date 1, H2 = Horsham 1999 - Sowing Date 2, M = Minnipa 1998, B = Birchip 1999, L = Lameroo 1998, T = Tintinara 1998

5.4 Discussion

5.4.1 Lameroo, Tintinara and Minnipa, 1998

The results of this series of experiments agree with other studies that have shown a decrease in grain weight with an increase in mean temperature during the grain filling period (eg. Wardlaw *et al*. 1980, 1989b). As the mean temperature from anthesis until maturity increased between sites (Tintinara < Lameroo < Minnipa), kernel weight decreased. This, in conjunction with a decrease in the number of kernels per m^2, led to a decrease in grain yield between sites (Tintinara > Lameroo > Minnipa). It is acknowledged that temperature was not the only factor to vary between these sites, and other environmental conditions, such as rainfall and soil type, also influence grain yield. Nevertheless, this decrease in yield with an increase in temperature was a consistent finding across all seven varieties. If 7.5 kg Zn ha^{-1} at sowing is taken to represent an adequate supply of Zn, the average kernel weight across all varieties was 32.6 mg at Minnipa, where the average daily maximum temperature during grain filling was 26.6°C; 35.2 mg at Lameroo (25.6°C during grain filling); and 38.5 mg at Tintinara (23.9°C during grain filling).

All varieties produced a higher grain yield at Tintinara than at Lameroo, and a higher grain yield at Lameroo than at Minnipa. Meering, a variety previously classified as thermosensitive on the basis of its reduced grain weight under heat stress (Stone and Nicolas 1995a), showed the largest increase in yield of all genotypes, with a 50% increase from Minnipa to Lameroo and another 35% increase from Lameroo to Tintinara. Conversely Halberd, a tall variety with a lower yield potential, showed the most stable pattern of grain yield between the three sites, with a 15% increase from Minnipa to Lameroo and a 10% increase from Lameroo to Tintinara. Halberd has previously been classified as a thermotolerant variety, however this was on the basis of its protein content and dough strength under heat stress, rather than its grain yield response (Blumenthal *et al.* 1991b, Wrigley *et al*. 1994),

The increase in grain yield of Meering from Minnipa to Lameroo was due solely to an

increase in the number of grains per m^2, whereas the increase between Lameroo and Tintinara was due both to an increase in grain number and an increase in grain weight. This suggests that the pre-anthesis growth of Meering was affected at Minnipa, and, to a lesser extent, at Lameroo, resulting in a smaller number of grains per m^2. The optimum daily maximum temperature for the pre-anthesis growth of wheat has been found to range from 15 to 18ºC, with a 3% decrease in grain number for each 1ºC rise in temperature above this optimum during booting (Wardlaw *et al.* 1989a). In the present experiments average daily maximum temperatures prior to anthesis were highest at Minnipa (18.2ºC from July to September), lower at Lameroo (17.6ºC) and lowest at Tintinara (16.9ºC). Pre-anthesis growth did not contribute to the increase in grain yield between sites in the thermotolerant variety Halberd however, with the number of grains per m^2 not differing between sites in this variety. The increase in grain yield of Halberd between Minnipa and Lameroo, and between Lameroo and Tintinara, was due solely to an increase in grain weight.

The application of Zn increased the grain yield in all varieties at Tintinara and in five of the seven varieties at Lameroo. These yield increases were due to an increase in the number of grains per m^2, with no effect of Zn fertilisation on grain weight. A decrease in the size of the wheat head, resulting in a lower number of grains per m^2, has been previously described under Zn deficiency (Sharma *et al.* 1979), and may be attributed to the micronutrient deficiency in the period between single ridge and stem elongation, when spikelets are being formed. Flowering and seed production are also known to be depressed by Zn deficiency and this is attributed to (1) enhanced formation of abscissic acid in the plant, causing premature abscission of flower buds, and (2) disruption of the development and physiology of anthers and pollen grains (Brown *et al.* 1993). Small anthers with abnormal pollen grains have been found to develop under moderate Zn deficiency in wheat (Sharma *et al.* 1979), resulting in a reduced number of grains per spikelet.

Most of the yield increases due to Zn in the present experiments (with the exception of Goldmark at Lameroo) occurred with the soil application of Zn alone, with no further increase in yield with the application of the two Zn foliar sprays. This is consistent with

the findings of Yilmaz *et al.* (1997), who reported no difference between the grain yield of wheat grown with soil applied Zn supplemented with a Zn foliar spray and the yield of wheat grown with the soil applied Zn alone. However, it is not clear why the grain weight at Tintinara in the present experiments was reduced with the foliar Zn treatment, while there was only a small, non-significant, increase in grain number in this treatment compared with the soil applied Zn alone. At Lameroo, the grain weight of the foliar treatment was reduced in two varieties compared with the soil only treatment, but in each case this was due to a larger number of smaller grains being produced with the foliar treatment.

Yilmaz *et al.* (1997) also found that the concentration of Zn in the grain of plants supplied with soil and foliar Zn fertilisation was significantly higher than that of plants supplied with soil Zn fertilisation alone. This was also observed in the present study. The Zn treatment of 7.5 kg Zn ha^{-1} applied at sowing did not significantly increase the grain Zn concentration at either Lameroo or Tintinara. However, the supplementation of this treatment with a foliar spray of Zn at tillering and at stem elongation significantly increased the grain Zn concentration at both sites, by 167% and 93%, respectively. The Zn treatment of 22.5 kg Zn ha^{-1} at sowing also boosted the grain Zn concentration, by 41% at Lameroo and by 23% at Tintinara.

Grain Zn content increased with Zn fertilisation at all three sites in 1998, including Minnipa. There was a marked increase in grain Zn content with the Zn foliar spray at Lameroo and Tintinara, and it is interesting to note that those bread wheat varieties that accumulated high amounts of Zn in the grain with the foliar spray, Frame, Halberd and Trident, are those that have the *Glu-D1d* allele at the *Glu-1* high molecular weight glutenin subunit locus, coding for glutenin protein subunits 5+10. Those varieties with less Zn in the grain, Goldmark and Meering, and also Excalibur at Tintinara, are those with the *Glu-D1a* allele at the *Glu-D1* locus, coding for glutenin protein subunits 2+12. Cultivars with the *Glu-D1a* allele have been found to be more susceptible to the dough weakening effect of high temperatures during grainfilling than those with the *Glu-D1d* allele (Blumenthal *et al.* 1995b, Panozzo and Eagles 2000). Further analysis will be conducted to determine whether elevated levels of grain Zn can alter the protein

composition of wheat grain, and thereby perhaps provide some level of protection against the adverse effects of high temperatures on grain quality (see Chapter 7).

The lack of responses to Zn observed at Minnipa confirms the fact that this trial was not planted on a Zn deficient soil. However, the grain produced at this site does differ in Zn content and was formed under higher temperature conditions than either of the other two sites. This grain will therefore be used in later experiments, in conjunction with the grain from Lameroo and Tintinara, to determine whether changes in grain quality due to high temperature can be lessened by adequate Zn nutrition.

5.4.2 Waite Institute, 1998

The duration of grain dry matter accumulation at the Waite Institute was reduced at the second sowing date, which is likely to be a response to the increased temperature during grain filling. However the decrease in grain filling duration was compensated by an increase in the rate of grain filling, resulting in no significant decrease in grain yield between the two sowing dates. This agrees with many other studies which have shown that temperatures above optimal cause a reduction in the duration of grain filling in wheat, which may or may not be compensated for by an increase in rate (Sofield *et al.* 1977, Tashiro and Wardlaw 1989, Jenner 1994).

Grain yield data from the Waite Institute showed no effect of sowing date or Zn fertilisation on yield, kernel weight or grain number of the main stem ears. The only exception to this was the kernel weight of Excalibur, which was significantly lower at SD 2 than at SD 1. This may be because the anthesis date of Excalibur was 2 days later than the other bread wheat varieties at SD 2, thus exposing this genotype to a different suite of high temperature events during grain filling. The average daily maximum temperature during grain filling was 27.6ºC for Excalibur at SD 2, 0.5ºC above that of the other bread wheat varieties. Furthermore, Excalibur experienced temperatures above 30ºC at 2, 3, 8 and 9 DAA, and temperatures above 35ºC at 16-18 DAA, whereas the other bread wheat varieties experienced these temperatures at 4, 5, 10 and 11 DAA

and 18-20 DAA, for 30ºC and 35ºC, respectively. In a controlled environment experiment involving two varieties of wheat subjected to short period of high temperature at 5-day intervals throughout grain filling, Stone and Nicolas (1995b) found that the mature individual kernel mass was most sensitive to heat stress applied early in grain filling, and becomes progressively less sensitive throughout the grain filling period. Heat-induced reductions of mature individual kernel mass were 1% for each 2 day delay in the start of heat treatment. Another possible explanation for the lower kernel weight of Excalibur at SD 2 is that this genotype may be somewhat sensitive to high temperature stress, since little is known about the heat tolerance of Excalibur.

The kernel weight of grains of the central spikelets was significantly lower in the –Zn plants at the second sowing date than in the plants that had received the supplementary foliar spray of Zn. When all the grains of the main stem were considered a similar trend was observed, however this was not a statistically significant difference. This was an unexpected result, since the grains of the central spikelets are generally less affected by environmental stresses than those of the more distal spikelets (Rawson and Evans 1970, Calderini and Ortiz-Monasterio 2003). The present results would suggest that plants with supplementary Zn nutrition were able to sustain a higher rate and/or a longer duration of grain filling during the warmer grain filling period of SD 2 than those that did not receive supplementary Zn. However there was no significant effect of Zn fertilisation on either the maximum rate of grain filling or the duration of grain filling, despite there being large increases in grain filling duration with Zn fertilisation in some varieties. The duration of grain filling was increased by 36% in Excalibur and by 28% in Goldmark with the application of Zn at SD 2. This is in contrast to the duration of grain filling in Frame and Meering, which increased by only 6% and 2% with supplementary Zn, respectively. This would suggest that a Zn by genotype interaction may exist with respect to duration of grain filling under high temperatures, and further work is necessary to clarify these results.

The decline in the chlorophyll fluorescence ratio (Fv/Fm) caused by high temperature was reduced by elevated Zn fertilisation in this experiment, at least in a Zn inefficient wheat genotype. Temperatures in the field above 35ºC decreased Fv/Fm in the Zn-

inefficient variety, Goldmark, but only in plants that had not received the supplementary foliar spray of Zn at anthesis. At 40ºC however, the Fv/Fm of both –Zn and +Zn plants was reduced, with no difference between the two Zn treatments. In the Zn efficient variety, Frame, temperatures above 35ºC did not lower the Fv/Fm ratio in either the –Zn or +Zn plants, but at 40ºC this ratio was reduced in both Zn treatments.

The reduction in the Fv/Fm of Goldmark under high temperature and no supplementary Zn fertilisation was due primarily to an increase in chlorophyll initial fluorescence, Fo, rather than any effect on chlorophyll maximum fluorescence, Fm. This suggests that a temperature of 37ºC was causing a separation of the chlorophyll *a*/b light harvesting complex or PSII (LHCII) from the PSII core complex in the –Zn plants of Goldmark (Yamane *et al.* 1997), associated with increased lipid fluidity of the thylakoid membranes at elevated temperature (Armond *et al.* 1980, Berry and Björkman 1980, Gounaris *et al.* 1984, Sundby *et al.* 1986). However thylakoid membrane integrity appears to have been maintained at this temperature in the +Zn plants of Goldmark and in both Zn treatments in Frame. It is therefore possible that the protective effect of Zn on Fo in Goldmark is associated with the function of Zn in the preservation of membrane integrity (Chvapil 1973, Von Glós and Boursnell 1981). At higher temperatures (40ºC) however, this protective effect of Zn was ineffective in both varieties and Fo increased.

Although a lack of Zn fertilisation resulted in a decline in the Fv/Fm of Goldmark at 37ºC, this did not result in a significant decrease in the kernel weight of the –Zn plants. This may be because the supplementary Zn provided protection of the photosynthetic apparatus during a narrow temperature window, perhaps from 35 to < 40ºC for example, and above and below these temperatures there was no difference in the Fv/Fm of the –Zn and +Zn treatments. Furthermore deprivation of assimilate does not generally account for the high temperature reduction of kernel weight in wheat (Wardlaw *et al.* 1980, Nicolas *et al.* 1984, Bhullar and Jenner 1986); more commonly it is the reduction in the activity of the soluble starch synthase enzyme that limits grain filling under high temperature, as the conversion of sucrose to starch is impaired (Jenner 1994). Although some researchers (Al-Khatib and Paulsen 1990, Blum *et al.* 1994, Reynolds *et al.* 2000)

have demonstrated a strong correlation between photosynthetic activity and grain yield of plants exposed to heat stress during grain filling, others suggest that both diminished source activity and sink capacity are of equal importance in reducing productivity (Harding *et al.* 1990b).

5.4.3 Birchip and Horsham, 1999

Results of these experiments in 1999 showed a higher grain yield across all varieties at Birchip than at Horsham. The average grain yield at 7.5 kg Zn ha^{-1} was 318 g m^{-2} at Birchip and 226 g m^{-2} at Horsham for the average of both sowing dates. However despite the average temperature during grain filling being higher at Horsham (24ºC, compared with 23ºC at Birchip), the higher yield at Birchip was due solely to an increase in the number of grains per m^2, not to an increase in grain weight. This indicates that temperature during grain filling had no effect on grain yield, and instead it was pre-anthesis growth conditions that produced the differences in yield between the sites. Since there was little difference in average daily temperatures prior to anthesis between Birchip and Horsham, it must be some other environmental factor, such as an isolated temperature event during booting or anthesis, or the drier than average spring in 1999 at Horsham, that produced the smaller number of grains per m^2 at Horsham.

Zinc fertilisation increased grain yield in all varieties at Birchip, and again these yield increases were due to an increase in the number of grains per m^2, as at Lameroo and Tintinara in 1998. The Zn treatment involving soil applied Zn plus a single foliar spray of Zn at anthesis produced a similar yield as the soil applied Zn alone, which would be expected since the majority of grains per m^2 would have been established by flowering time. Unlike Lameroo and Tintinara however, the Zn treatment involving soil applied Zn plus a foliar spray of Zn at tillering and at stem elongation did marginally increase the yield at Birchip, by 4% compared with the soil application of Zn alone. This may be because Birchip was a more Zn-responsive site than either Lameroo or Tintinara. The DTPA-extractable Zn at Birchip was similar to that of the 1998 sites (0.30 mg kg^{-1}), but differences in shoot dry matter production between Zn treatments were observed at

Birchip during stem elongation, whereas the mid-season dry matter harvest at Lameroo showed no differences between Zn treatments.

No yield responses to Zn application were observed at Horsham, at either sowing date. The number of grains per m^2 increased marginally at the first sowing date with the Zn treatment involving two foliar sprays of Zn, but this did not result in an increase in grain yield. This suggests that this site, like Minnipa in 1998, was not deficient in native Zn. Furthermore, analysis of other nutrient concentrations in the grain from Horsham revealed no other major nutrient deficiencies or toxicities that may have limited the Zn response. Brennan (1992) examined the relationship between critical DTPA-extractable Zn and the properties of 42 south-western Australian soils which were responsive to applied Zn, and found that the critical level of DTPA-extractable Zn for maximum wheat growth ranged from 0.12 mg kg^{-1} to 0.27 mg kg^{-1}. At the Horsham field trial site in 1999 the DTPA-extractable soil Zn was found to be 0.52 mg kg^{-1}; much higher than the critical range reported by Brennan (1992), which provides an explanation for the poor response to Zn fertilisation at this site.

Analysis of the grain Zn concentration from these sites again showed that plants supplied with soil and foliar Zn fertilisation produced grain with a significantly higher Zn concentration than those supplied with soil Zn alone. As in 1998, the Zn treatment of 7.5 kg Zn ha^{-1} applied at sowing did not increase the grain Zn concentration above that of the plants with no supplementary Zn fertilisation. However, the supplementation of this treatment with two foliar sprays of Zn increased the grain Zn concentration at both sites, by 71% at Birchip, and by 51% at Horsham (average of both sowing dates). Although Horsham was not a Zn responsive site in terms of grain yield, supplementary Zn in the form of a foliar spray increased the grain Zn concentration from 20 mg kg^{-1} in the nil Zn plants to 26 mg kg^{-1} for the single spray treatment, and to 32 mg kg^{-1} in the two-spray treatment. In a pot experiment, Rengel and Graham (1995b) also observed that large increases in grain Zn concentration could be achieved with supplementary Zn fertilisation, even when there is no significant change in grain yield. Higher levels of Zn in cereal grains has important consequences, not only for the growth of seedlings, but also for human populations where a high dependence on grains for food may result

in Zn deficiencies in susceptible humans (Welch 1993). In countries that have serious Zn deficiency problems in plants and humans combined applications of Zn to both the soil and leaves of cereals may be part of the solution to Zn deficiencies in plants and humans (Welch 1993, Yilmaz *et al.* 1997).

Plant leaf temperature is affected by radiational, convectional and transpirational processes, and the degree of cooling reflects the rate of evapotranspiration at the surface of the plant canopy, relative to the air temperature. Canopy temperature depression (CTD) is a physiological trait that has been shown to be significantly correlated with yield in warm environments and can be used as a powerful and robust selection criterion for heat tolerance (Amani *et al.* 1997, Reynolds *et al.* 1998). In the present experiments, supplementary Zn fertilisation at Birchip was found to increase the CTD of the Zn-inefficient bread wheat variety Goldmark, and also the two durum wheat varieties, Daki-Cyn and Kronos. Since CTD indirectly reflects transpiration at the whole crop level (Royo *et al.* 2002), these results suggest an improvement in some physiological trait with supplementary Zn nutrition in these varieties, such as better root growth, greater soil moisture extraction and/or higher stomatal conductance, which results in a cooler canopy (Pinter *et al.* 1990, Fischer *et al.* 1998). These results therefore support the suggestion that supplementary Zn nutrition can improve the thermotolerance of Zn inefficient varieties. No increase in CTD was found in the Zn-efficient varieties of Trident and Frame, or in the heat tolerant variety Halberd, which also appears to be quite Zn-efficient based on the yield results of the 1998 experiments.

Two unexpected results were obtained when measuring CTD in the present study; the increase in CTD in the Zn-efficient variety Excalibur, and the lack of CTD increase in the thermosensitive variety, Meering. This suggests that Meering is perhaps unresponsive to Zn, while Excalibur, as well as not being as Zn-efficient as once thought (Yusuf Genc, pers. commun.), may also be somewhat heat sensitive. This is in agreement with the results obtained at the Waite Institute, where Excalibur was the only variety to show a decrease in kernel weight at the second sowing date, which also suggests that Excalibur is sensitive to high temperature stress.

5.4.4 Yield stability across sites

The results of the Finlay-Wilkinson regression analysis have shown that some varieties, such as Meering, will be more sensitive to the stresses of low yielding environments (such as high temperature and drought) when also grown under conditions of low Zn availability. Meering appears to be a Zn efficient genotype (McDonald *et al.* 2001 and this chapter), but is also known to be thermosensitive with respect to grain weight (Stone and Nicolas 1995a). The present study has shown that Meering will have average yield stability across environments when grown with supplementary Zn. When grown without adequate Zn fertilisation however, the variety will produce low yields in low yielding environments but higher yields under more favourable conditions. In other words varieties such as Meering will be more sensitive to changes in the environment under conditions of Zn deficiency. The addition of supplementary Zn fertilisation to these varieties in such harsh environments will therefore increase yield stability.

5.5 General conclusion

The results presented here add some support to the hypothesis that Zn has the ability to provide wheat plants with tolerance to high temperature stress. The improvement in chlorophyll fluorescence under heat stress with supplementary Zn fertilisation observed under controlled environment conditions (Chapter 4) was reproduced in these experiments in the field. In addition, supplementary Zn was found to improve canopy temperature depression and thus provide a cooler canopy, possibly through better exploitation of soil moisture and/or a higher stomatal conductance. These results were seen largely in the Zn-inefficient varieties, with additional Zn having less of an effect in Zn-efficient varieties. Supplementary Zn fertilisation also increased the grain weight of the central spikelets of plants grown under warm conditions at the Waite Institute. However the results suggest that the improvement in photosynthetic activity under heat stress with additional Zn may be unrelated to the improvement observed in kernel weight under these conditions. Further work is therefore necessary to confirm this relationship between elevated Zn nutrition and tolerance of the photosynthetic apparatus

to high temperature stress, and to establish whether there is a link between increased thermotolerance of photosynthetic activity and the improvement in kernel weight under high temperatures and supplementary Zn.

This study has also shown that certain wheat varieties, particularly those that are heat sensitive, will be more vulnerable to the environmental stresses of low yielding sites (such as heat or water stress) when also grown under conditions of low Zn availability. The addition of supplementary Zn to these varieties will increase yield stability, thus again supporting the hypothesis that Zn has the ability to provide wheat plants with some tolerance to environmental stress.

Finally, while it seems likely that adequate levels of Zn have the ability to maintain photosynthetic activity, and possibly grain yield, under high temperature conditions, it is unknown whether grain quality can also be maintained by adequate Zn nutrition. These experiments have produced grain under a number of different temperature regimes, and with a wide range of Zn concentrations in the grain. This grain will be used to determine whether changes in protein content and composition due to high temperature can be ameliorated by adequate Zn nutrition.

CHAPTER 6

EFFECTS OF ZINC SUPPLY AND HEAT STRESS DURING GRAIN FILLING ON THE GROWTH, PHOTOSYNTHESIS AND YIELD OF WHEAT

6.1 Introduction

High temperature stress during grain filling is a major cause of yield loss in cereal crops throughout many of the world's cereal growing areas, including India (Chinoy 1947), the USA (Wiegand and Cuellar 1981) and Australia (Marcellos and Single 1972, Wardlaw *et al.* 1989a). In wheat, an average post-anthesis temperature of 15ºC is close to optimum for the attainment of maximum kernel weight (Chowdhury and Wardlaw 1978), with a general reduction in yield per ear of 3-4% for each 1ºC rise above this optimum (Wardlaw *et al.* 1989a). High temperatures are known to cause deleterious changes in a number of physiological processes in plants, including disruption of membranes, denaturation of enzymes and reduced photosynthesis (Nguyen and Joshi 1993). The photosystem II (PSII) complex on the chloroplast thylakoid membrane is particularly sensitive to elevated temperatures, and it is the damage to this system that is primarily responsible for the decrease in photosynthesis during a period of heat stress (Schreiber and Berry 1977).

The primary purpose of this study was to determine whether zinc (Zn) nutrition has a role to play in alleviating the adverse effects of post-anthesis heat stress in wheat. Zinc is an essential micronutrient that is also involved in the maintenance of photosynthetic activity and membrane integrity (Brown *et al.* 1993). The studies described in Chapter 4 showed that adequate Zn nutrition could reduce the adverse effects of a short period of high temperature on photosynthesis in wheat seedlings. The aim of the present study was to assess whether these effects of Zn on photosynthesis during early growth could also be detected within an episode of high temperature during grain filling, and further, to determine whether these effects of Zn during heat stress can influence grain yield.

6.2 Materials and methods

Laffer sand was collected and prepared as described in Chapter 3 (Section 3.3), and 3 kg was weighed into polythene-lined cardboard cartons (270 mm x 95 mm x 95 mm) of 2 L volume. Basal nutrients were applied as described in Chapter 3, together with two Zn treatments, 0.2 and 2 mg Zn kg^{-1} soil, applied as $ZnSO_4.7H_20$ and designated as $Zn_{0.2}$ and Zn_2. Nanopure water was added to each pot to bring the soil water content to field capacity (12% w/w), and this was maintained by daily watering to weight. Solutions of NH_4NO_3 (120 mg N kg^{-1} dry soil) and K_2SO_4 (54 mg K kg^{-1} dry soil) were applied to each pot at four and six weeks after sowing, respectively.

Four wheat genotypes were chosen for this study on the basis of previously reported differences in their Zn efficiency or tolerance to high temperature (Chapter 3, Section 3.2). These cultivars were Frame (moderately Zn efficient); Goldmark (Zn inefficient); Halberd (heat tolerant) and Meering (heat sensitive). Further information regarding the Zn efficiency and thermotolerance of these varieties has been derived from the field experiments described in Chapter 5. Frame appears to be a relatively thermotolerant cultivar, and Goldmark moderately thermosensitive, based on the photosynthetic activity of these varieties under elevated temperatures. Both Meering and Halberd appear to be Zn efficient genotypes with respect to grain yield, with Halberd also showing Zn efficiency with respect to canopy temperature depression.

All genotypes used in this experiment had similar seed Zn contents: 540, 530, 550 and 500 ng/seed for Frame, Goldmark, Halberd and Meering, respectively. This seed was obtained from the field experiment at Tintinara (Chapter 5) in which plants were grown without applied Zn. Seeds were prepared and germinated as described in Chapter 3 (Section 3.4). Ten uniformly germinated seeds with radicle emerged were sown into each pot, and following emergence, plants were thinned to give a final population of eight uniform plants per pot. A layer of acid washed black polypropylene beads was placed over the soil surface of each pot to a depth of approximately 10 mm to prevent excess soil moisture loss by evaporation.

Plants were grown in a growth room at 22/16ºC day/night temperature, 14 h

photoperiod and 600 µmol s^{-1} m^{-2} light intensity at the plant canopy level. Heat treatments were applied for a duration of 3 days starting from 10 days after anthesis (DAA). Heat-treated plants were moved during the night cycle to a second growth room in which the night temperature of 20ºC was maintained for 10 h and the peak of 40ºC day temperature was kept for 8 h. The rate of heating to, and cooling from, the day temperature maximum was 7˚C h^{-1}. The light intensity in this growth room was 810 µmol s^{-1} m^{-2} at the plant canopy level. These differences in light intensity between growth rooms is not ideal, as under certain circumstances high light intensity may exacerbate the effects of Zn deficiency (Zhang and Wu 1989, Cakmak *et al.* 1995, 1998b). However these studies have compared Zn deficiency symptoms under a much wider range of light intensities than that used in the present experiment, and also subjected plants to these different light intensities for a much longer duration than that used here. There is little information available in the literature about the effect of such little difference in light intensity as that used in the present experiments. Heat-treated plants were watered to weight four times daily to ensure an adequate level of soil moisture was maintained and water stress did not confound the effect of heat stress. No visible symptoms of stress were evident during the heat treatment period.

The youngest emerged leaf blade (YEB) was sampled from one plant of each replicate at 38 DAS, and analysed for Zn and other nutrient concentrations by ICP spectrometry as described in Chapter 3 (Section 3.6). Each main stem ear was labelled with the date of the first anther exsertion (Zadoks stage 60, Zadoks *et al.* 1974) and the date of all subsequent operations was related to the average anthesis date for each genotype. Plants for which a variation of greater than 3 days in anthesis date was recorded were excluded from the experiment. Flag leaf area was determined at 10 DAA using a Paton Electronic Planimeter (Paton Industries, Pty Ltd., South Australia). One plant from each pot was harvested at 10, 13, 20, 35 and 50 DAA, and a total of four grains were removed from the *a* and *b* florets of the central spikelets on each side of the main stem ear (Figure 6.1). These were oven dried, along with the rest of the grains from the main stem ear and tillers, at 80ºC for 48 h, before being weighed to determine their dry mass.

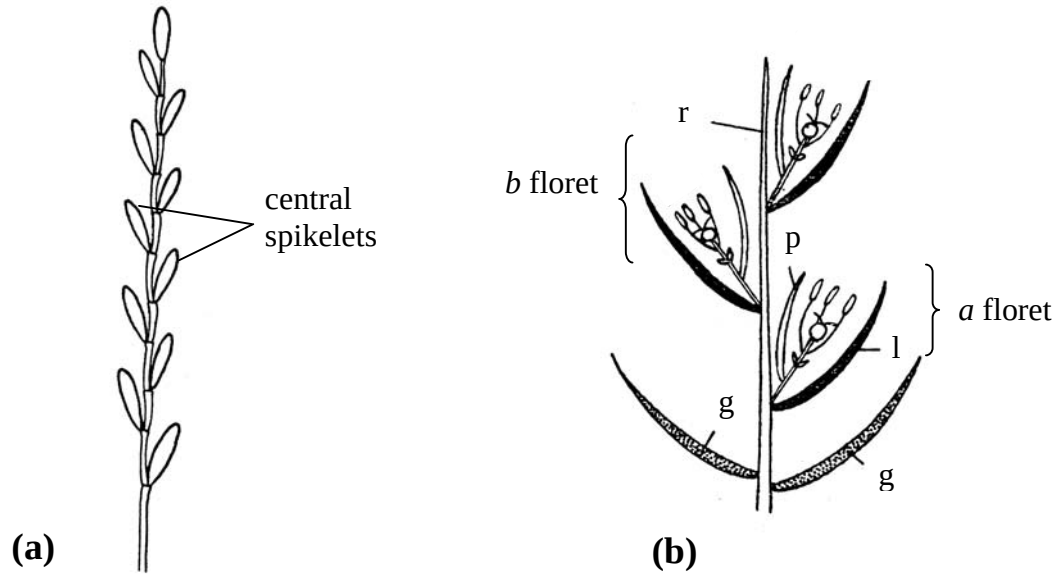


Figure 6.1. (a) Diagram of the wheat spike (ear), showing the location of the central spikelets. (b) Structure of a typical spikelet, showing the location of the *a* and *b* florets. r = rachilla, p = palea, l = lemma, g = glume Adapted from Gill and Vear (1958).

Chlorophyll fluorescence measurements were made on the flag leaves of 2 plants per pot at regular intervals from 5 days prior to anthesis until the leaves began to senesce (Chapter 3, Section 3.5.1). Each measurement was recorded at the same time of day for each genotype; 4-5 h into the light period. Leaf chlorophyll content was measured on the flag leaves of 2 plants per pot at two-day intervals from 9 DAA until leaf senescence (Chapter 3 Section 3.5.2). At maturity (50 DAA), two plants per pot were harvested from each replicate, oven dried at 80ºC for 48 h, and weighed to determine the total above ground biomass. Spikes were threshed, and the number of grains spike^{-1}, grain weight and grain yield were recorded. The harvest index was calculated as the ratio of grain weight to the total weight of above ground parts (grain + straw). Grain Zn concentration was determined by ICP spectrometry (Chapter 3, Section 3.6).

The experiment was set up in a factorial completely randomised block design (4 genotypes x 2 Zn levels x 2 temperature treatments) with four replicates. Results were statistically analysed as explained in Chapter 3 (Section 3.7).

6.3 Results

6.3.1 Zinc concentration in the youngest emerged leaf blades

Zinc concentration in the YEBs increased significantly with the application of Zn (Table 6.1). At 38 DAS the mean Zn concentration was 17 mg Zn kg^{-1} for the $Zn_{0.2}$ treatment and 51 mg Zn kg^{-1} for the Zn_2 treatment, with no significant differences between genotypes. These Zn values for plants grown at the low Zn supply, $Zn_{0.2}$, were within the reported critical deficiency range of 14-19 mg Zn kg^{-1} for YEBs of wheat seedlings (Bansal *et al.* 1990, Riley *et al.* 1992, Wilhelm *et al.* 1993, Brennan and Bolland 2002). In contrast, at adequate Zn supply, Zn_2, Zn concentrations in the YEBs were within the reported adequate range.

Table 6.1. Effects of zinc fertilisation (mg kg^{-1} soil) on the zinc concentration (mg kg^{-1} D.W.) in the YEBs of wheat genotypes at 38 DAS.

Genotype	Zn 0.2	Zn 2	*Mean*
Frame	17.9	58.2	*38.1*
Goldmark	17.1	46.9	*32.0*
Halberd	12.7	48.2	*30.5*
Meering	19.5	48.5	*34.0*
Mean	*16.8*	*50.5*	
$LSD_{0.05}$			
Genotype	**n.s.**		
Zinc	**3.8**		
Genotype x Zinc	**n.s.**		
CV (%)	**9.7**		

n.s. = non-significant

6.3.2 Concentration of other nutrients

Potassium (K) concentration in the YEBs varied with genotype (Table 6.2), however the concentrations of K in all genotypes, ranging from 14975 to 29250 mg K kg^{-1}, were much lower than reported critical deficiency values for wheat seedlings of 32000 mg K kg^{-1} for the top two leaves at 75 days after emergence (Rao 1986), and 38000 to 41000 mg K kg^{-1} for whole shoots at 30 and 45 days after emergence, respectively (Cox 1981,

Rao 1986). This indicated that the plants in the present experiment were K-deficient, and this was corrected with an application of K_2SO_4 solution to each pot (54 mg K kg^{-1} dry soil), at 42 DAS.

Table 6.2. Effects of zinc fertilisation (mg kg^{-1} soil) on the concentration of potassium, copper, calcium and phosphorus (mg kg^{-1} D.W.) in the YEBs of wheat genotypes at 38 DAS.

Genotype	Potassium concentration			Copper concentration		
	Zn 0.2	**Zn 2**	***Mean***	**Zn 0.2**	**Zn 2**	***Mean***
Frame	23500	20250	*21875*	12.3	14.8	*13.6*
Goldmark	30500	28000	*29250*	11.9	12.2	*12.0*
Halberd	15200	14750	*14975*	12.5	14.1	*13.3*
Meering	26000	24500	*25250*	11.6	12.6	*12.1*
Mean	*23800*	*21875*		*12.1*	*13.4*	
$LSD_{0.05}$						
Genotype		**4997**			**0.9**	
Zinc		**n.s.**			**0.6**	
Genotype x Zinc		**n.s.**			**n.s.**	
CV (%)		**13.4**			**4.3**	

Genotype	Calcium concentration			Phosphorus concentration		
	Zn 0.2	**Zn 2**	***Mean***	**Zn 0.2**	**Zn 2**	***Mean***
Frame	6550	7800	*7175*	5400	5050	*5225*
Goldmark	9000	8450	*8725*	5450	5200	*5325*
Halberd	9350	9500	*9425*	4400	4700	*4550*
Meering	6500	5900	*6200*	5450	5650	*5550*
Mean	*7850*	*7913*		*5175*	*5150*	
$LSD_{0.05}$						
Genotype		**1717**			**n.s.**	
Zinc		**n.s.**			**n.s.**	
Genotype x Zinc		**n.s.**			**n.s.**	
CV (%)		**13.4**			**11.3**	

n.s. = non-significant

Zn fertilisation significantly affected the concentration of copper (Cu) in the YEBs of the wheat seedlings (Table 6.2). Across genotypes, the mean Cu concentration was 12.1 mg Cu kg^{-1} for the $Zn_{0.2}$ treatment, and 13.4 mg Cu kg^{-1} for the Zn_2 treatment, both of

which are well within the reported adequate range for the YEBs of wheat seedlings (Nambiar 1976, Weir and Cresswell 1994). The concentration of other nutrients, including calcium, magnesium, manganese, molybdenum and sodium, was found to vary with genotype (Tables 6.2 and 6.3), however the concentration of these and all other nutrients were within the reported adequate range for the YEBs of wheat seedlings (Reuter *et al.* 1997a).

Table 6.3. Effects of zinc fertilisation (mg kg^{-1} soil) on the concentration of magnesium, manganese, molybdenum and sodium (mg kg^{-1} D.W.) in the YEBs of wheat genotypes at 38 DAS.

Genotype	Magnesium concentration			Manganese concentration		
	Zn 0.2	**Zn 2**	***Mean***	**Zn 0.2**	**Zn 2**	***Mean***
Frame	1730	1920	*1825*	80.8	91.4	*86.1*
Goldmark	2195	2150	*2173*	129.0	133.7	*131.4*
Halberd	1535	1545	*1540*	73.6	85.4	*79.5*
Meering	2100	2055	*2078*	100.8	112.4	*106.6*
Mean	*1890*	*1918*		*96.1*	*105.7*	
LSD$_{0.05}$						
Genotype		**288**			**25.3**	
Zinc		**n.s.**			**n.s.**	
Genotype x Zinc		**n.s.**			**n.s.**	
CV (%)		**9.3**			**15.4**	
Genotype	**Molybdenum concentration**			**Sodium concentration**		
	Zn 0.2	**Zn 2**	***Mean***	**Zn 0.2**	**Zn 2**	***Mean***
Frame	21.8	24.3	*23.1*	34.9	34.4	*34.7*
Goldmark	31.7	32.1	*31.9*	15.7	13.5	*14.6*
Halberd	21.6	21.2	*21.4*	18.9	24.0	*21.5*
Meering	20.7	21.6	*21.2*	15.5	22.2	*18.8*
Mean	*24.0*	*24.8*		*21.2*	*23.5*	
LSD$_{0.05}$						
Genotype		**7.5**			**8.6**	
Zinc		**n.s.**			**n.s.**	
Genotype x Zinc		**n.s.**			**n.s.**	
CV (%)		**19.2**			**23.6**	

n.s. = non-significant

6.3.3 Flag leaf area

Flag leaf area at 10 DAA differed significantly between wheat genotypes (Figure 6.2). Halberd, an older, tall-strawed variety, released in 1969, had significantly smaller flag leaves than the other, more recently released, varieties. Meering had significantly larger flag leaves than the other genotypes, and the leaf area of Meering also responded to Zn fertilisation, with the leaves of the Zn deficient plants being 24% smaller than their Zn sufficient counterparts.

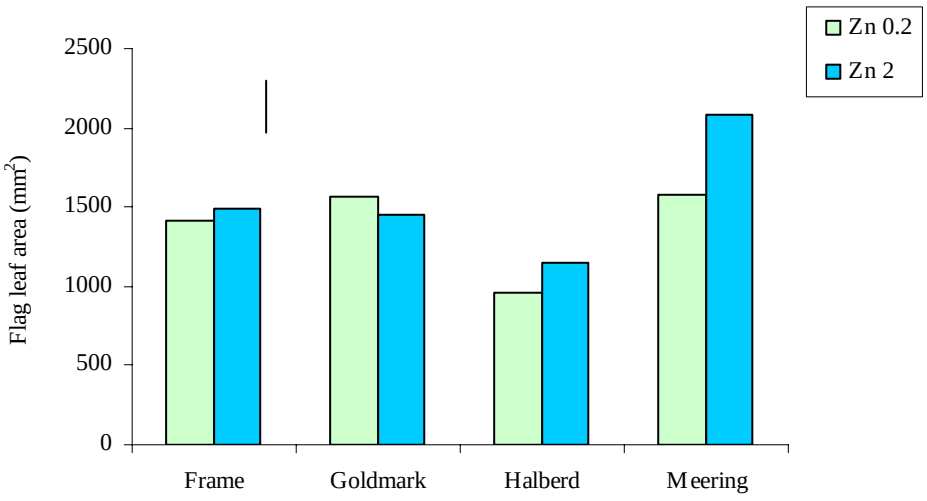


Figure 6.2. Effects of Zn fertilisation (mg kg^{-1} soil) on the flag leaf area of wheat genotypes at 10 DAA. The vertical bar represents the $LSD_{0.05}$ for the Genotype x Zinc interaction.

6.3.4 Chlorophyll fluorescence

The chlorophyll fluorescence ratio (Fv/Fm) decreased significantly in all varieties during the high temperature treatment and ensuing 24 hours (Figure 6.3). This reduction in Fv/Fm by high temperature was significantly greater in the thermosensitive variety, Meering, and significantly lower in the thermotolerant variety, Halberd. At 10 DAA, the first day of high temperature stress, the Fv/Fm ratio was lowest under Zn

deficiency and heat stress in all genotypes, with an average value of 0.765, as compared with 0.774 for the adequate Zn plants and 0.822 for the plants not subjected to heat stress. At 11 DAA there was no significant Zn effect on the Fv/Fm ratio, while at 12 DAA there was a significant difference between varieties. By this 3rd day of heat stress Fv/Fm was significantly lower in the Zn deficient plants of Frame and Halberd under the high temperature conditions. This trend was also clearly observed in Goldmark. Conversely, the Meering plants supplied with adequate Zn showed a significantly lower Fv/Fm ratio during the heat stress than the Zn deficient plants.

The lower Fv/Fm of Meering under high temperature was due mainly to a greater reduction in chlorophyll maximum fluorescence (Fm) in this variety (Figure 6.4), rather than any effect on chlorophyll initial fluorescence (Fo) (Figure 6.5). Goldmark was also found to have a low Fm under high temperature, but the higher Fo in this cultivar resulted in an Fv/Fm ratio that was higher than that of thermosensitive Meering under heat stress. In contrast to Meering, the thermotolerance of the Fv/Fm of Halberd was a result of a stable Fo during the high temperature treatment (Figure 6.5), with little difference in Fm between this and other varieties (Figure 6.4).

The lower Fv/Fm ratio under high temperature and Zn deficiency at 10 DAA was a result of a decrease in Fm (Figure 6.4), with no effect on Fo. The observed values for chlorophyll variable fluorescence (Fv) were similar to those observed for Fm (Figure 6.6). Fm decreased by 16.5% in the Zn adequate plants under high temperature stress and by 19% in the Zn deficient plants at 10 DAA. By the third day of high temperature some acclimation had occurred, and the reduction in Fm had decreased to 16%, with no difference between the Zn treatments.

The decrease in the Fv/Fm ratio at 12 DAA under high temperature and low Zn was therefore a result of an increase in Fo (Figure 6.5). Fo was significantly higher in the Zn deficient plants of Frame and Halberd under the high temperature conditions at 12 DAA, and also higher in the Zn deficient plants of Goldmark under high temperature at 11 DAA. As with the Fv/Fm ratio, the Zn adequate plants of Meering showed a more deleterious Fo reading during the heat stress than the Zn deficient plants.

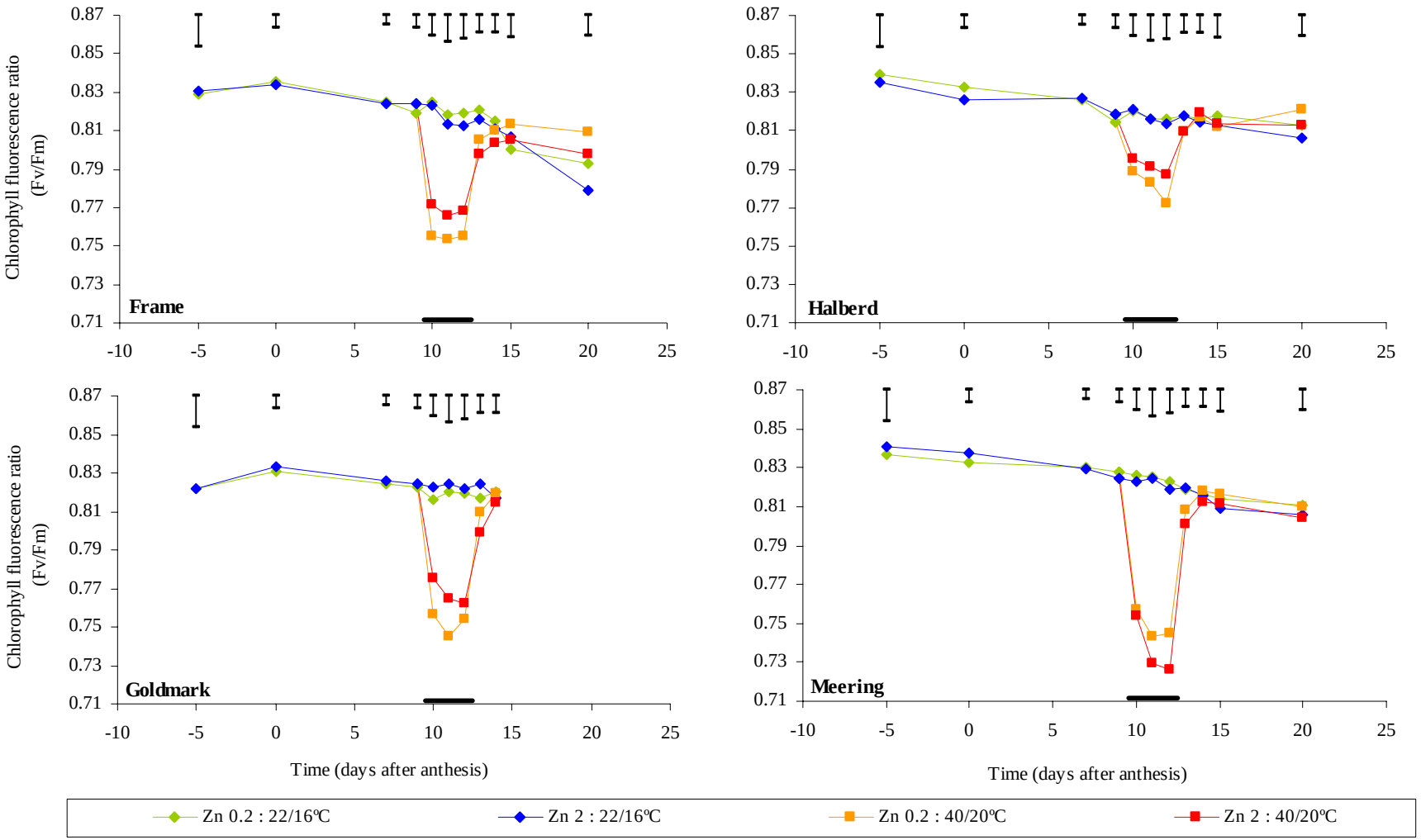


Figure 6.3. Effects of Zn supply (mg kg^{-1} soil) and temperature treatment on the chlorophyll fluorescence ratio, Fv/Fm, of wheat flag leaves from 5 days before anthesis. Vertical bars represent the $LSD_{0.05}$ for the interaction of Genotype x Zinc x Temperature. Horizontal bars on x-axes represent the period of high temperature treatment.

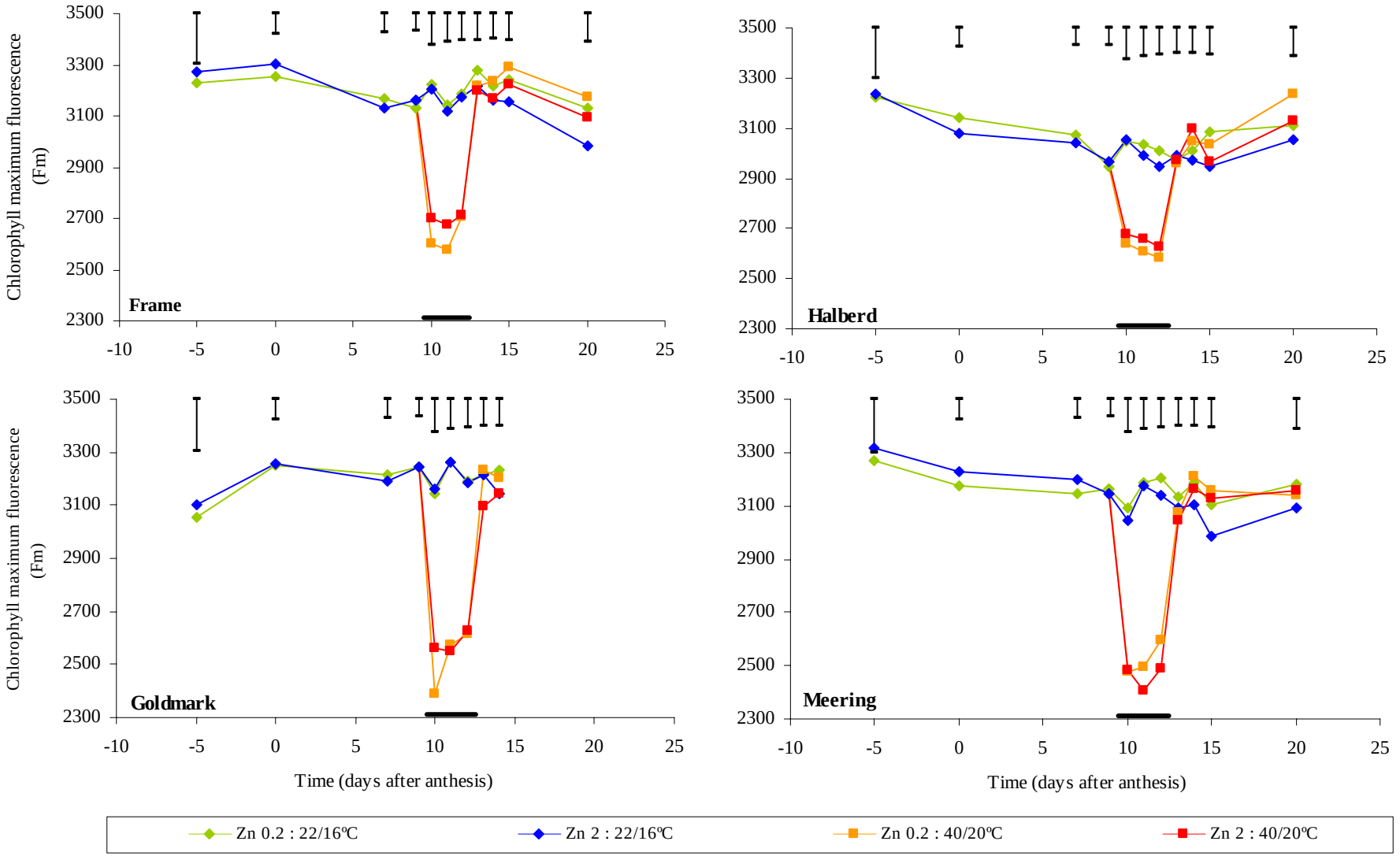


Figure 6.4. Effects of Zn supply (mg kg^{-1} soil) and temperature treatment on the chlorophyll maximum fluorescence, Fm, of wheat flag leaves from 5 days before anthesis. Vertical bars represent the $LSD_{0.05}$ for the interaction of Genotype x Zinc x Temperature. Horizontal bars on x-axes represent the period of high temperature treatment.

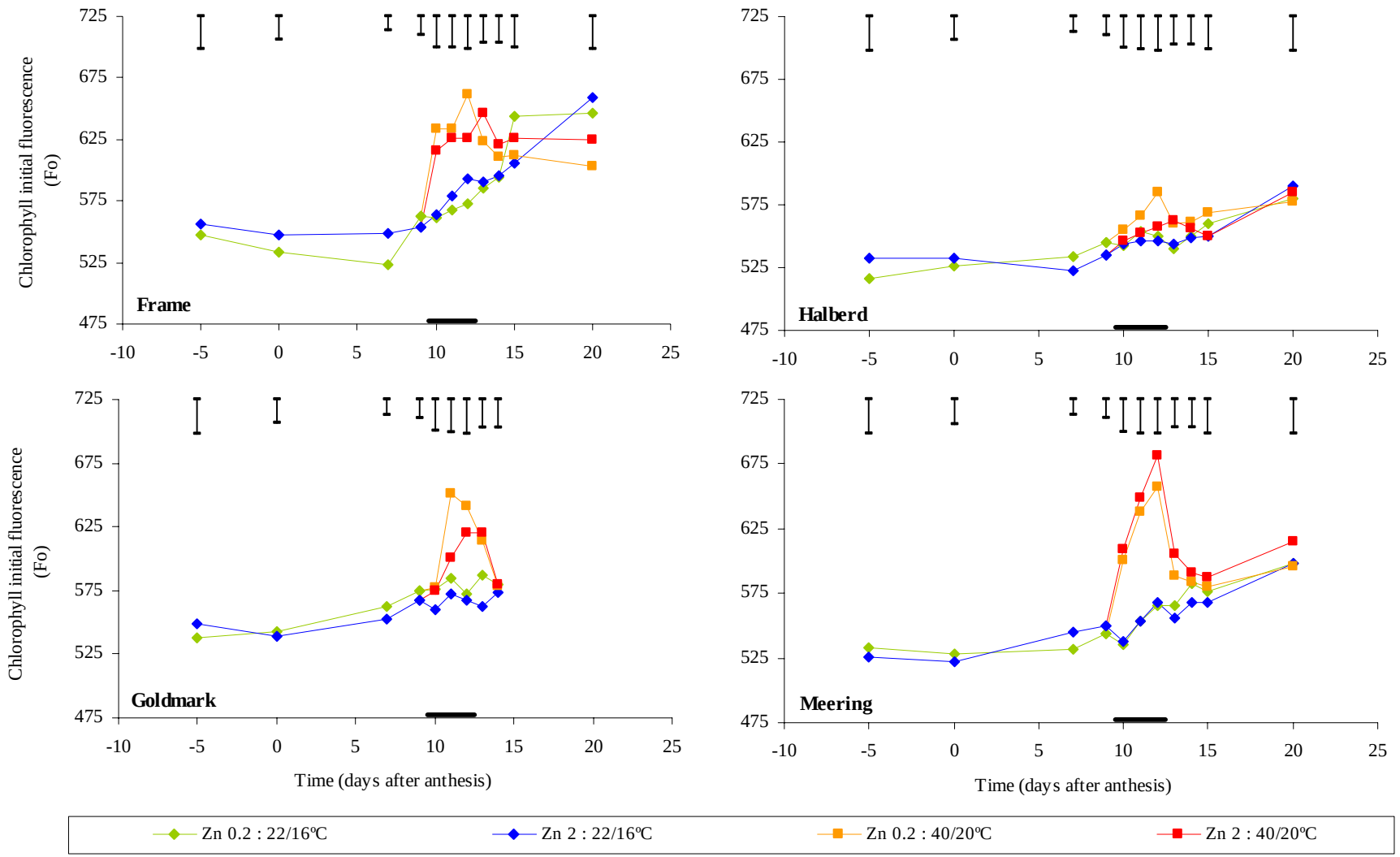


Figure 6.5. Effects of Zn supply (mg kg^{-1} soil) and temperature treatment on chlorophyll initial fluorescence, Fo, of wheat flag leaves from 5 days before anthesis. Vertical bars represent the $LSD_{0.05}$ for the interaction of Genotype x Zinc x Temperature. Horizontal bars on x-axes represent the period of high temperature treatment.

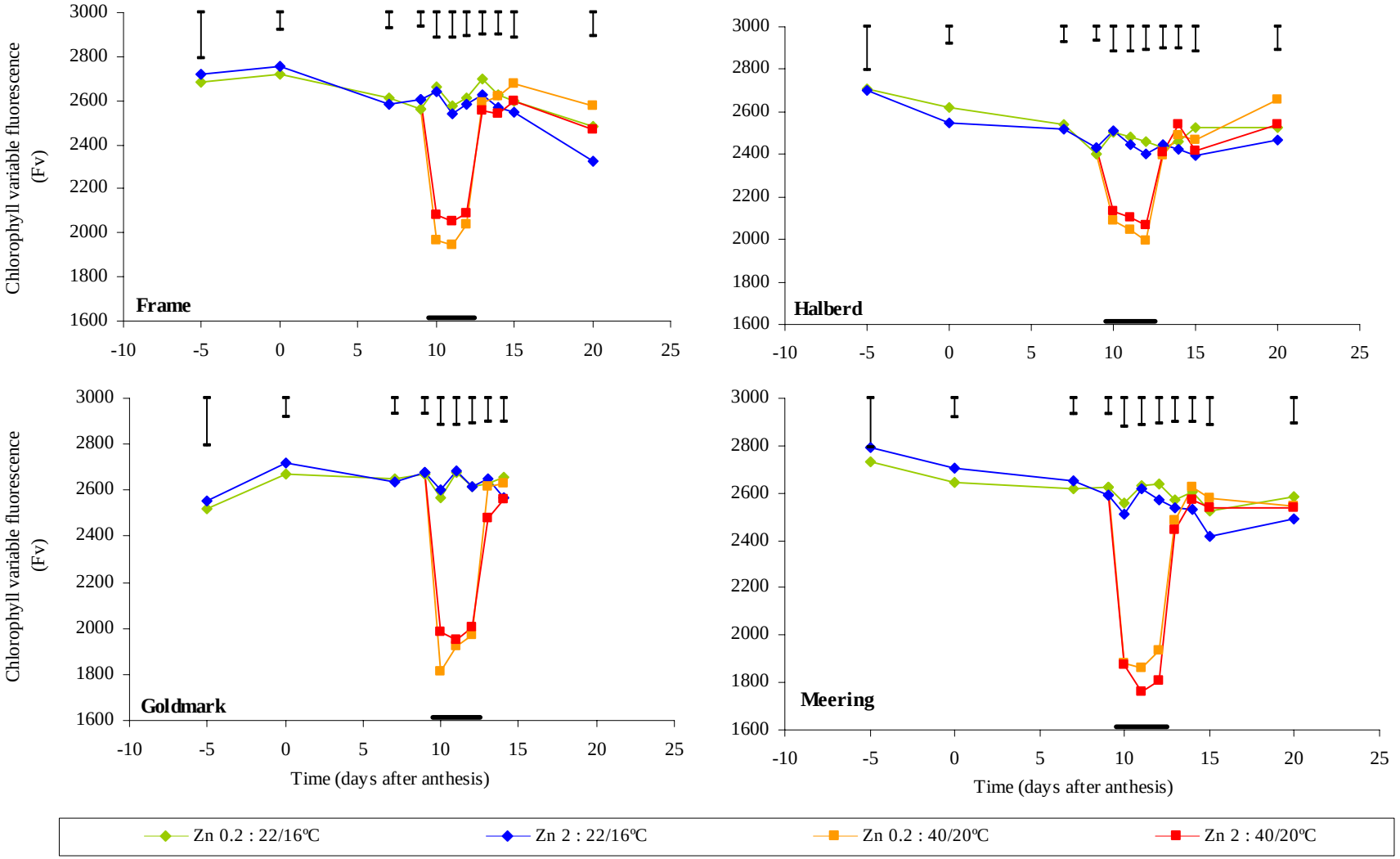


Figure 6.6. Effects of Zn supply (mg kg^{-1} soil) and temperature treatment on the chlorophyll variable fluorescence, Fv, of wheat flag leaves from 5 days before anthesis. Vertical bars represent the $LSD_{0.05}$ for the interaction of Genotype x Zinc x Temperature. Horizontal bars on x-axes represent the period of high temperature treatment.

6.3.5 Chlorophyll content

High temperature significantly reduced the chlorophyll content in the flag leaves of two varieties during the heat treatment, the Zn-inefficient variety, Goldmark, and the heat sensitive variety, Meering. Furthermore this lower chlorophyll content of the high temperature plants was maintained for up to 2 weeks following the high temperature treatment (Figure 6.7), particularly in Goldmark, which appeared to be very sensitive to the heat stress treatment.

Zinc deficiency further reduced the chlorophyll content of Goldmark under high temperature stress, with a significant interaction between Zn fertilisation and temperature treatment observed at 13 DAA. At 13 DAA, the chlorophyll content of Zn deficient Goldmark plants subjected to high temperature was only 33% of that of the Zn adequate plants grown at 22/16ºC. In comparison, the chlorophyll content of the Zn adequate plants under high temperature was 59% of that of the Zn adequate, 22/16ºC, plants, while the chlorophyll content of the Zn deficient and Zn adequate plants grown at 22/16ºC differed by less than 1%.

From 31 DAA the chlorophyll content of the Zn_2 plants was significantly lower than that of the $Zn_{0.2}$ plants, in all varieties. Thus Zn deficiency delayed flag leaf senescence for 10 days until 41 DAA, when again there was no difference between Zn treatments on chlorophyll content.

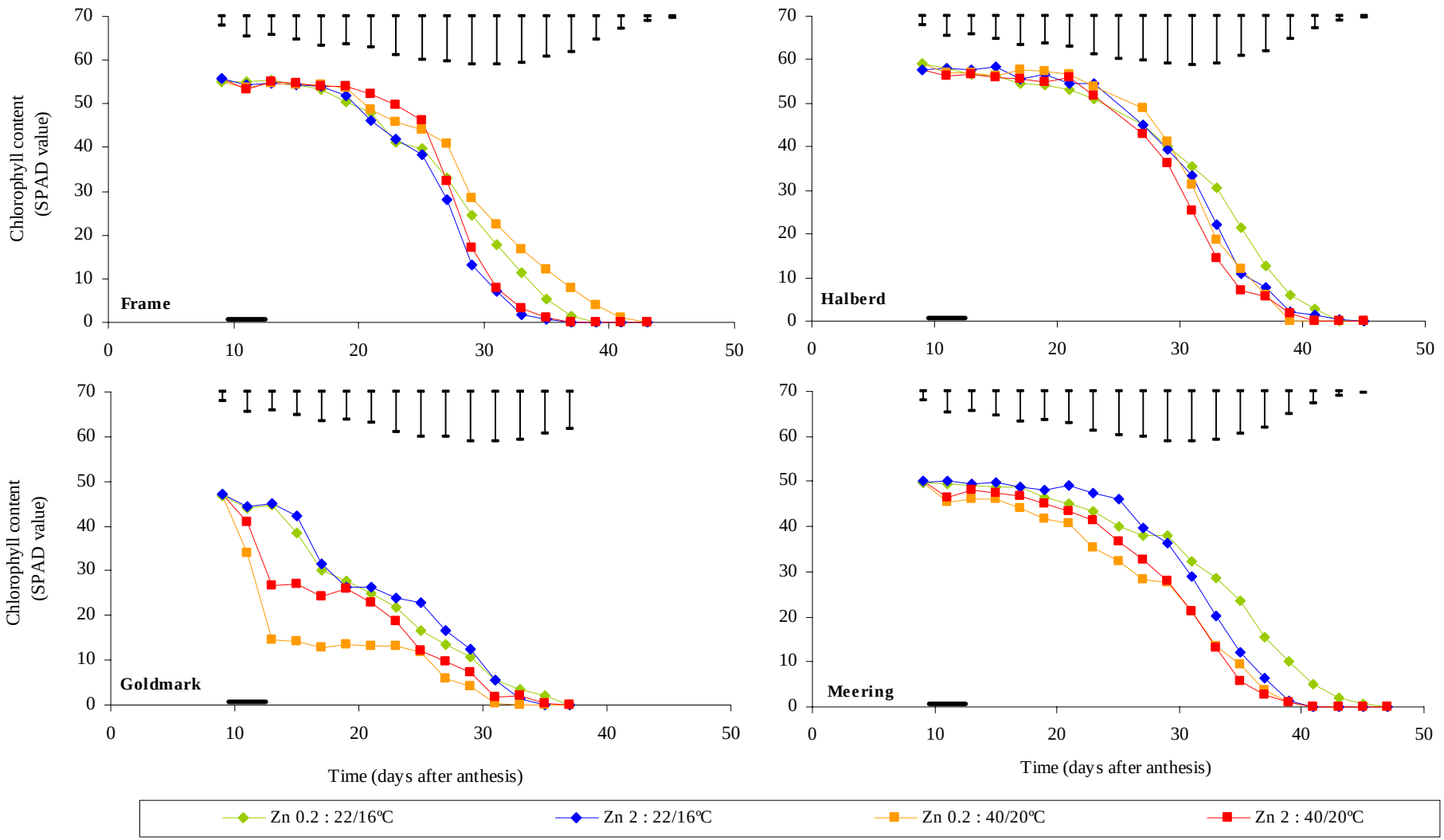


Figure 6.7. Effects of Zn supply (mg kg^{-1} soil) and temperature treatment on chlorophyll content of wheat flag leaves from 9 DAA. Vertical bars represent the $LSD_{0.05}$ for the interaction of Genotype x Zinc x Temperature. Horizontal bars on x-axes represent the period of high temperature treatment.

An integration of values under each of the chlorophyll content curves in Figure 6.7 was performed to give an estimate of green area duration of the flag leaf after anthesis. High temperature significantly reduced the duration of green area in three of the four varieties studied; Goldmark, Meering and Halberd (Figure 6.8). There was no significant effect of Zn fertilisation on this parameter, however.

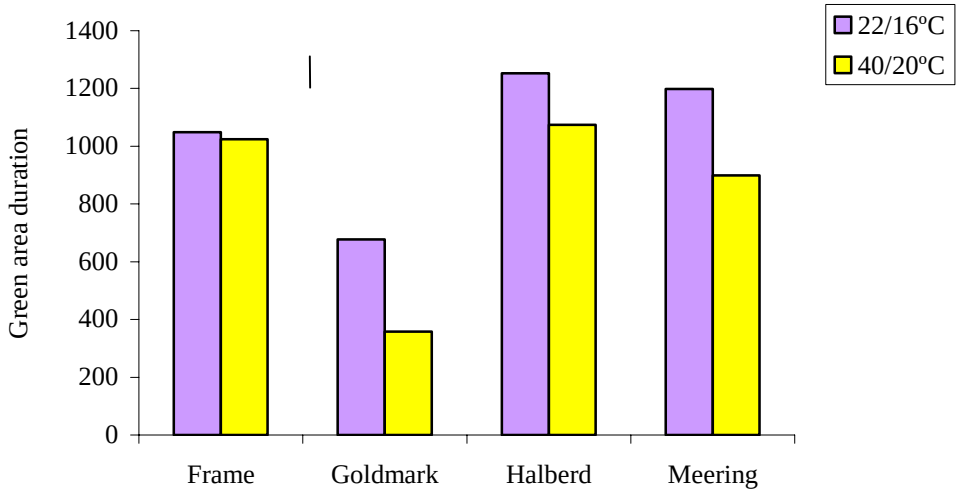


Figure 6.8. Effects of temperature treatment on the green area duration (derived from an integration of values under the chlorophyll content curves, Figure 6.7) of the flag leaves of wheat genotypes following anthesis. The vertical bar represents the $LSD_{0.05}$ for the Genotype x Temperature interaction.

6.3.6 Shoot dry matter production

Shoot dry matter production at maturity was not significantly affected by either Zn fertilisation or high temperature treatment (Appendix 6.1). Genotypes differed in dry matter production however, with Frame and Halberd producing significantly more above ground biomass than Meering, which in turn produced a greater amount of dry matter than Goldmark.

6.3.7 Harvest index

The high temperature treatment of 3 days at 40/20ºC significantly reduced the harvest index in 3 of the 4 varieties studied (Figure 6.9). Frame, Goldmark and Meering were all less effective at partitioning assimilates when exposed to the heat stress conditions, whereas the harvest index of Halberd, which was significantly lower than the other varieties, did not differ between temperature treatments.

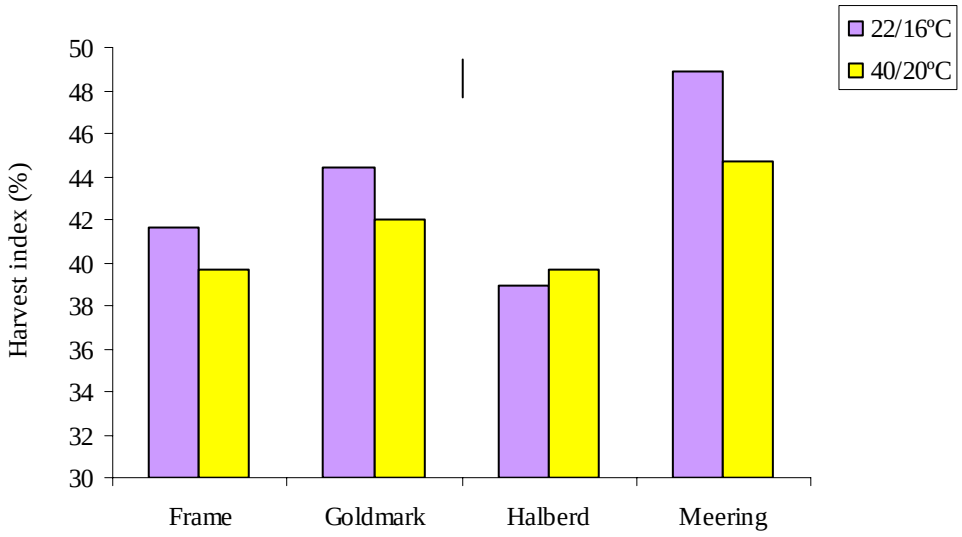


Figure 6.9. Effects of temperature treatment on the harvest index of wheat genotypes. The vertical bar represents the $LSD_{0.05}$ for the Genotype x Temperature interaction.

Zn fertilisation also influenced the harvest index (Figure 6.10), with all varieties being less efficient at partitioning assimilates under low soil Zn conditions. There was no significant interaction between Zn fertilisation and temperature treatment on harvest index.

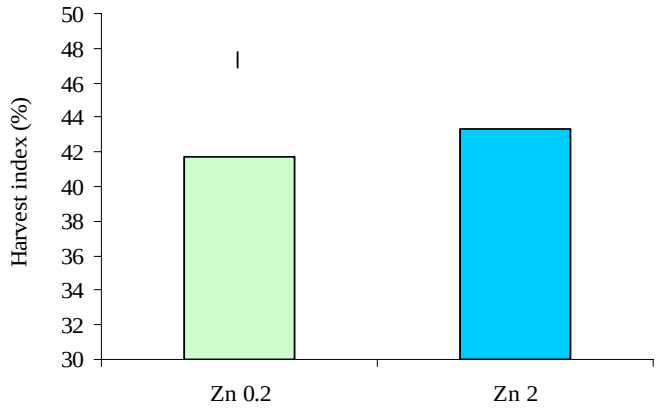


Figure 6.10. Effects of Zn fertilisation (mg kg^{-1} soil) on the harvest index of wheat. The vertical bar represents the $LSD_{0.05}$ for the main effect of Zinc.

6.3.8 Grain growth

Exposure to high temperature for 3 days from 10 DAA significantly altered the dynamics of grain growth in two of the varieties studied, Goldmark and Meering (Figure 6.11). Plants of Goldmark showed a reduced rate of grain filling from 20-35 DAA, compared with the control (Figure 6.11b and f). Exposure to high temperature also caused a reduction in the duration of grain growth in Goldmark, such that there was no addition to grain dry matter after 35 DAA. Grains of the central spikelets of Meering show a similar response to high temperature as Goldmark, that is, a reduced rate of grain filling from 20-35 DAA, and a reduced duration of grain growth, with a cessation of dry matter accumulation from 35 DAA (Figure 6.11d). When all grains of the main culm ear of Meering are considered however, it appears that the linear phase of grain growth ceased between 20 and 35 DAA, with dry matter accumulating at a much lower rate than the control from 20 DAA (Figure 6.11h).

Zinc fertilisation also affected the dynamics of grain growth, with no significant difference among varieties (Figure 6.12). At low Zn supply, $Zn_{0.2}$, there was a small reduction in the rate of dry matter accumulation between 10 and 13 DAA. As a result individual kernel mass was significantly lower in the Zn deficient plants at 13, 20, 35 and 50 DAA. There was no difference in the duration of grain filling between Zn deficient and Zn adequate plants. Furthermore there was no significant interaction between Zn fertilisation and temperature treatment on grain growth.

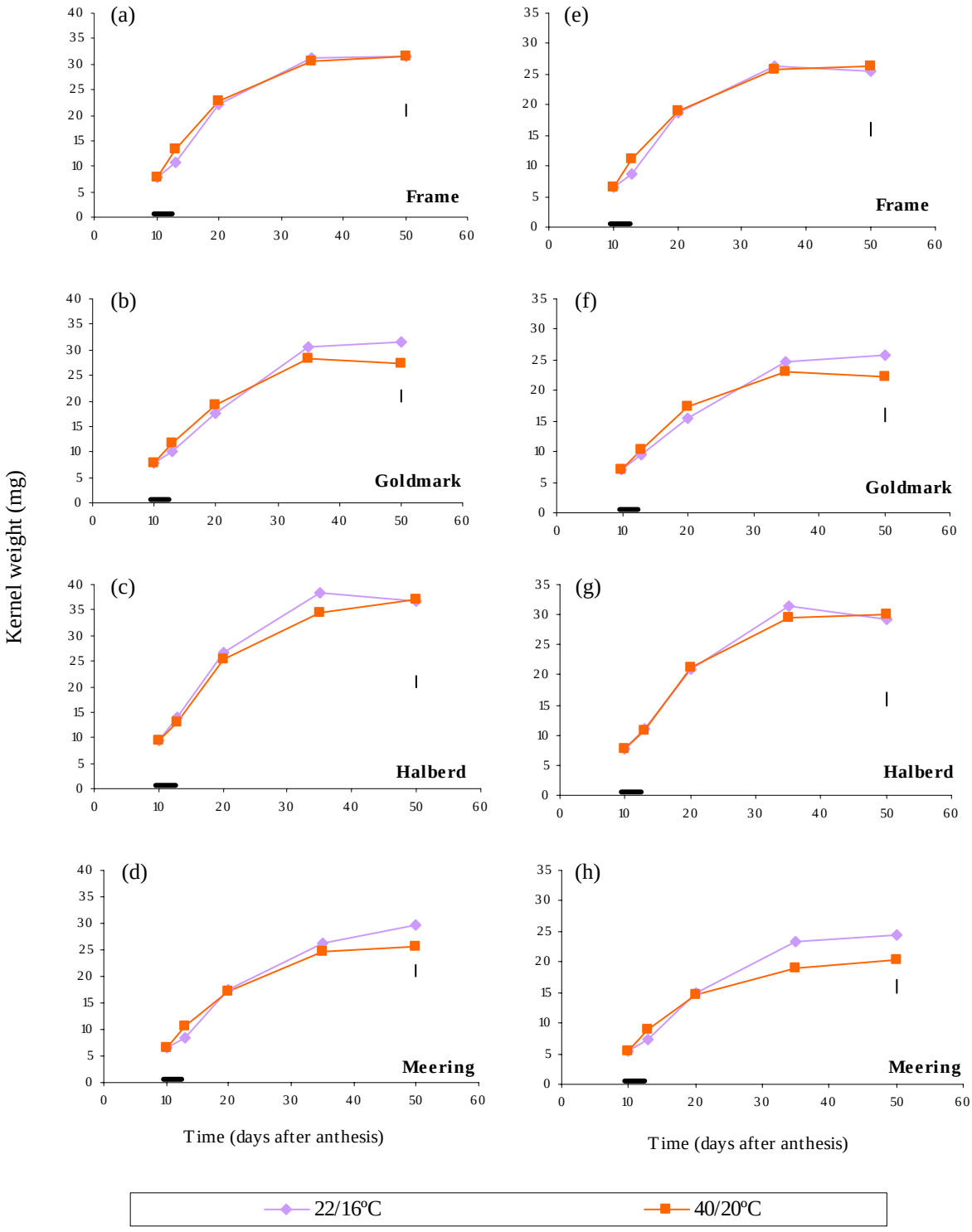


Figure 6.11. Effects of temperature treatment on the individual kernel mass (a-d, grains of the *a* and *b* florets of the central spikelets of the main culm ear; e-h, all grains of the main culm ear) of wheat genotypes from 9 DAA. Vertical bars represent the $LSD_{0.05}$ for the interaction of Genotype x Temperature at 50 DAA. Horizontal bars on x-axes represent the period of high temperature stress.

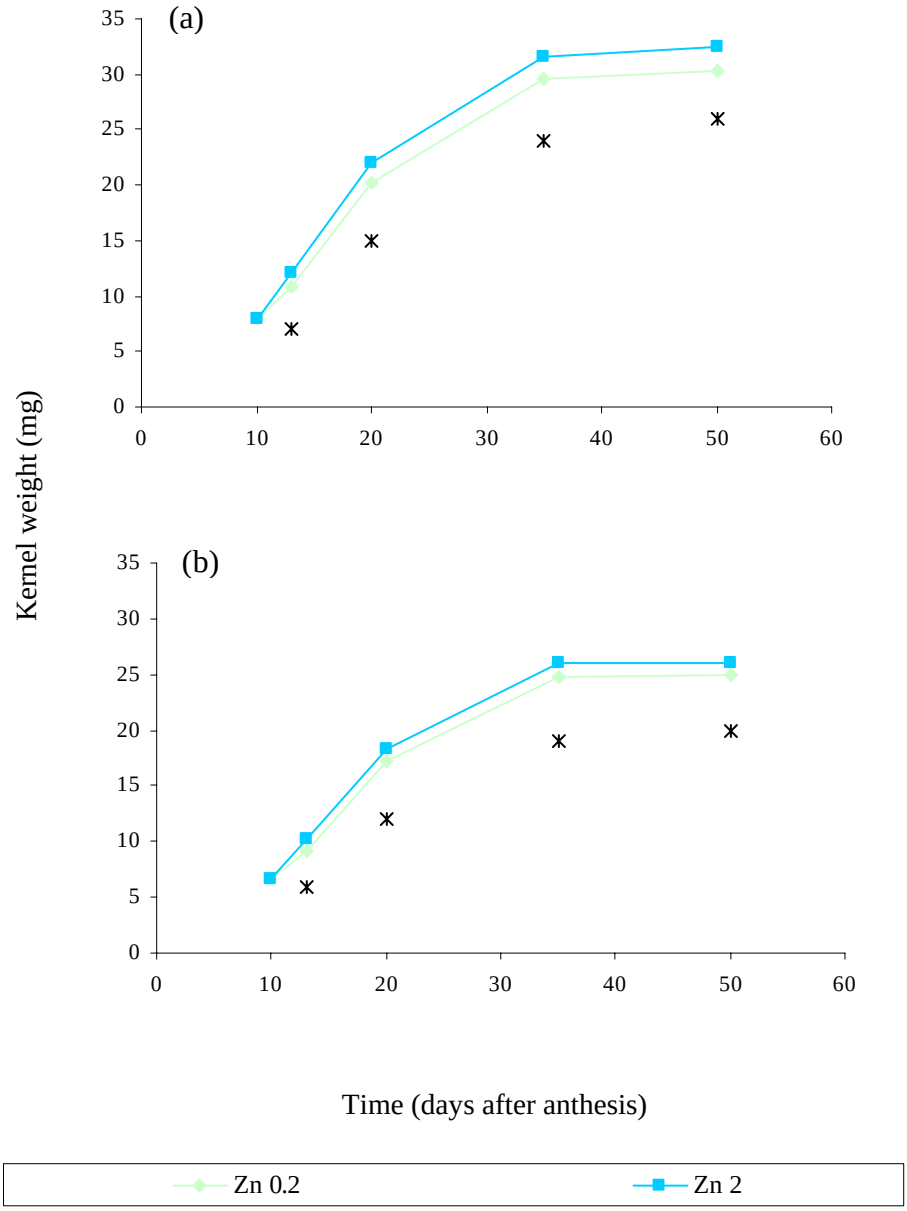


Figure 6.12. Effects of zinc fertilisation (mg kg^{-1} soil) on the individual kernel mass (a, grains of the *a* and *b* florets of the central spikelets of the main culm ear; b, all grains of the main culm ear) of wheat from 9 DAA. Asterisks indicate a significant difference; P < 0.05.

6.3.9 Grain yield

Neither the high temperature treatment nor the Zn fertilisation treatment had any effect on the grain yield of wheat plants in this experiment (Table 6.4). However the three-day exposure to high temperature from 10 DAA did significantly reduce the grain yield of the main culm in two of the four varieties, Goldmark and Meering, by 21 and 22%, respectively (Table 6.5). Zn deficiency also reduced the grain yield of the main culm, by an average of 11%, with no significant difference between genotypes (Table 6.5). There was no significant interaction between Zn fertilisation and temperature treatment on grain yield.

6.3.9.1 Kernel weight

Mature individual kernel weight was reduced by the three-day exposure to high temperature by 15% in Goldmark and 17% in Meering (Table 6.4). There was no significant effect of high temperature on the kernel weight of Frame or Halberd, however. When the grains of the main stem were considered alone a similar result was observed, with kernel weight reduced by the high temperature treatment by 14% in Goldmark and 17% in Meering (Table 6.5). Zn deficiency had no effect on the weight of kernels in the whole plant, but significantly reduced the grain weight of main stem kernels by 4%, with no significant difference between varieties (Table 6.5). Again there was no significant interaction between Zn fertilisation and temperature treatment on kernel weight.

6.3.9.2 Kernel number

The number of kernels per plant was unaffected by the high temperature treatment or Zn fertilisation (Table 6.4), but the number of grains per main stem ear was significantly reduced by Zn deficiency in all varieties, by an average of 7% (Table 6.5). This reduction in kernel number under low Zn fertilisation was due to both a decrease in the number of spikelets per spike (Figure 6.13) and a reduced number of grains per spikelet (Figure 6.14).

Table 6.4. Effects of temperature treatment and Zn fertilisation (mg kg^{-1} soil) on the grain yield (mg $plant^{-1}$), kernel weight (mg) and kernel number of wheat plants.

Genotype	Grain Yield (mg $plant^{-1}$)			Kernel Weight (mg $plant^{-1}$)			Kernel Number (per plant)		
	22/16ºC	**40/20ºC**	***Mean***	**22/16ºC**	**40/20ºC**	***Mean***	**22/16ºC**	**40/20ºC**	***Mean***
Frame	2051	2000	*2026*	27.0	27.8	*27.4*	76.9	75.2	*76.1*
Goldmark	1681	1618	*1649*	25.8	22.0	*23.9*	66.2	74.2	*70.2*
Halberd	1948	1996	*1972*	30.6	30.7	*30.6*	64.2	65.3	*64.8*
Meering	2246	1869	*2057*	24.9	20.6	*22.7*	91.1	91.1	*91.1*
Mean	*1982*	*1870*		*27.1*	*25.2*		*74.6*	*76.5*	
	Zn 0.2	**Zn 2**	***Mean***	**Zn 0.2**	**Zn 2**	***Mean***	**Zn 0.2**	**Zn 2**	***Mean***
Frame	2019	2032	*2026*	27.1	27.7	*27.4*	75.3	76.8	*76.1*
Goldmark	1619	1679	*1649*	24.3	23.5	*23.9*	67.7	72.8	*70.2*
Halberd	1847	2098	*1972*	29.1	32.2	*30.6*	63.8	65.7	*64.8*
Meering	1991	2122	*2057*	22.1	23.4	*22.7*	90.6	91.6	*91.1*
Mean	*1869*	*1983*		*25.6*	*26.7*		*74.3*	*76.7*	
$LSD_{0.05}$									
Genotype	**190**			**1.5**			**7.5**		
Zinc	**n.s.**			**n.s.**			**n.s.**		
Temperature	**n.s.**			**1.1**			**n.s.**		
Genotype x Zinc	**n.s.**			**n.s.**			**n.s.**		
Genotype x Temperature	**n.s.**			**2.2**			**n.s.**		
Zinc x Temperature	**n.s.**			**n.s.**			**n.s.**		
Genotype x Zinc x Temp	**n.s.**			**n.s.**			**n.s.**		
CV (%)	**19.9**			**11.8**			**20.0**		

n.s. = non-significant

Table 6.5. Effects of temperature treatment and Zn fertilisation (mg kg^{-1} soil) on the grain yield (mg plant^{-1}), kernel weight (mg) and kernel number of the main stem of wheat plants.

Genotype	Grain Yield (mg plant^{-1})			Kernel Weight (mg plant^{-1})			Kernel Number (per plant)		
	22/16ºC	**40/20ºC**	***Mean***	**22/16ºC**	**40/20ºC**	***Mean***	**22/16ºC**	**40/20ºC**	***Mean***
Frame	1045	1045	*1045*	25.4	26.2	*25.8*	41.4	40.5	*41.0*
Goldmark	1073	847	*960*	25.7	22.1	*23.9*	42.1	38.7	*40.4*
Halberd	1053	1093	*1073*	29.3	30.1	*29.7*	36.0	36.4	*36.2*
Meering	1097	858	*978*	24.5	20.3	*22.4*	44.8	42.0	*43.4*
Mean	*1067*	*961*		*26.2*	*24.7*		*41.1*	*39.4*	
	Zn 0.2	**Zn 2**	***Mean***	**Zn 0.2**	**Zn 2**	***Mean***	**Zn 0.2**	**Zn 2**	***Mean***
Frame	1036	1053	*1045*	25.5	26.1	*25.8*	40.8	41.3	*41.0*
Goldmark	882	1038	*960*	24.2	23.7	*23.9*	37.0	43.8	*40.4*
Halberd	996	1150	*1073*	28.1	31.3	*29.7*	35.5	36.9	*36.2*
Meering	907	1048	*978*	21.7	23.1	*22.4*	41.5	45.3	*43.4*
Mean	*955*	*1072*		*24.9*	*26.0*		*38.7*	*41.8*	
LSD$_{0.05}$									
Genotype	**72**			**1.4**			**2.8**		
Zinc	**51**			**1.0**			**2.0**		
Temperature	**51**			**1.0**			**n.s.**		
Genotype x Zinc	**n.s.**			**n.s.**			**n.s.**		
Genotype x Temperature	**102**			**2.0**			**n.s.**		
Zinc x Temperature	**n.s.**			**n.s.**			**n.s.**		
Genotype x Zinc x Temp	**n.s.**			**n.s.**			**n.s.**		
CV (%)	**14.3**			**11.4**			**14.1**		

n.s. = non-significant

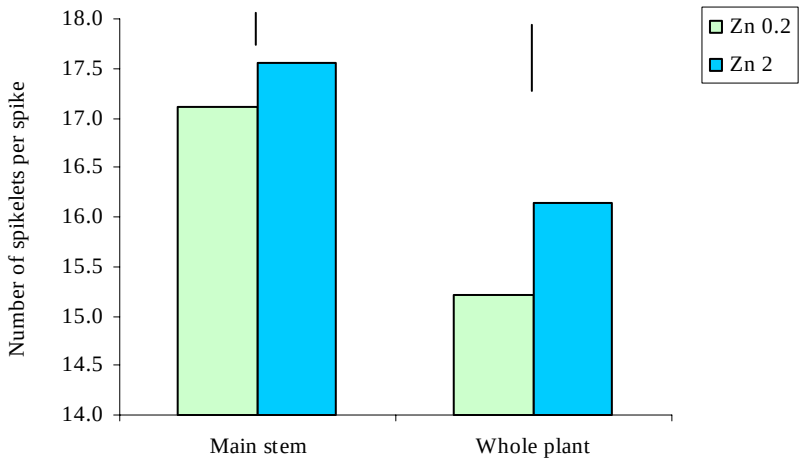


Figure 6.13. Effects of Zn fertilisation (mg kg^{-1} soil) on the number of spikelets per spike of wheat plants. The vertical bar represents the $LSD_{0.05}$ for the main effect of Zinc.

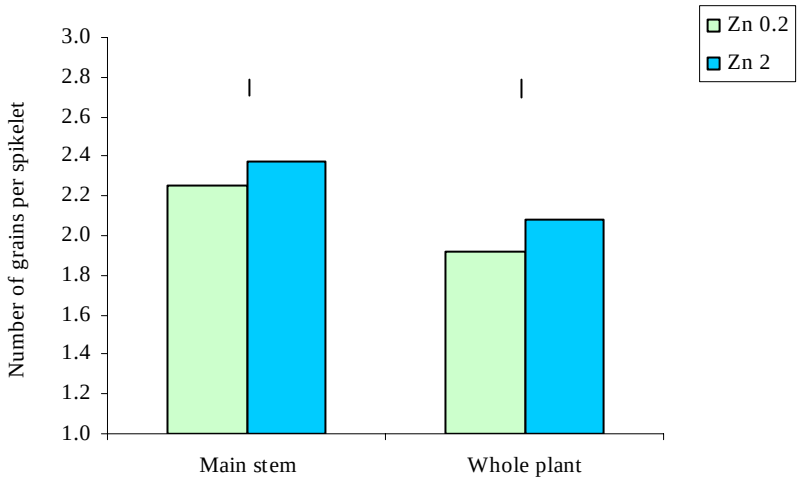


Figure 6.14. Effects of Zn fertilisation (mg kg^{-1} soil) on the number of grains per spikelet of wheat plants. The vertical bar represents the $LSD_{0.05}$ for the main effect of Zinc.

6.3.10 Grain zinc concentration

Concentrations of Zn in the grain were an average 248% higher in plants supplied with adequate Zn than in plants with inadequate Zn (Figure 6.15). Genotypes differed in their grain Zn concentrations, but to a lesser degree when Zn supply was inadequate. Halberd was found to have the highest grain Zn concentration at both low and adequate Zn supply (15.6 and 55.4 mg kg^{-1} respectively), followed by Goldmark (13 and 46.2 mg kg^{-1}), Frame (12.5 and 40.2 mg kg^{-1}) and Meering (11.2 and 39.8 mg kg^{-1}). The critical level of Zn in wheat grain has been reported to be approximately 10 mg Zn kg^{-1} (Riley *et al.* 1992), although a range of 7.6-13 mg Zn kg^{-1} has also been found in Zn deficient wheat plants (Graham *et al.* 1992), while 15-16 mg Zn kg^{-1} has been reported as marginal (Tiwari and Dwivedi 1990).

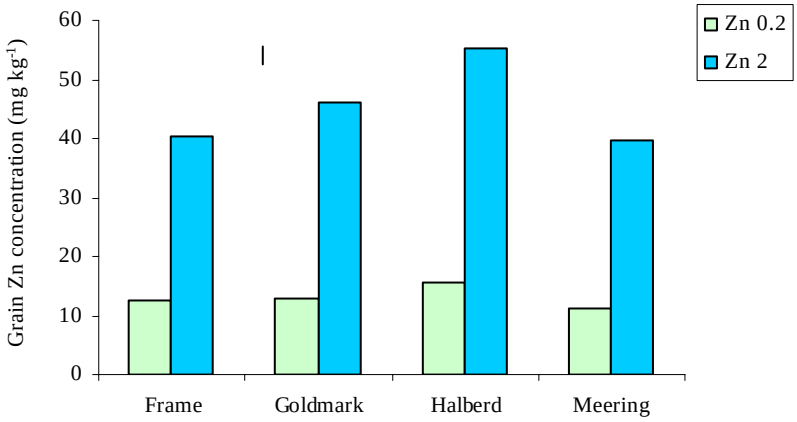


Figure 6.15. Effects of Zn fertilisation (mg kg^{-1} soil) on the grain zinc concentration of wheat genotypes. The vertical bar represents the $LSD_{0.05}$ for the Genotype x Zinc interaction.

Grain Zn concentration was also affected by the three day period of high temperature stress. The concentration of Zn in the grain of Halberd was 19% lower in those plants exposed to the high temperature treatment (Figure 6.16). In Goldmark this result was reversed, with the plants exposed to high temperature having a significantly higher grain Zn concentration. The Zn content of the grain of Goldmark did not differ significantly between temperature treatments however (Figure 6.17), indicating the smaller grain size of the 40/20ºC treatment.

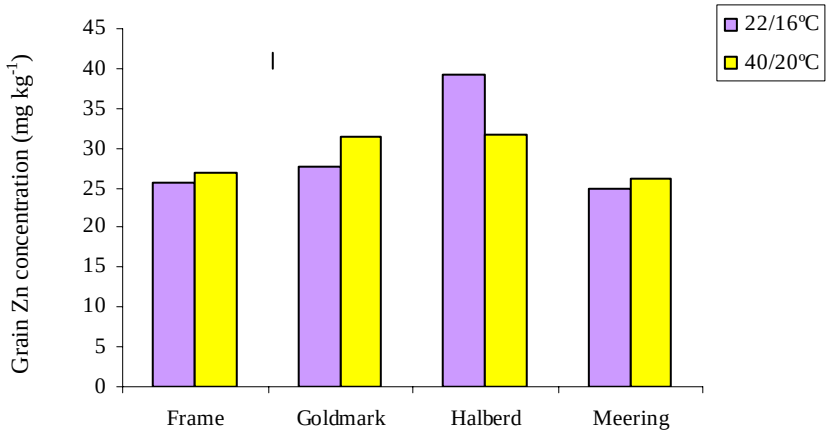


Figure 6.16. Effects of temperature treatment on the grain zinc concentration of wheat genotypes. The vertical bar represents the $LSD_{0.05}$ for the Genotype x Temperature interaction.

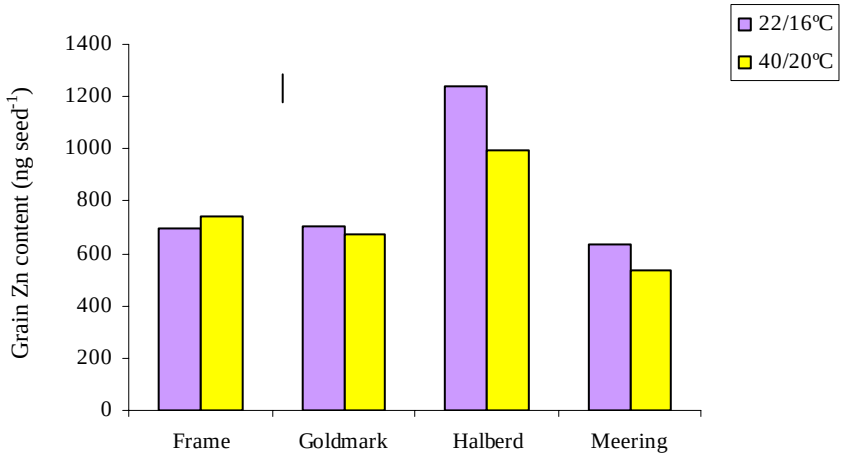


Figure 6.17. Effects of temperature treatment on the grain zinc content of wheat genotypes. The vertical bar represents the $LSD_{0.05}$ for the Genotype x Temperature interaction.

6.4 Discussion

The results of this study demonstrated that the two environmental stresses of low soil Zn availability and high temperature following anthesis can significantly affect the growth and yield of wheat plants, both individually and in combination. Individually, Zn deficiency reduced the rate of grain filling in all four varieties studied, while the 3-day period of high temperature stress significantly reduced photosynthetic activity in all varieties, and the rate and duration of grain filling in two of the four varieties. In combination, Zn deficiency and high temperature stress lowered the chlorophyll maximum fluorescence (Fm) and increased the chlorophyll initial fluorescence (Fo) of wheat leaves significantly further than high temperature stress alone, in three of the four varieties studied. This significant interaction between Zn fertilisation and temperature treatment on chlorophyll fluorescence during grain filling again indicates that Zn can play a role in maintaining photosynthetic activity during a period of heat stress, as indicated in the seedling experiments of Chapter 4.

Zinc deficiency reduced the flag leaf area in only one of the varieties studied, Meering, and did not affect the total dry matter production of any variety. This result, together with the observed Zn concentration in the YEBs at 38 DAS of 16.8 mg Zn kg^{-1}, and grain Zn concentrations of 11.2–15.6 mg Zn kg^{-1}, indicates that the plants fertilised with 0.2 mg Zn kg^{-1} soil in this experiment were grown under conditions of marginal, rather than severe, Zn deficiency.

Grain yield is known to be depressed to a relatively greater extent by Zn deficiency than total dry matter production (Marschner 1995). The present results demonstrated that grain yield reduction in the main stem under low Zn supply was due both to a decrease in grain weight and to a decrease in the number of grains per spike. It was observed that Zn deficiency reduced the rate of dry matter accumulation in the grain between 10 and 13 DAA. This period is within the phase of cell division, when the maximum number of endosperm cells is fixed. There is a high degree of association between endosperm cell number and final grain weight (Brocklehurst 1977, Jenner *et al.* 1991). The reduction in the rate of grain filling by Zn deficiency during the cell division phase in the present experiment resulted in a lower kernel weight from this period onward.

Furthermore the amount of substrate available for grain filling is also limited by Zn deficiency, resulting in grains being less well filled than with adequate Zn (Rengel and Graham 1995b). Carbohydrates are known to accumulate in the leaves of Zn-deficient plants as a result of lowered sink activity (Marschner 1995), and are also preferentially partitioned to the roots to increase the surface area available for Zn uptake (Pearson and Rengel 1997). This may explain, in part, the lower harvest index of the plants grown under low Zn supply. The net result is fewer small grains produced with $Zn_{0.2}$ fertilisation for approximately the same amount of dry matter production.

The main contributor to the reduced grain yield of the main stem under low Zn supply in the present experiment was a smaller number of grains per spike. This was due both to a lower number of spikelets per spike and a reduced number of grains per spikelet. This result supports that of the field experiments (Chapter 5), where yield increases in response to Zn fertilisation were due to an increase in the number of grains per m^2, rather than an increase in grain weight. As discussed in Chapter 5, a decrease in the number of spikelets per spike has been previously described under Zn deficiency (Sharma *et al.* 1979), and can be attributed to a lack of Zn nutrition in the period between single ridge and stem elongation, when spikelets are being formed. Zinc deficiency also affects a plant's ability to initiate flower buds and set seed (Rengel and Graham 1995b), which results in a reduced number of grains per spikelet (Sharma *et al.* 1979).

Grain yield of the main stem was reduced by the 3-day exposure to high temperature from 10 DAA in two of the four wheat varieties studied, Goldmark and Meering. This is in agreement with the field experiments of 1998 (Chapter 5), which showed that the grain yield of Meering was reduced at the warmer sites, whereas the yield of Halberd, the thermotolerant genotype, was more stable between sites. In the present experiment the decrease in grain yield was a result of a reduced kernel weight under high temperature; kernel number was unaffected by the temperature treatment. Since the period of 1-7 DAA is the time after anthesis during which grain number is the most heat sensitive (Tashiro and Wardlaw 1990a,b), floret and grain abortion have been avoided in the present experiment by starting the heat treatment at 10 DAA.

Large varietal differences in the yield response of wheat to periods of high to very high temperature have been widely reported (Sofield *et al.* 1977, Wardlaw *et al.* 1989a, Al-Khatib and Paulsen 1990, Hunt *et al.* 1991, Stone and Nicolas 1995a, Wardlaw and Moncur 1995, Fokar *et al.* 1998). Individual kernel weight in the main stem and whole plant was reduced by the high temperature treatment by 17% in Meering and 15% in Goldmark, while Frame and Halberd were unaffected. In a similar study of 75 wheat cultivars subjected to a high temperature treatment of 40/15ºC day/night for 3 days from 10 DAA, Stone and Nicolas (1995a) describe a similar result in that Meering showed a decrease in kernel mass of 20% from the control plants grown at 20/15ºC, whereas Halberd did not show a significant decline in kernel weight.

The reduction in the mature kernel weight of Goldmark and Meering in the current experiment was a result of a reduction in both the rate and duration of grain filling. This is consistent with the findings of Stone and Nicolas (1995b) who reported a significant reduction in the rate and duration of grain filling of a heat sensitive variety, Oxley, when subjected to a 5-day high temperature treatment of 40/19ºC from 15 DAA. In the current experiment both Meering and Goldmark showed a reduced rate of grain filling from 20-35 DAA compared with the control, and a cessation of growth from approximately 35 DAA. This is also consistent with the reports of Stone and Nicolas (1995b), who describe a reduced rate of grain filling of Oxley from 15-25 DAA, and no significant addition to grain dry matter after 30 DAA.

While both the rate and duration of grain filling were reduced under high temperature in Goldmark and Meering, these were unaffected by the high temperature treatment in Frame. In the other heat tolerant variety, Halberd, however, the rate of grain filling was slowed by the high temperature treatment, but this was compensated for by an increase in grain filling duration. Consequently the mature kernel weight of Halberd was unaffected by high temperature. This is also in agreement with the study by Stone and Nicolas (1995b), who found that the duration of grain growth of the heat tolerant variety, Egret, was less affected than that of the heat sensitive variety, Oxley. Stone and Nicolas (1995b) conclude that although changes in the rate of grain growth are significant, they are not as important as changes in the duration of grain filling in

explaining final grain weight under high temperature conditions. A similar conclusion was drawn by Hunt and co-workers (1991).

The present experiment has provided an opportunity to compare and contrast the responses of photosynthesis and grain development to a period of high temperature early in grain filling. Whereas photosynthetic activity declines rapidly during heat stress but recovers rapidly to pre-stress levels following removal of the stress, the response of kernel weight is quite different, and shows a delayed response that persists well after the stress has ended. The rate of grain growth in the heat-treated plants of Meering and Goldmark was reduced after, rather than during, the application of heat stress and resulted in a lower grain weight. This is consistent with other results (Jenner 1991). It is commonly accepted that endosperm cell weight, rather than cell number, is reduced by high temperature (Hoshikawa 1962, Wardlaw 1970, Nicolas *et al.* 1984), although these studies have only observed endosperm cell numbers under moderately high temperatures (27-30ºC). It is also possible that the generation of endosperm cells is limited under higher temperature regimes during the cell division phase (up to 20 DAA), as in shading and drought experiments (Nicolas *et al.* 1984, Jenner *et al.* 1991).

Genotypic differences in the rate of grain growth under high temperatures in wheat have been generally related to differing sensitivities of starch synthesising enzymes, particularly soluble starch synthase (Caley *et al.* 1990, Jenner 1991, Jenner 1994). However studies of the effect of high temperature on the photosynthetic apparatus suggest that the availability of photosynthate may also play a role in the response of the grain to high temperature (Al-Khatib and Paulsen 1984, 1990, Blum *et al.* 1994, Reynolds *et al.* 2000). Moffatt *et al.* (1990) showed that the productivity of six wheat cultivars grown under controlled environment conditions was directly related to the stability of the photosynthetic apparatus during a high temperature treatment of 37/25ºC from 5 DAA. It is possible however, that these are correlated responses to high temperature stress, and that both the production of photosynthate and the conversion of sucrose to starch are affected under elevated temperatures. Since the deprivation of assimilates in wheat does not account for the reduction in kernel weight under high temperature conditions (Wardlaw *et al.* 1980, Nicolas *et al.* 1984, Bhullar and Jenner

1986), it appears that the reduction in activity of soluble starch synthase is the major limitation to grain filling in wheat during a period of heat stress.

Disturbance of photosynthetic activity was again assessed in this experiment by chlorophyll fluorescence yield, a sensitive indicator of changes in thylakoid membrane integrity caused by environmental stress (Schreiber and Berry 1977). The chlorophyll fluorescence ratio, Fv/Fm, was significantly lowered by high temperature in all varieties in the present experiment, when measured on flag leaves during exposure to the heat treatment of 40/20ºC, and up to 24 h after this exposure. This decrease in Fv/Fm was greatest in the thermosensitive variety, Meering, and lowest in the thermotolerant variety, Halberd. As discussed above, Meering was also found to have the largest decrease in grain weight due to the high temperature treatment, while the grain weight of Halberd was unaffected by high temperature. In a study of 10 genotypes from major world wheat producing regions grown under moderately high temperature (32/27ºC, day/night) from anthesis to maturity, Al-Khatib and Paulsen (1990) found that genotypes that were most tolerant of high temperature had stable rates of photosynthetic activity, high kernel weights and high harvest indices, all of which were evident in Halberd in the present experiment.

Chlorophyll fluorescence parameters have been used previously to distinguish high temperature tolerance among wheat genotypes (Moffatt *et al.* 1990, Al-Khatib and Paulsen 1990). The greater heat tolerance of Fv/Fm in Halberd in the present experiment was due to a more stable Fo under heat stress. Since an increase in Fo by heat stress can be attributed to a separation of the chlorophyll *a/b* light harvesting complex of PSII (LHCII) from the PSII core complex (Enami *et al.* 1994, Yamane *et al.* 1997), it is possible that the thermostability of photosynthetic activity in Halberd may involve a more stable binding of LHCII to the core PSII complex. Conversely, the greater heat sensitivity of Fv/Fm in Meering appeared to be due to an effect on Fm, suggesting that the greater thermosensitivity of this genotype may be due to a greater thermosensitivity of the chlorophyll proteins (Yamane *et al.* 1997).

Zinc deficiency and heat stress in combination was found to lower the Fv/Fm ratio

further than heat stress alone in all varieties during the first day of the high temperature treatment. This was due to a reduction in Fm, rather than an increase in Fo, and resulted in a corresponding decrease in the Fv component of chlorophyll fluorescence. A decline in Fm under heat stress has been attributed to denaturation of the chlorophyll-protein complexes (Yamane *et al.* 1997). It is feasible that adequate Zn fertilisation can restrict the decrease in Fm during heat stress, possibly through the role of Zn as a component of the Cu/Zn-superoxide dismutase (Cu/Zn-SOD) enzyme. This enzyme is important in the detoxification of reactive oxygen species (ROS), which are produced to excess under stress conditions, and can damage many cellular components, including proteins and chlorophyll. In addition, tolerance of photosynthetic activity to high temperature stress has previously been reported to be associated with an increase in Cu/Zn-SOD expression (Sen Gupta *et al.* 1993a,b).

In the present experiment the protective effect of Zn on the Fv/Fm ratio continued beyond the first day of high temperature stress (10 DAA) in three of the four varieties studied. At 12 DAA, Fv/Fm was significantly lower in the Zn deficient plants of Frame and Halberd under high temperature stress, and a similar trend was observed in Goldmark. However, in contrast to the situation at 10 DAA, the reduction in Fv/Fm under Zn deficiency and high temperature at 12 DAA was primarily due to an increase in Fo, rather than any effect on Fm. Physical dissociation of the LHCII from the PSII core complex, which results in an increase in Fo, is associated with increases in the fluidity of the thylakoid membrane lipids at elevated temperatures (Armond *et al.* 1980, Berry and Björkman 1980, Gounaris *et al.* 1984, Sundby *et al.* 1986). It is therefore feasible that adequate Zn supply can limit the increase in Fo during heat stress through its role in the preservation of membrane integrity. A number of mechanisms can be hypothesised for this, including (1) the physical presence of Zn as a stabilising influence on membrane components; (2) the role of Zn as a component of the Cu/Zn-SOD enzyme, since ROS also have the potential to damage cellular membranes; or (3) the inhibitory effect of Zn on the generation of ROS by NADPH-oxidase (Chvapil 1973, Von Glós and Boursnell 1981, Cakmak and Marschner 1988b,c).

Interestingly in the most heat-sensitive variety, Meering, the Fv/Fm ratio at 12 DAA

was significantly lower in the plants supplied with adequate Zn, than in the Zn deficient plants, under heat stress conditions. This again suggests that Meering is unresponsive to Zn fertilisation, as observed in the lack of an increase in canopy temperature depression with Zn fertilisation, as discussed in Chapter 5. Further work is required to elucidate this unexpected response of lowered chlorophyll fluorescence in the leaves thermosensitive Meering under heat stress but adequate Zn nutrition.

Flag leaf chlorophyll content was reduced by high temperature in all varieties, and markedly reduced under heat stress and Zn deficiency in the Zn inefficient variety, Goldmark. The results suggest that adequate Zn nutrition can limit the loss of chlorophyll in wheat leaves following a high temperature event, at least in a Zn inefficient variety. A loss of chlorophyll in plants subjected to heat stress is associated with irreversible damage to the photosynthetic apparatus (Al-Khatib and Paulsen 1984, Harding *et al.* 1990a, Havaux and Tardy 1999), which seems to have been exacerbated in Goldmark by low Zn fertilisation. As outlined above, adequate Zn fertilisation may reduce the damage to the photosynthetic apparatus under heat stress through its various roles in maintaining membrane stability.

While high temperature was found to hasten flag leaf senescence in three varieties, Halberd, Meering and Goldmark, Zn deficiency was observed to delay senescence from 31 DAA in all varieties. This may be due to the fact that senescence is a co-ordinated, deteriorative growth process, involving the systematic breakdown of chloroplast proteins (Camp *et al.* 1982, Peoples and Dalling 1988, Davies *et al.* 1990). Environmental conditions that influence photosynthesis have been found to interfere with senescence and protein catabolism in wheat leaves (Ferguson *et al.* 1993, Herrman and Feller 1998), and it is possible that Zn deficiency may disrupt some aspects of normal senescence. Not only is there an impaired export of photosynthates under Zn deficiency as a result of low sink activity (Marschner and Cakmak 1989), but Zn is also the metal component of the carboxypeptidase enzyme, an enzyme involved in the degradation of proteins during senescence (Peoples and Dalling 1988, Marschner 1995), and thus may cause the delay in flag leaf senescence seen in the present experiment.

Table 6.6. Effects of zinc deficiency and 3 days of high temperature from 10 DAA on the physiology of a Zn inefficient wheat variety, Goldmark.

	Zinc deficiency ONLY (Low temperature)	Zinc deficiency AND Heat stress	Heat stress ONLY (Adequate zinc)
Chlorophyll fluorescence			
Fv/Fm		↓↓	↓
Fo		↑↑↑	↑
Fm		↓↓	↓
Fv		↓↓	↓
Chlorophyll content		↓↓↓	↓
Grain filling and yield			
Rate of grain filling	↓	↓	↓
Duration of grain filling		↓	↓
Harvest index	↓	↓	↓
Kernel number	↓	↓	
Kernel weight	↓	↓	↓
Grain yield	↓	↓	↓

NB: The number of arrows is indicative of the magnitude of the response.

6.5 Conclusion

The results of this experiment suggest that the two environmental stresses of heat stress and Zn deficiency act independently on photosynthetic activity and grain growth. The improvement in chlorophyll fluorescence under heat stress conditions with adequate Zn fertilisation observed at the seedling stage (Chapter 4) was reproduced in this experiment during grain filling. However supplementary Zn did not prevent the decline in kernel weight or grain yield in the main stem of the Zn inefficient or thermosensitive genotypes under high temperature. Nevertheless, the results suggest that the detrimental effects of low Zn availability and heat stress, particularly in Zn inefficient cultivars, will be most damaging when these stresses occur in combination (Table 6.6). Further work is necessary to determine the mechanism(s) by which elevated Zn nutrition can provide the photosynthetic apparatus with tolerance to high temperature stress.

CHAPTER 7

EFFECT OF ZINC SUPPLY AND ELEVATED TEMPERATURE ON THE GRAIN QUALITY OF WHEAT: PROTEIN CONTENT AND PROTEIN COMPOSITION

7.1 Introduction

Wheat quality can be defined as its ability to produce a product that is suitable for the end-user, and is determined by a number of variables, including grain size, protein percentage and composition, and starch content and composition (Panozzo and Eagles 2000). Environmental conditions exert a significant influence on these end-use quality characteristics, with several studies showing that environmental variation associated with quality traits often exceeds genotypic variation (Lukow and McVetty 1991, Peterson *et al.* 1992, Borghi, *et al.* 1997, Panozzo and Eagles 2000).

Proteins are the most important components of wheat grains governing baking quality, and variations in both protein content and composition significantly modify flour quality for breadmaking (Weegels *et al.* 1996). Moderately high temperatures during grain growth (*ca* 25-32ºC) tend to improve the quality of wheat for breadmaking through a suppression of starch synthesis and corresponding rise in grain protein concentration (Bhullar and Jenner 1985, Jenner *et al.* 1991, Keeling *et al.* 1993), which results in an improvement in important characters such as dough strength, extensibility and loaf volume (Randall and Moss 1990, Rao *et al.* 1993, Correll *et al.* 1994, Wrigley *et al.* 1994). By contrast, very high temperatures (*ca* 33-40ºC) tend to reduce the breadmaking quality of wheat despite the increase in grain protein percentage (Randall and Moss 1990, Blumenthal *et al.* 1991b, Borghi *et al.* 1995, Corbellini *et al.* 1998).

Glutenins and gliadins are the major components of the storage protein in wheat and make a significant contribution to dough rheology and baking quality (Payne 1987, Weegels *et al.* 1996). The proportions of both components are influenced by both

genotype and environment. At high temperatures (eg. maximum daily temperatures >35ºC) the percentage of gliadin in total protein increases with increasing flour protein, resulting in an increase in the gliadin:glutenin ratio, and a corresponding increase in dough extensibility and a decrease in dough strength (Blumenthal *et al.* 1993, Daniel and Triboï 2000, Panozzo and Eagles 2000). High temperatures have been found to decrease the proportion of high molecular weight (HMW) glutenins in grain protein, which also results in a decrease in dough strength (Blumenthal *et al.* 1994, 1995a, Ciaffi *et al.* 1996, Wardlaw *et al.* 2002).

Another environmental factor known to produce modifications of wheat grain quality is soil fertility. Hemantaranjan and Garg (1988) have shown that increasing supplies of iron (Fe) and zinc (Zn), up to an optimum concentration, will significantly increase the starch and protein content of wheat grains, while Sadras *et al.* (2002) have reported a 0.26% increase in grain protein concentration with every mg of Zn per kg of topsoil. Huebner and Bietz (1988) were among the first to suggest that soil mineral composition could cause quantitative differences in gliadin synthesis, while Bonfil *et al.* (1997) found that various deficiencies of phosphorus (P), potassium (K) or magnesium (Mg) could alter the percentage of HMW glutenins within the wheat seed, with little change in total grain protein content.

While only a few studies have considered the relationship between soil nutrient availability and grain protein composition, still fewer have examined that between grain/flour nutrient concentration (with the exception of N and S), and protein composition. An early study by Bequette and colleagues (1963) showed that the concentration of P and Mn in the grain were negatively correlated with baking quality, while calcium (Ca) exerted a positive influence on baking quality. Another study conducted by Douglas and Dyson (1985) also found a negative relationship between P and baking quality, as well as negative relationships between K, Mg and molybdenum (Mo) and baking quality.

It has been suggested that nutrient deficiency may affect a number of processes involved in the accumulation of grain storage proteins, such as folding, assembly,

transport or deposition (Bonfil *et al.* 1997). Further evidence for the role of Zn in the accumulation of wheat grain proteins has been provided by the labelling studies of Starks and Johnson (1985), which showed that the majority of ^{65}Zn applied at anthesis is incorporated into grain protein, and furthermore, that the glutenins have the greatest level of ^{65}Zn incorporation. It therefore appears that Zn is in some way associated with the seed storage proteins of wheat, and it can be hypothesised that Zn may be able to limit some of the deleterious alterations to protein composition that occur under high temperature during grain filling, perhaps through its essential role in protein synthesis (Marshner 1995).

In an earlier study (Chapter 5), a number of wheat genotypes differing in Zn efficiency and thermotolerance were grown at six field sites where different levels of Zn deficiency and high temperature stress were encountered. These experiments produced grain with a wide range of Zn concentrations, under a number of different temperature regimes. This grain was analysed for protein content and composition in the study described here, which had the following aims: (1) to assess what effect various grain Zn concentrations have on the protein content and composition of wheat grain; (2) to determine whether Zn nutrition can affect the increase in the gliadin:glutenin ratio that occurs under high temperature; and (3) to ascertain whether the decrease in the proportion of HMW glutenins caused by high temperature during grain filling can be altered by Zn nutrition. Furthermore, the protein content and composition of grain produced under controlled environment conditions (Chapter 6) was also analysed in the present study, in order to assess these attributes of grain quality in grain generated under more precise conditions of high temperature and Zn deficiency.

7.2 Materials and methods

7.2.1 Cultivars

Four bread wheat cultivars were chosen for analysis of grain protein content and composition on the basis of previously reported differences in their Zn efficiency or

thermotolerance (Chapter 3, Section 3.2). These cultivars were Frame (moderately Zn efficient), Goldmark (Zn inefficient), Halberd (heat tolerant) and Meering (heat sensitive). Further information regarding the Zn efficiency and thermotolerance of these varieties has been derived from previous field and controlled environment studies (Chapters 5 and 6). Frame appears to be a relatively thermotolerant cultivar, based on its photosynthetic activity and kernel weight under high temperature conditions, while Goldmark appears to be moderately thermosensitive with respect to chlorophyll content and grain weight. Both Meering and Halberd appear to be Zn efficient genotypes with respect to grain yield, with Halberd also showing Zn efficiency with respect to shoot growth and canopy temperature depression. The HMW (*Glu-1*) allelic compositions of these cultivars are given in Table 7.1, using the designations of Payne and Lawrence (1983).

Table 7.1 Allelic compositions of the *Glu-1* locus (after Payne and Lawrence 1983) of the cultivars analysed in these experiments.

Cultivar	*Glu-A1*		*Glu-B1*		*Glu-D1*	
	Allele	**Subunits**	**Allele**	**Subunits**	**Allele**	**Subunits**
Frame	a	1	b	7+8	d	5+10
Goldmark	b	2*	i	17+18	a	2+12
Halberd	a	1	c, e	7+9, 20	d	5+10
Meering	b	2*	b	7+8	a	2+12

7.2.2 Growth conditions

7.2.2.1 Field experiments

The four cultivars were grown at six sites across South Australia and Victoria in 1998 and 1999. A combination of location, sowing time and Zn fertilisation was used to obtain various environments, which ranged in yield from 2.21 to 3.87 t ha^{-1}. Details of the environments and experimental designs were given in Chapter 5.

7.2.2.2 Controlled environment experiment

The four wheat cultivars were also grown to maturity under controlled environment conditions, with two Zn treatments applied as 0.2 and 2 mg Zn kg^{-1} soil and designated as $Zn_{0.2}$ and Zn_2, respectively. A high temperature treatment of 40/20ºC was also applied for 3 days, beginning at 10 days after anthesis. Control plants were maintained at 22/16ºC throughout the experiment. Further details of plant culture and the experimental design were presented in Chapter 6.

7.2.3 Grain protein concentration

Grain samples from the field experiments of approximately 10 g were milled in a UDY cyclone sample mill (UDY Corporation, Fort Collins, CO, USA) equipped with a 0.5 mm screen and wholemeal flour was obtained. The flour protein percentage was determined by a standard micro-Kjeldahl method (Bradstreet 1954, Isaac and Johnson 1976). Flour samples of approximately 0.5 g were digested with 7.5 mL of concentrated sulphuric acid at 390 °C in order to convert the N in the sample into ammonium sulphate. A catalyst tablet containing 3.5 mg of selenium was added to promote the oxidation of the organic matter. The catalyst tablet also contained 3.5 g of potassium sulphate, which raises the temperature of the digest and therefore increases the rate of reaction. Following digestion, 25 mL of reverse osmosis (RO) water was added to each sample. Ammonia was released from the digest by steam distillation with sodium hydroxide using the Kjeltec Auto 1030 Analyzer (Tecator AB, Höganäs, Sweden). Ammonia was collected in a 2% boric acid solution containing 1% of methyl red/bromocresol green indicator solution, which was then titrated with 0.1 M hydrochloric acid. A conversion factor of 5.7 was used to convert the ammonia nitrogen into protein, and this was expressed on flour dry weight basis.

7.2.4 Grain protein composition

Grain protein composition was determined by size-exclusion high-performance liquid chromatography (SE-HPLC), using the two-step method of Gupta *et al.* (1993) and Stone *et al.* (1996). The first step of this method extracts those proteins soluble in dilute SDS (glutenins, gliadins and albumins/globulins), while the second extract contains proteins soluble only after sonication (glutenins and smaller amounts of gliadins, albumins and globulins). For the first extraction, 11 mg of flour was suspended in 1 mL of 0.5% SDS-phosphate buffer (pH 6.9) by stirring with a stainless steel wire and vortexing for 5 s. Samples were then stirred for 5 min at 2000 rpm and centrifuged for 30 min at 10,000 *g* to obtain a supernatant of SDS-extractable protein. This extract was then filtered through a 0.45 µm filter before being used for HPLC analysis. The pellet remaining after the supernatant was removed was then resuspended in 1 mL SDS-phosphate buffer by stirring with a stainless steel wire, and sonicated for 30 s with a Branson Sonifier®, model B-12 (Branson Ultrasonics Corporation, Danbury, CT, USA) at power setting 5, output 40 W. This sample was then centrifuged for 30 min at 10,000 *g* to obtain a supernatant of SDS-unextractable protein, which was filtered through 0.45 µm filter before being used for HPLC analysis.

The HPLC analysis was performed on a Waters® Protein Pak 300 sw column (Millipore Corporation, Milford, MA, USA) using a Waters® liquid chromatography system equipped with two model 510 pumps, a WISP model 712 automatic sampler and a Lambda-Max model 481 UV-visible detector. Pump control and data acquisition were achieved using Waters Millennium® 32 software package. Separation was attained in 40 min by loading 20 µL of sample into an eluent of 50% (v/v) acetonitrile and water containing 0.1% (v/v) trifluroacetic acid at a flow rate of 0.5 mL min^{-1}. Proteins were detected by UV absorbance at 214 nm.

The elution profile was divided into three main peaks (Figure 7.1). Peak I contained mainly glutenin plus a small amount of aggregated albumin and globulin, peak II contained mainly gliadin, while peak III contained mainly albumin and globulin (Batey *et al.* 1991, Gupta *et al.* 1993, Jia *et al.* 1996). Furthermore, the SDS-unextractable

protein fraction has been shown to correlate well with the percentage of HMW glutenin subunits present in the wheat grain (Gupta *et al.* 1993, Carceller and Aussenac 1999). The percentages of extractable and unextractable glutenins in the total amount of glutenin were calculated by:

$$\frac{\text{peak I area (extractable fraction)}}{\text{[peak I area (extractable fraction) + peak I area (unextractable fraction)]}} \times 100$$

and

$$\frac{\text{peak I area (unextractable fraction)}}{\text{[peak I area (extractable fraction) + peak I area (unextractable fraction)]}} \times 100$$

respectively (Gupta *et al.* 1993). By dividing peak I area (unextractable) by flour protein percentage, a relative value (arbitrary units) for the content of unextractable glutenins in the total protein was obtained.

Analysis of grain protein composition was restricted to two cultivars only, Goldmark and Frame, due to time constraints. These varieties were shown previously to differ widely in both Zn efficiency and thermotolerance, and were selected on this basis.

7.2.5 Statistical analyses

Results from each site were analysed separately by standard analysis of variance using the routines of the Genstat 5 statistical program (Chapter 3, Section 3.7). Principal component analysis (PCA) was used to classify and group the results from the six field sites in 1998 and 1999. PCA was conducted using a correlation matrix to reduce the variables to a common scale (Manly 1986) and was also performed using the Genstat 5, Release 4.1 statistical package (Lawes Agricultural Trust 1998).

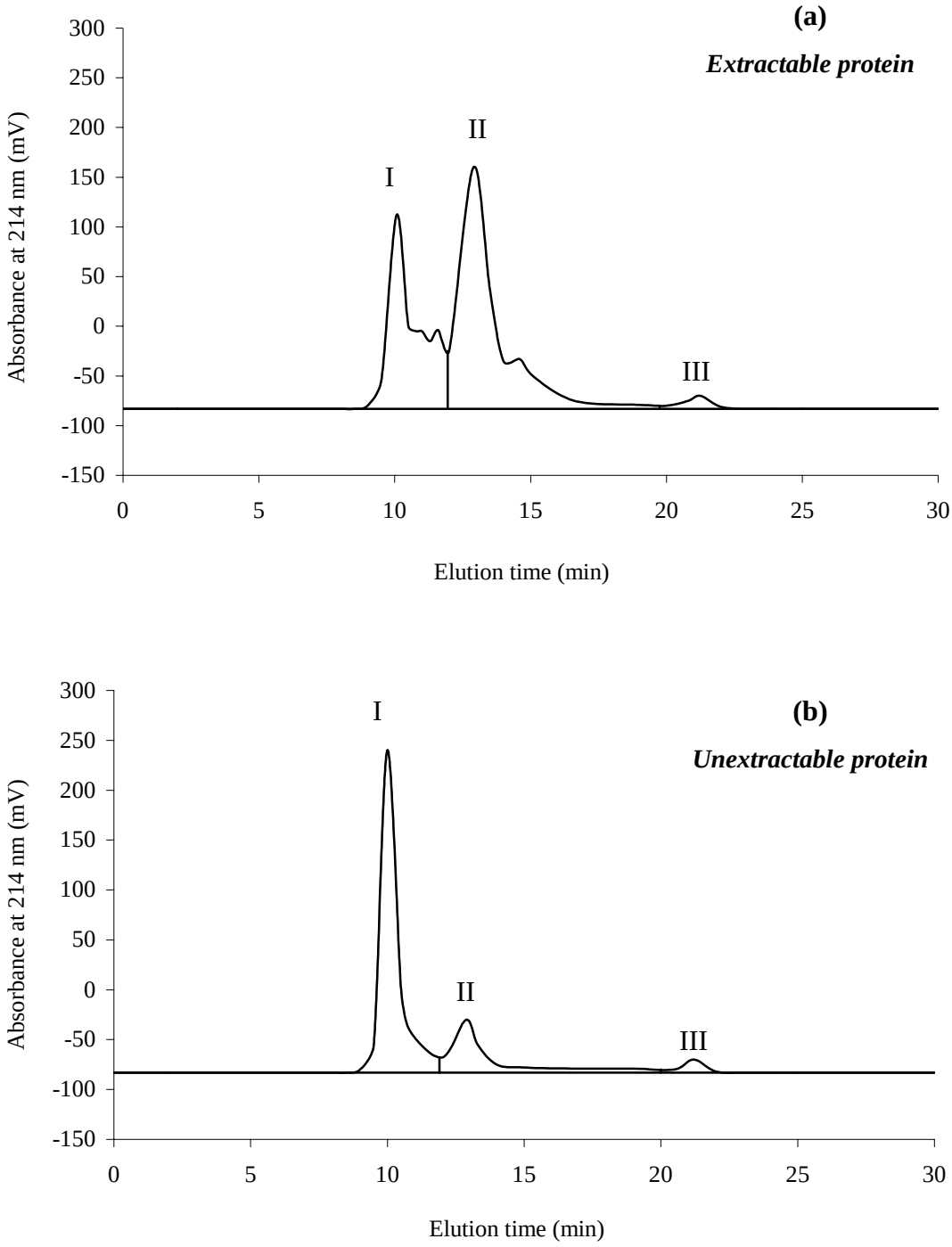


Figure 7.1. Size-exclusion high-performance liquid chromatographic profiles of the wheat cultivar Spear. (a) Wheat storage proteins extracted with 0.5% SDS-phosphate buffer (pH 6.9). (b) Storage proteins extracted with 0.5% SDS-phosphate buffer (pH 6.9) followed by sonication. Chromatograms for extractable and unextractable proteins were divided into 3 peaks, as indicated. Peak I was defined as aggregated glutenin, peak II was defined as monomeric gliadin and peak III as albumins and globulins (Batey *et al.* 1991, Gupta *et al.* 1993, Panozzo and Eagles 2000).

7.3 Results

7.3.1 Flour protein concentration

7.3.1.1 Lameroo, Tintinara and Minnipa, 1998

A combined ANOVA across sites showed that flour protein concentration was significantly higher at Minnipa (site average of 16.5%) than at Lameroo (15.6%), which was significantly higher than Tintinara (14.4%) (Table 7.2). This result correlates well with the higher temperatures recorded during grain filling at Minnipa compared to Lameroo, and at Lameroo compared to Tintinara (Figure 7.2). The mean maximum temperature during grain filling was 23.9ºC at Tintinara, 25.6ºC at Lameroo and 26.6ºC at Minnipa (Chapter 5). Furthermore, the magnitude of this response, illustrated in Figure 7.2, is comparable to other published reports of protein concentration responses to an increase in mean maximum daily temperatures during grain filling (Daniel and Triboi 2000). An analysis of the data of Randall and Moss (1990) revealed a similar linear relationship between grain protein percentage and mean maximum daily temperature after anthesis, for four cultivars of wheat grown at three sites in four seasons, as that obtained in the present experiments.

For the individual varieties in the present experiment there was also a positive correlation between the average maximum daily temperature during grain filling and flour protein percentage, with three of the four varieties being significant at the 10% level (Figure 7.3). It is acknowledged that the statistical power of these regression analyses is diminished due to the small number of data points. Nevertheless, certain trends can be identified. Three varieties, Frame, Halberd and Meering, showed a similar relationship between grain filling temperature and flour protein percentage, while Goldmark appeared quite different, and showed much less of an increase in flour protein concentration with an increase in temperature.

This lack of an increase in flour protein concentration with an increase in temperature during grain filling in Goldamark was also seen in the analysis of variance across sites (Table 7.2). Goldmark a Zn inefficient variety, did not show an increase in flour protein

concentration between sites, while Frame, a Zn efficient variety, showed a large significant increase in flour protein percentage between sites (11% between Tintinara and Lameroo and 10% between Lameroo and Minnipa). Halberd and Meering showed a similar increase in flour protein percentage between Tintinara and Lameroo as Frame, with the exception of the nil Zn treatment, which did not increase between the two sites. Between Lameroo and Tintinara however, the flour protein percentage of Halberd and Meering did not increase significantly, with the exception of the nil Zn treatment, which increased by 8 and 9% for each variety, respectively (Table 7.2).

Table 7.2. Effects of site, genotype and zinc fertilisation (kg ha^{-1}) on the flour protein (%) of wheat grown at Lameroo, Tintinara and Minnipa in 1998.

Genotype	Flour Protein (%)											
	Lameroo				Tintinara				Minnipa			
	0	7.5	22.5	*Mean*	0	7.5	22.5	*Mean*	0	7.5	22.5	*Mean*
Frame	15.9	15.8	15.3	*15.7*	14.3	14.3	13.9	*14.2*	17.1	17.8	16.9	*17.3*
Goldmark	15.0	14.7	14.2	*14.7*	13.9	14.8	13.8	*14.2*	15.3	14.5	15.3	*15.1*
Halberd	15.9	16.0	16.0	*16.0*	14.9	14.6	14.7	*14.7*	17.2	16.9	16.6	*16.9*
Meering	15.5	16.7	16.4	*16.2*	14.8	14.0	14.7	*14.5*	16.9	16.7	16.8	*16.8*
Mean	*15.6*	*15.8*	*15.5*	*15.6*	*14.5*	*14.4*	*14.3*	*14.4*	*16.6*	*16.5*	*16.4*	*16.5*
LSD$_{0.05}$												
Site	**0.9**											
Genotype	**0.5**											
Zinc	**n.s.**											
Site x Variety	**n.s.**											
Site x Zinc	**n.s.**											
Genotype x Zinc	**n.s.**											
Site x Genotype x Zinc												
(a)	**1.1**											
(b)	**0.7**											
(c)	**1.2**											
CV (%)	**2.8**											

(a) For comparisons within the same site and zinc treatment

(b) For comparisons within the same site and genotype

(c) For other comparisons

n.s. = non-significant

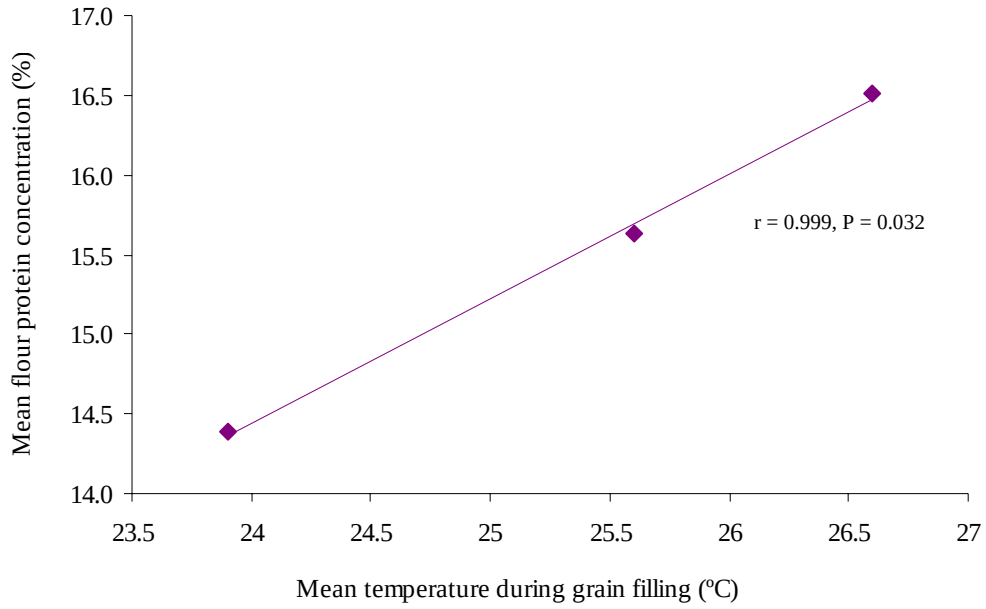


Figure 7.2. Relationship between the mean maximum temperature during grain filling (ºC) and the mean flour protein concentration (%) of wheat genotypes grown at 3 sites in 1998. Each data point is the mean of all genotypes and zinc treatments. Regression equation is presented in Appendix 7.1

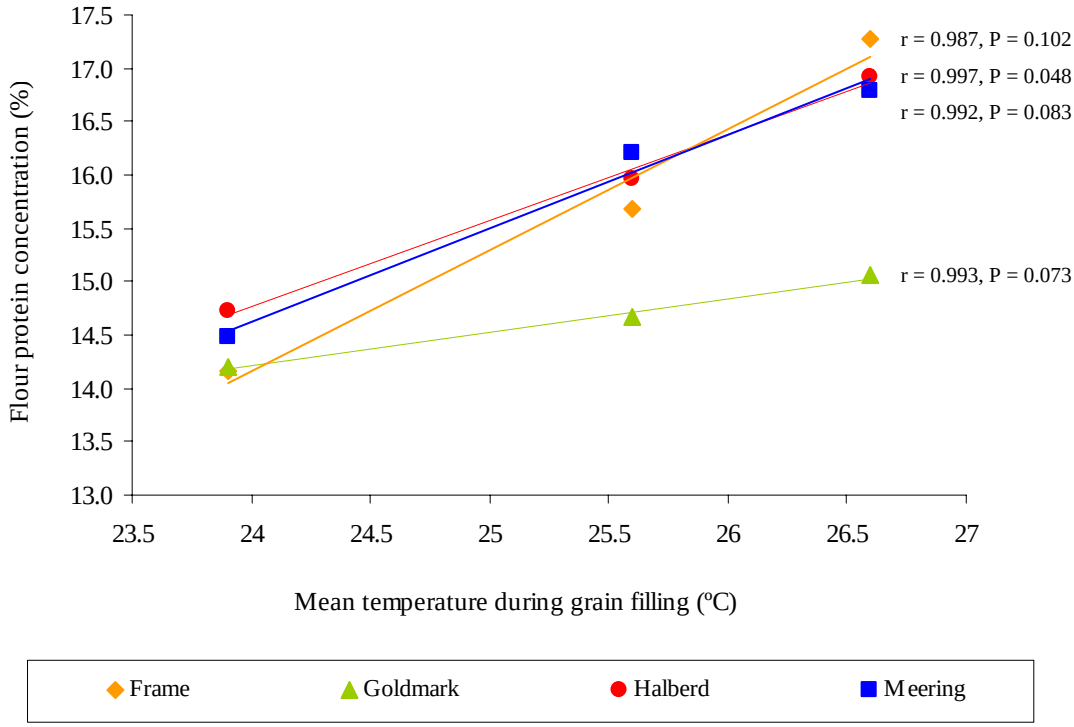


Figure 7.3. Relationship between the mean maximum temperature during grain filling (ºC) and flour protein concentration (%) of four wheat genotypes grown at 3 sites in 1998. Each data point is the mean of all zinc treatments. Regression equations are presented in Appendix 7.1

Flour protein concentration showed a negative correlation with the mean grain yield at each site in 1998 (Figure 7.4). As grain yield increased, flour protein percentage decreased, and this was significant at the 10% level. Frame, a variety with the *Glu-D1d* allele at the Glu-D1 locus, coding for HMW glutenin subunits 5+10, showed a large significant decrease in flour protein concentration for an increase in grain yield (Figure 7.5). Conversely Goldmark, with the *Glu-D1a* allele at the Glu-D1 locus, coding for HMW glutenin subunits 2+12, showed a much smaller significant decrease in flour protein concentration for an increase in grain yield.

Flour protein concentration was not as strongly correlated with kernel weight as with yield (Figure 7.6). A significant decrease in flour protein percentage with an increase in kernel weight was observed in Halberd only (Figure 7.7).

When each site was analysed individually, the only significant effect of Zn fertilisation on flour protein concentration was a significant interaction between genotype and Zn treatment at Tintinara (Table 7.3). The flour protein concentration of the Zn inefficient variety, Goldmark was approximately 7% lower than that of either Halberd or Meering in the treatment that had received no supplementary Zn fertilisation. Furthermore the flour protein concentration of Goldmark increased from 13.9% to 14.8% with the application of either 7.5 kg Zn ha^{-1} at sowing, or 7.5 kg Zn ha^{-1} at sowing plus 2 foliar sprays of Zn.

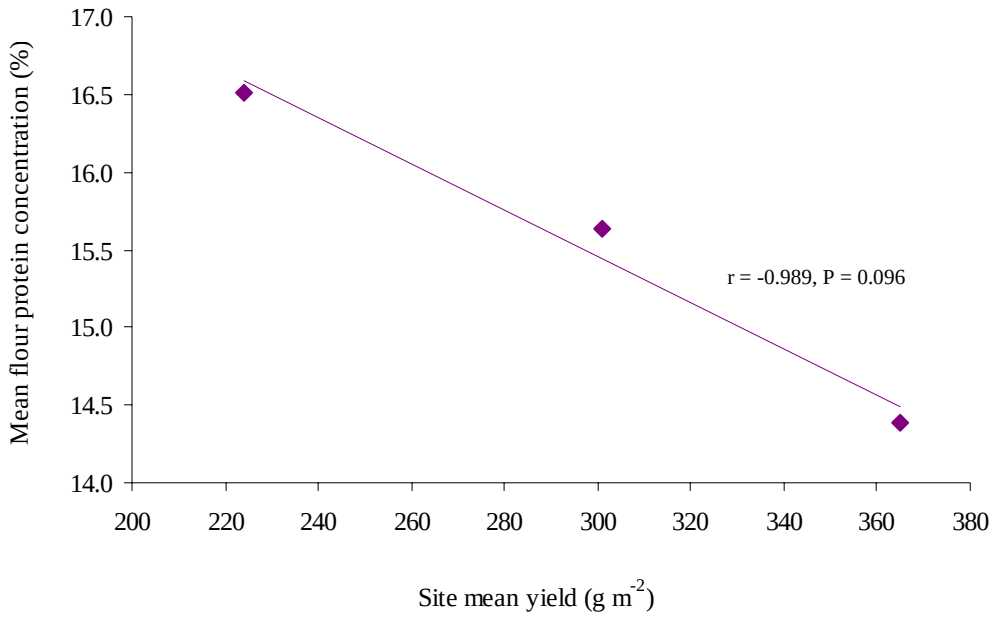


Figure 7.4. Relationship between the mean grain yield (g m^{-2}) and the mean flour protein concentration (%) of wheat genotypes grown at 3 sites in 1998. Each data point is the mean of all genotypes and zinc treatments. Regression equation is presented in Appendix 7.1

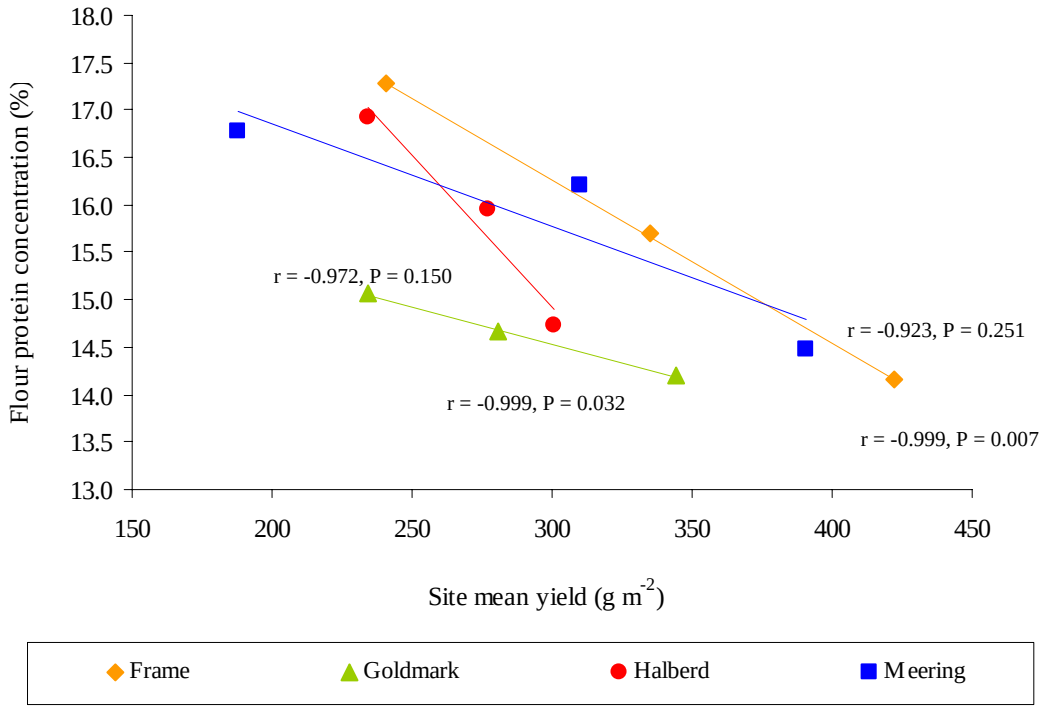


Figure 7.5. Relationship between the mean grain yield (g m^{-2}) and flour protein concentration (%) of four wheat genotypes grown at 3 sites in 1998. Each data point is the mean of all zinc treatments. Regression equations are presented in Appendix 7.1

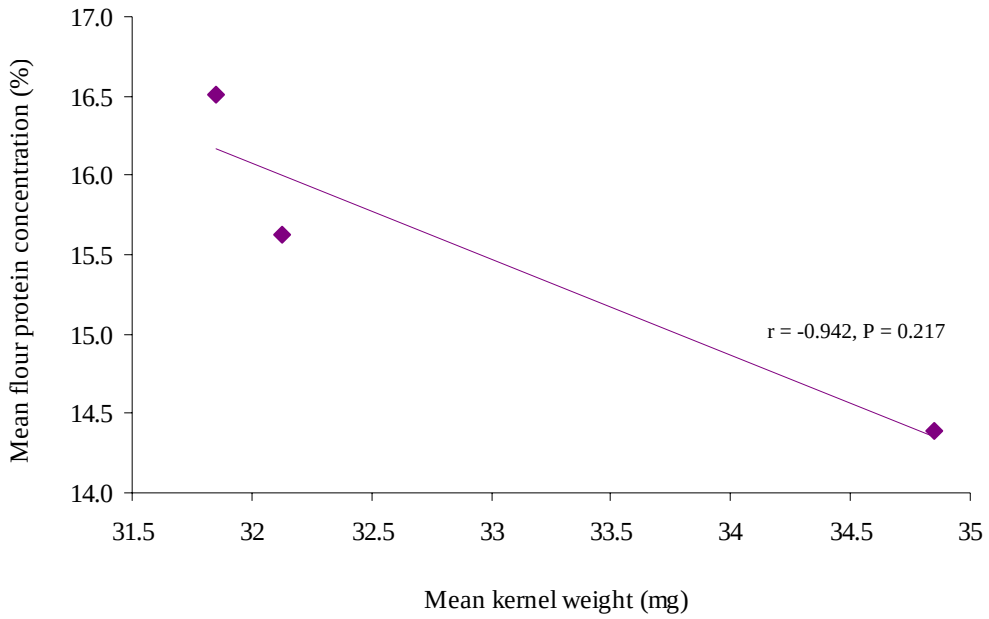


Figure 7.6. Relationship between the mean kernel weight (mg) and the mean flour protein concentration (%) of wheat genotypes grown at 3 sites in 1998. Each data point is the mean of all genotypes and zinc treatments. Regression equation is presented in Appendix 7.1

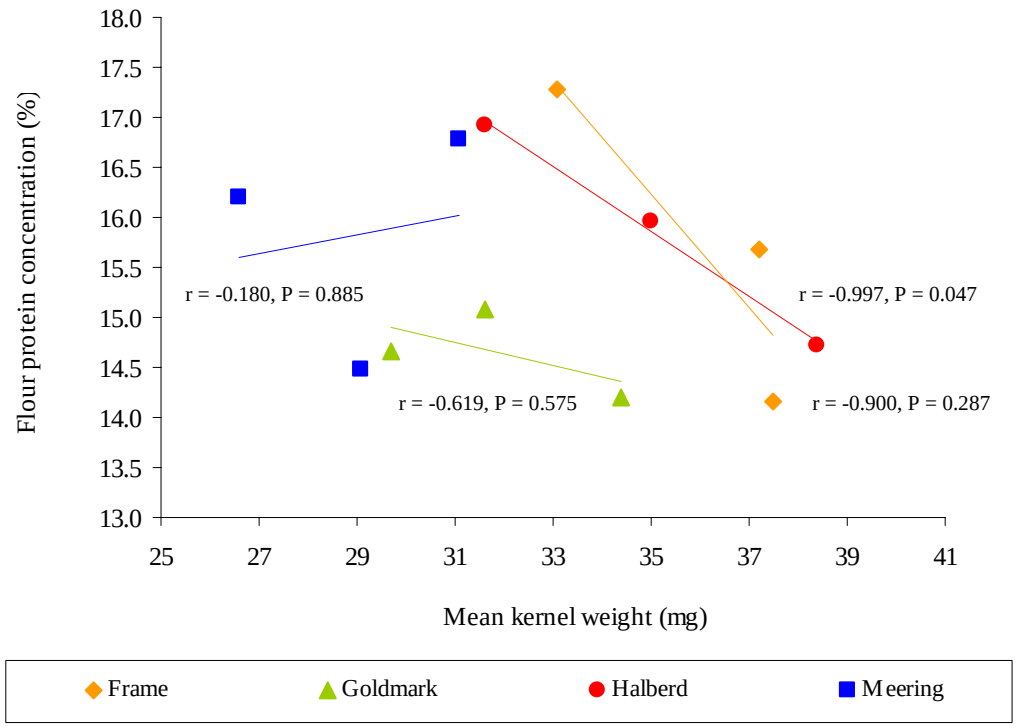


Figure 7.7. Relationship between the mean kernel weight (mg) and flour protein (%) of four wheat genotypes grown at 3 sites in 1998. Each data point is the mean of all zinc treatments. Regression equations are presented in Appendix 7.1

Table 7.3. Effects of genotype and zinc fertilisation (kg ha^{-1}) on the flour protein concentration (%) of wheat grown at Lameroo, Tintinara and Minnipa in 1998.

Genotype	Flour Protein (%)													
	Lameroo					Tintinara					Minnipa			
	0	7.5	7.5 + spray	22.5	*Mean*	0	7.5	7.5 + spray	22.5	*Mean*	0	7.5	22.5	*Mean*
Frame	15.9	15.8	15.1	15.3	*15.6*	14.3	14.3	13.7	13.9	*14.0*	17.1	17.8	16.9	*17.3*
Goldmark	15.0	14.7	14.9	14.2	*14.7*	13.9	14.8	14.8	13.8	*14.4*	15.3	14.5	15.3	*15.1*
Halberd	15.9	16.0	15.9	16.0	*16.0*	14.9	14.6	14.9	14.7	*14.8*	17.2	16.9	16.6	*16.9*
Meering	15.5	16.7	15.7	16.4	*16.1*	14.8	14.0	14.5	14.7	*14.5*	16.9	16.7	16.8	*16.8*
Mean	*15.6*	*15.8*	*15.4*	*15.5*		*14.5*	*14.4*	*14.5*	*14.3*		*16.6*	*16.5*	*16.4*	
LSD$_{0.05}$														
Genotype			**n.s.**					**n.s.**				**1.1**		
Zinc			**n.s.**					**n.s.**				**n.s**		
Genotype x Zinc			**n.s.**									**n.s.**		
(a)								**0.6**						
(b)								**0.9**						
CV (%)			**3.0**					**2.6**				**2.8**		

(a) For comparisons within the same genotype

(b) For other comparisons

n.s. = non-significant

7.3.1.2 Waite Institute, 1998

Flour protein concentration differed significantly between varieties grown at the Waite Institute in 1998 (Table 7.4). Meering (15.1% protein) and Goldmark (15.2%) had the lowest flour protein percentages, followed by Halberd (16.1%), while Frame (17.0%) had the highest flour protein concentration. Sowing date had a significant effect on the flour protein concentrations of the two thermosensitive varieties, Meering and Goldmark, which both showed a significant increase between sowing date 1 (SD 1) and sowing date 2 (SD 2). The mean daily maximum temperature during grain filling was 26.7ºC for SD 2 plants, 2.9ºC higher than for SD 1 plants. The thermotolerant varieties, Frame and Halberd, showed no significant difference in flour protein concentration between sowing dates, however. This is in contrast to the other field sites of 1998, where Frame showed the largest increase in flour protein percentage with an increase in temperature between sites, and Goldmark the lowest. However flour protein concentrations were generally higher at the Waite Institute than at the other sites in 1998, particularly in Frame. The flour protein concentration of Frame at the Waite Institute (SD 1) was 20% greater than at Tintinara, which experienced a similar mean daily maximum temperature during grain filling (23.9ºC). There was no significant effect of Zn treatment on the flour protein percentage of any variety at either SD 1 or SD 2.

7.3.1.3 Horsham and Birchip, 1999

A combined ANOVA of 1999 sites showed that flour protein concentration was significantly higher at Birchip (14.7%) than at Horsham (13.2% and 13.9%, for SD 1 and SD 2, respectively) (Table 7.5). This result was consistent for all varieties. Mean maximum temperatures during grain filling differed little between sites and sowing dates in 1999, being 23.0ºC at Birchip and 23.9ºC and 24.0ºC at Horsham for SD 1 and SD 2, respectively. Thus the highest flour protein concentration was produced at the coolest site in 1999, however this may be due to the drier than average spring that occurred throughout Victoria in 1999 and resulted in low grain yields (Chapter 5), therefore preventing any meaningful comparisons between sites.

The main effect of Zn fertilisation was also significant for flour protein concentration across sites in 1999 (Table 7.5). The flour protein concentration of the Zn treatment involving 7.5 kg Zn ha^{-1} plus two foliar sprays of Zn did not differ from that of the nil Zn treatment, however both of these treatments had a significantly higher flour protein percentage than the treatment that received 7.5 kg Zn ha^{-1} at sowing only (no foliar spray). This result was also seen in Meering at Tintinara in 1998 (Tables 7.2 and 7.3) but was not related to any grain yield parameters (Chapter 5). It is therefore difficult to draw any definite conclusions about the effect of Zn fertilisation on flour protein concentration from either the 1998 or 1999 field trials.

Table 7.4. Effects of sowing date, zinc fertilisation and genotype on the flour protein (%) of wheat grown at the Waite Institute in 1998.

Genotype	**Flour Protein (%)**					
	Sowing Date #1			**Sowing Date #2**		
	- Zn	**+ Zn**	***Mean***	**- Zn**	**+ Zn**	***Mean***
Frame	16.9	16.8	*16.8*	17.1	17.3	*17.2*
Goldmark	14.6	13.6	*14.1*	16.0	16.7	*16.3*
Halberd	15.5	15.5	*15.5*	16.2	17.0	*16.6*
Meering	13.8	14.4	*14.1*	15.8	16.4	*16.1*
Mean	*15.2*	*15.1*		*16.3*	*16.9*	
$LSD_{0.05}$						
Sowing Date			**n.s.**			
Zinc			**n.s.**			
Genotype			**0.5**			
Sowing Date x Zinc			**n.s.**			
Sowing Date x Genotype						
(a)			**0.8**			
(b)			**1.1**			
Zinc x Genotype			**n.s.**			
Sowing Date x Zinc x Genotype			**n.s.**			
CV (%)			**1.7**			

(a) For comparisons within the same sowing date

(b) For other comparisons

n.s. = non-significant

Table 7.5. Effects of site, genotype and zinc fertilisation (kg ha^{-1}) on the flour protein (%) of wheat grown at Birchip and Horsham in 1999.

Genotype	Flour Protein (%)											
	Birchip				Horsham (sowing date #1)				Horsham (sowing date #2)			
	0	7.5	7.5 + 2 sprays	*Mean*	0	7.5	7.5 + 2 sprays	*Mean*	0	7.5	7.5 + 2 sprays	*Mean*
Frame	15.5	15.0	15.4	*15.3*	13.3	13.3	13.7	*13.4*	13.5	13.5	14.2	*13.7*
Goldmark	14.6	14.8	14.3	*14.6*	13.8	13.1	13.7	*13.5*	14.4	13.5	14.5	*14.2*
Halberd	14.3	13.5	14.6	*14.1*	13.1	13.0	13.2	*13.1*	14.5	14.2	14.1	*14.3*
Meering	14.8	15.0	14.8	*14.9*	12.8	13.0	12.7	*12.8*	14.0	13.3	13.4	*13.6*
Mean	*14.8*	*14.6*	*14.8*	*14.7*	*13.2*	*13.1*	*13.3*	*13.2*	*14.1*	*13.6*	*14.1*	*13.9*
LSD$_{0.05}$												
Site						**0.8**						
Genotype						**n.s.**						
Zinc						**0.3**						
Site x Genotype						**n.s.**						
Site x Zinc						**n.s.**						
Genotype x Zinc						**n.s.**						
Site x Genotype x Zinc						**n.s.**						
CV (%)						**3.8**						

n.s. = non-significant

7.3.2 Grain protein composition

7.3.2.1 Lameroo, Tintinara and Minnipa, 1998

Cultivar means across sites for glutenin were 36.3% of flour protein for Frame and 37.5% of flour protein for Goldmark, whereas for gliadin these were 47.0% and 44.2% for Frame and Goldmark, respectively. These proportions are similar to those reported by Panozzo and Eagles (2000) for trials across the wheat-growing regions of Victoria. Together the glutenin and gliadin classes accounted for a mean of 82.5% of flour protein.

There were no significant differences between cultivars for any of the protein characteristics, except for the percentage of glutenin in flour protein at Tintinara, which was marginally higher in Goldmark than in Frame (Table 7.7). However when all 3 sites were analysed together the combined ANOVA showed that the proportion of gliadin in flour protein was significantly lower in Goldmark (44.7%) than in Frame (47.8%) (Appendix 7.2).

A number of the protein composition characteristics, with the exception of the proportion of glutenin in flour protein, were significantly affected by the Zn treatment of two foliar sprays at Lameroo (Table 7.6) and Tintinara (Table 7.7). This treatment, which consisted of 7.5 kg Zn ha^{-1} applied at sowing plus 2 foliar sprays of 334 g Zn ha^{-1} at tillering and stem elongation, significantly decreased both the proportion of gliadin in flour protein and the proportion of unextractable glutenin in total glutenin in both varieties at both sites. This treatment also significantly reduced kernel weight at both Lameroo and Tintinara, while concomitantly increasing the number of grains per m^2 (Chapter 5).

The proportion of gliadin in flour protein was reduced in the foliar Zn treatment by 5 percentage units at Lameroo and by 4 percentage units at Tintinara, relative to the nil Zn control treatment. Relative to the Zn treatment that had received 7.5 kg Zn ha^{-1} at sowing only, the proportion of gliadin in flour protein was reduced by approximately 4

percentage units at both Lameroo and Tintinara. This resulted in a significant increase in the glutenin:gliadin ratio of an average 16% relative to the nil Zn control, and an average 14% relative to the soil only Zn treatment.

The proportion of unextractable glutenin in flour protein decreased with the application of the foliar Zn treatment in both varieties at Tintinara, but only in Goldmark at Lameroo. However the proportion of extractable glutenin in total protein increased with this treatment in both varieties at both sites, and the net result was no significant change to the total amount of glutenin in the flour protein in either variety at either site. The proportion of unextractable glutenin in total glutenin was reduced by approximately 12 percentage units relative to the nil Zn control treatment at both sites, and by approximately 13 percentage units relative to the soil only Zn treatment at both sites.

There was no foliar Zn treatment applied to plots at Minnipa, and no effect of Zn treatment on any of the protein composition characteristics at this site (Table 7.8). There were no significant effects of cultivar on protein composition at Minnipa either, with both Frame and Goldmark producing similar proportions of glutenins and gliadins at this site.

7.3.2.2 Waite Institute, 1998

The Zn treatment applied to plots at the Waite Institute in 1998 was in the form of a Zn foliar spray, applied at anthesis, which delivered 570 g Zn ha^{-1}. However there was no significant effect of Zn fertilisation on any of the protein composition characteristics of the four cultivars grown at this site.

Cultivar means for glutenin at the Waite Institute ranged from 43.3% of flour protein for Halberd up to 47.0% of flour protein for Meering (Table 7.9). The cultivars with the *Glu-D1d* allele, Frame and Halberd, had a significantly lower proportion of glutenin in flour protein than the cultivars with the *Glu-D1a* allele, Meering and Goldmark. Conversely cultivars with the *Glu-D1d* allele had a significantly higher proportion of gliadin in flour protein than cultivars with the *Glu-D1a* allele, and a significantly lower

glutenin:gliadin ratio. There was no effect of sowing date on the proportion of glutenin or gliadin in flour protein, with the exception of Frame, which had a significantly higher proportion of gliadin in the flour protein at SD 1 (43.4%) than at SD 2 (40.9%).

The proportion of unextractable glutenin in flour protein was influenced by genotype, with Meering having the highest proportion (27.1%), followed by Goldmark (25.8%), Frame (24.2%) and Halberd (21%). Halberd also had the highest amount of extractable glutenin in flour protein (22.2%), significantly higher than Goldmark (20.4%), Meering (19.9%) and Frame (19.6%). This protein component, the proportion of extractable glutenin in flour protein, was also affected by sowing date, being significantly higher at SD 2.

The proportion of unextractable glutenin in the total amount of glutenin was also affected by genotype and sowing date. Halberd had a significantly lower percentage of unextractable glutenin in total glutenin than the other 3 varieties, and this protein fraction was also significantly lower at the SD 2 compared with SD 1.

7.3.2.3 Horsham and Birchip, 1999

There was no significant effect of Zn treatment on any protein components at either Birchip or Horsham in 1999 (Tables 7.10, 7.11 and 7.12). There were significant differences between the cultivars however, with Goldmark (*Glu-D1a* allele) again having a lower proportion of gliadin in flour protein than Frame (*Glu-D1d* allele). This was a consistent observation at both sites and both sowing dates and led to a significantly higher glutenin:gliadin ratio in the cultivar Goldmark. Goldmark was also found to have a significantly higher proportion of glutenin in flour protein than Frame at Horsham. There was no difference between cultivars in the proportions of glutenin in total flour protein or glutenin in total glutenin at either site in 1999, however.

Table 7.6. Effect of genotype and zinc fertilisation (kg ha^{-1}) on the protein composition characteristics of wheat grown at Lameroo in 1998.

Genotype	0	7.5	7.5 + 2 sprays	22.5	*Mean*	0	7.5	7.5 + 2 sprays	22.5	*Mean*	0	7.5	7.5 + 2 sprays	22.5	*Mean*
	Glutenin (% total protein)					*Gliadin (% total protein)*					*Glutenin : Gliadin ratio*				
Frame	33.8	33.9	39.0	35.2	*35.5*	48.4	46.3	42.3	46.0	*45.8*	0.71	0.73	0.92	0.77	*0.78*
Goldmark	34.8	35.0	34.7	34.5	*34.8*	44.0	43.4	40.1	41.5	*42.3*	0.79	0.81	0.87	0.83	*0.83*
Mean	*34.3*	*34.5*	*36.9*	*34.8*		*46.2*	*44.9*	*41.2*	*43.8*		*0.75*	*0.77*	*0.89*	*0.80*	
LSD$_{0.05}$															
Genotype			**n.s.**					**n.s.**					**n.s.**		
Zinc			**n.s.**					**2.1**					**0.07**		
Genotype x Zinc			**n.s.**					**n.s.**					**n.s.**		
CV (%)			**5.7**					**3.9**					**7.3**		

	Unextractable Glutenin (% total protein)					*Extractable Glutenin (% total protein)*					*Unextractable Glutenin (% total glutenin)*				
Frame	17.7	17.7	16.2	17.7	*17.3*	16.1	16.2	22.8	17.5	*18.1*	52.6	52.4	41.6	50.4	*49.2*
Goldmark	17.0	18.3	12.2	17.0	*16.1*	17.8	16.7	22.6	17.5	*18.6*	49.0	52.7	34.9	49.4	*46.5*
Mean	*17.4*	*18.3*	*14.2*	*17.3*		*16.9*	*16.4*	*22.7*	*17.5*		*50.8*	*52.5*	*38.3*	*49.9*	
LSD$_{0.05}$															
Genotype			**n.s.**					**n.s.**					**n.s.**		
Zinc			**1.3**					**2.0**					**3.4**		
Genotype x Zinc								**n.s.**					**n.s.**		
(a)			**1.8**												
(b)			**2.0**												
CV (%)			**6.1**					**8.7**					**6.0**		

(a) For comparisons within the same genotype

(b) For other comparisons

n.s. = non-significant

Table 7.7. Effect of genotype and zinc fertilisation (kg ha^{-1}) on the protein composition characteristics of wheat grown at Tintinara in 1998.

Genotype	0	7.5	7.5 + 2 sprays	22.5	*Mean*	0	7.5	7.5 + 2 sprays	22.5	*Mean*	0	7.5	7.5 + 2 sprays	22.5	*Mean*
	Glutenin (% total protein)					*Gliadin (% total protein)*					*Glutenin : Gliadin ratio*				
Frame	37.8	37.5	39.6	37.1	*38.0*	47.0	46.4	41.6	45.8	*45.2*	0.81	0.81	0.95	0.82	*0.84*
Goldmark	38.8	40.3	40.4	39.1	*39.7*	44.8	45.1	41.8	44.9	*44.1*	0.87	0.90	0.97	0.87	*0.90*
Mean	*38.3*	*38.9*	*40.0*	*38.1*		*45.9*	*45.8*	*41.7*	*45.4*		*0.84*	*0.85*	*0.96*	*0.84*	
LSD$_{0.05}$															
Genotype			**0.4**					**n.s.**					**n.s.**		
Zinc			**n.s.**					**2.0**					**0.06**		
Genotype x Zinc			**n.s.**					**n.s.**					**n.s.**		
CV (%)			**3.4**					**3.5**					**5.9**		
	Unextractable Glutenin (% total protein)					*Extractable Glutenin (% total protein)*					*Unextractable Glutenin (% total glutenin)*				
Frame	20.0	20.8	17.0	19.1	*19.2*	17.8	16.7	22.5	18.0	*18.7*	53.2	55.6	43.1	51.5	*50.9*
Goldmark	20.2	21.1	15.4	19.5	*19.1*	18.5	19.3	25.0	19.6	*20.6*	52.1	52.1	38.1	50.0	*48.1*
Mean	*20.1*	*20.9*	*16.2*	*19.3*		*18.2*	*18.0*	*23.8*	*18.8*		*52.6*	*53.8*	*40.6*	*50.8*	
LSD$_{0.05}$															
Genotype			**n.s.**					**n.s.**					**n.s.**		
Zinc			**2.3**					**2.5**					**5.9**		
Genotype x Zinc			**n.s.**					**n.s.**					**n.s.**		
CV (%)			**9.7**					**10.0**					**9.4**		

n.s. = non-significant

Table 7.8. Effect of genotype and zinc fertilisation (kg ha^{-1}) on the protein composition characteristics of wheat grown at Minnipa in 1998.

Genotype	0	7.5	22.5	*Mean*	0	7.5	22.5	*Mean*	0	7.5	22.5	*Mean*
	Glutenin (% total protein)				*Gliadin (% total protein)*				*Glutenin : Gliadin ratio*			
Frame	36.2	34.8	35.6	*35.5*	49.4	50.6	50.0	*50.0*	0.73	0.69	0.71	*0.71*
Goldmark	38.7	38.2	37.6	*38.1*	46.6	45.7	46.2	*46.2*	0.83	0.84	0.81	*0.83*
Mean	*37.4*	*36.5*	*36.6*		*48.0*	*48.2*	*48.1*		*0.78*	*0.76*	*0.76*	
$LSD_{0.05}$												
Genotype		**n.s.**				**n.s.**				**n.s.**		
Zinc		**n.s.**				**n.s.**				**n.s.**		
Genotype x Zinc		**n.s.**				**n.s.**				**n.s.**		
CV (%)		**2.3**				**2.6**				**4.3**		
	Unextractable Glutenin (% total protein)				*Extractable Glutenin (% total protein)*				*Unextractable Glutenin (% total glutenin)*			
Frame	19.0	17.7	17.9	*18.2*	17.2	17.1	17.7	*17.3*	52.5	50.9	50.3	*51.2*
Goldmark	19.5	18.8	18.1	*18.8*	19.2	19.4	19.5	*19.3*	50.6	49.4	48.4	*49.5*
Mean	*19.3*	*18.0*	*18.3*		*18.2*	*18.2*	*18.6*		*51.6*	*50.2*	*49.3*	
$LSD_{0.05}$												
Genotype		**n.s.**				**n.s.**				**n.s.**		
Zinc		**n.s.**				**n.s.**				**n.s.**		
Genotype x Zinc		**n.s.**				**n.s.**				**n.s.**		
CV (%)		**8.5**				**9.0**				**8.1**		

n.s. = non-significant

Table 7.9. Effect of sowing date, zinc fertilisation, and genotype on the protein composition characteristics of wheat grown at the Waite Institute in 1998.

Genotype	SD #1 - Zn	SD #1 + Zn	SD #2 - Zn	SD #2 + Zn	*Mean*	SD #1 - Zn	SD #1 + Zn	SD #2 - Zn	SD #2 + Zn	*Mean*	SD #1 - Zn	SD #1 + Zn	SD #2 - Zn	SD #2 + Zn	*Mean*
	Glutenin (% total protein)					*Gliadin (% total protein)*					*Glutenin : Gliadin ratio*				
Frame	42.5	44.0	44.0	44.5	*43.7*	44.4	42.5	41.1	40.7	*42.2*	0.96	1.04	1.07	1.09	*1.04*
Goldmark	46.2	46.5	45.8	46.4	*46.2*	38.1	36.7	38.5	38.4	*37.9*	1.22	1.27	1.20	1.21	*1.22*
Halberd	43.3	42.6	43.4	43.7	*43.3*	42.4	42.8	41.2	41.6	*42.0*	1.02	1.00	1.06	1.05	*1.03*
Meering	47.3	47.4	46.9	46.4	*47.0*	35.9	35.8	36.5	37.2	*36.3*	1.32	1.33	1.29	1.25	*1.30*
Mean	*44.8*	*45.1*	*45.0*	*45.3*		*40.2*	*39.4*	*39.3*	*39.5*		*1.13*	*1.16*	*1.16*	*1.15*	
$LSD_{0.05}$															
Sowing Date			**n.s.**					**n.s.**					**n.s.**		
Zinc			**n.s.**					**n.s.**					**n.s.**		
Genotype			**1.2**					**1.4**					**0.07**		
SD x Zinc			**n.s.**					**n.s.**					**n.s.**		
SD x Genotype			**n.s.**										**n.s.**		
(a)								**1.9**							
(b)								**2.0**							
Zinc x Genotype			**n.s.**					**n.s.**					**n.s.**		
SD x Zinc x Genotype			**n.s.**					**n.s.**					**n.s.**		
CV (%)			**3.0**					**4.1**					**6.8**		

(a) For comparisons within the same sowing date

(b) For other comparisons

n.s. = non-significant

Table 7.9. continued

Genotype	SD #1 - Zn	SD #1 + Zn	SD #2 - Zn	SD #2 + Zn	*Mean*	SD #1 - Zn	SD #1 + Zn	SD #2 - Zn	SD #2 + Zn	*Mean*	SD #1 - Zn	SD #1 + Zn	SD #2 - Zn	SD #2 + Zn	*Mean*
	Unextractable Glutenin (% total protein)					*Extractable Glutenin (% total protein)*					*Unextractable Glutenin (% total glutenin)*				
Frame	24.5	25.0	22.6	24.6	*24.2*	18.1	19.0	21.3	19.8	*19.6*	57.5	56.8	51.4	55.3	*55.3*
Goldmark	25.7	26.9	25.2	25.4	*25.8*	20.5	19.6	20.7	21.0	*20.4*	55.7	57.9	54.9	54.8	*55.8*
Halberd	21.8	21.5	20.5	20.6	*21.1*	21.5	21.1	22.9	23.1	*22.2*	50.3	50.4	47.2	47.1	*48.8*
Meering	28.3	27.8	26.8	25.6	*27.1*	19.0	19.7	20.1	20.9	*19.9*	59.8	58.4	56.8	54.9	*57.5*
Mean	*25.1*	*25.3*	*23.8*	*24.1*		*19.8*	*19.8*	*21.3*	*21.2*		*55.8*	*55.9*	*52.6*	*53.0*	
LSD$_{0.05}$															
Sowing Date			**n.s.**					**0.8**					**1.9**		
Zinc			**n.s.**					**n.s.**					**n.s.**		
Genotype			**1.2**					**0.7**					**2.1**		
SD x Zinc			**n.s.**					**n.s.**					**n.s.**		
SD x Genotype			**n.s.**					**n.s.**					**n.s.**		
Zinc x Genotype			**n.s.**					**n.s.**					**n.s.**		
SD x Zinc x Genotype			**n.s.**					**n.s.**					**n.s.**		
CV (%)			**6.0**					**6.2**					**4.6**		

n.s. = non-significant

Table 7.10. Effect of genotype and zinc fertilisation (kg ha^{-1}) on the protein composition characteristics of wheat grown at Birchip in 1999.

Genotype	0	7.5	7.5 + 2 sprays	*Mean*	0	7.5	7.5 + 2 sprays	*Mean*	0	7.5	7.5 + 2 sprays	*Mean*
	Glutenin (% total protein)				*Gliadin (% total protein)*				*Glutenin : Gliadin ratio*			
Frame	49.8	49.9	49.4	*49.7*	43.7	43.6	43.7	*43.7*	1.14	1.15	1.13	*1.14*
Goldmark	52.6	51.8	51.7	*52.0*	40.1	40.7	40.0	*40.3*	1.31	1.27	1.29	*1.29*
Mean	*51.2*	*50.8*	*50.5*		*41.9*	*42.1*	*41.9*		*1.23*	*1.21*	*1.21*	
$LSD_{0.05}$												
Genotype		**n.s.**				**0.3**				**0.07**		
Zinc		**n.s.**				**n.s.**				**n.s.**		
Genotype x Zinc		**n.s.**				**n.s.**				**n.s.**		
CV (%)		**1.3**				**1.7**				**2.7**		

	Unextractable Glutenin (% total protein)				*Extractable Glutenin (% total protein)*				*Unextractable Glutenin (% total glutenin)*			
Frame	26.4	27.5	26.5	*26.8*	23.4	22.4	22.9	*22.9*	53.0	55.1	53.7	*53.9*
Goldmark	26.9	24.7	27.8	*27.4*	25.7	24.4	23.9	*24.7*	51.1	52.9	53.8	*52.6*
Mean	*26.7*	*27.4*	*27.1*		*24.6*	*23.4*	*23.4*		*52.0*	*54.0*	*53.7*	
$LSD_{0.05}$												
Genotype		**n.s.**				**n.s.**				**n.s.**		
Zinc		**n.s.**				**n.s.**				**n.s.**		
Genotype x Zinc		**n.s.**				**n.s.**				**n.s.**		
CV (%)		**2.8**				**4.7**				**3.5**		

n.s. = non-significant

Table 7.11. Effect of genotype and zinc fertilisation (kg ha^{-1}) on the protein composition characteristics of wheat grown at Horsham (sowing date #1) in 1999.

Genotype	0	7.5	7.5 + 2 sprays	*Mean*	0	7.5	7.5 + 2 sprays	*Mean*	0	7.5	7.5 + 2 sprays	*Mean*
	Glutenin (% total protein)				*Gliadin (% total protein)*				*Glutenin : Gliadin ratio*			
Frame	49.6	50.0	50.1	*49.9*	43.5	43.3	42.9	*43.2*	1.14	1.15	1.17	*1.15*
Goldmark	51.2	51.5	51.3	*51.3*	41.3	40.5	40.9	*40.9*	1.24	1.27	1.26	*1.26*
Mean	*50.4*	*50.7*	*50.7*		*42.4*	*41.9*	*41.9*		*1.19*	*1.21*	*1.21*	
LSD$_{0.05}$												
Genotype		**n.s.**				**1.3**				**0.09**		
Zinc		**n.s.**				**n.s.**				**n.s.**		
Genotype x Zinc		**n.s.**				**n.s.**				**n.s.**		
CV (%)		**2.0**				**1.8**				**3.7**		
	Unextractable Glutenin (% total protein)				*Extractable Glutenin (% total protein)*				*Unextractable Glutenin (% total glutenin)*			
Frame	28.5	28.7	27.9	*28.4*	21.0	21.3	22.2	*21.5*	57.6	57.5	55.7	*56.9*
Goldmark	28.8	30.1	30.4	*29.8*	22.4	21.4	20.9	*21.6*	56.3	58.5	59.3	*58.0*
Mean	*28.7*	*29.4*	*29.2*		*21.7*	*21.3*	*21.6*		*56.9*	*58.0*	*57.5*	
LSD$_{0.05}$												
Genotype		**n.s.**				**n.s.**				**n.s.**		
Zinc		**n.s.**				**n.s.**				**n.s.**		
Genotype x Zinc		**n.s.**				**n.s.**				**n.s.**		
CV (%)		**3.3**				**3.8**				**2.5**		

n.s. = non-significant

Table 7.12. Effect of genotype and zinc fertilisation (kg ha^{-1}) on the protein composition characteristics of wheat grown at Horsham (sowing date #2) in 1999.

Genotype	0	7.5	7.5 + 2 sprays	*Mean*	0	7.5	7.5 + 2 sprays	*Mean*	0	7.5	7.5 + 2 sprays	*Mean*
	Glutenin (% total protein)				*Gliadin (% total protein)*				*Glutenin : Gliadin ratio*			
Frame	49.0	49.3	50.0	*49.5*	44.3	43.9	43.4	*43.9*	1.11	1.12	1.15	*1.13*
Goldmark	51.2	51.4	50.8	*51.1*	42.2	41.9	42.5	*42.2*	1.21	1.23	1.20	*1.21*
Mean	*50.1*	*50.3*	*50.4*		*43.3*	*42.9*	*43.0*		*1.16*	*1.18*	*1.17*	
LSD$_{0.05}$												
Genotype		**0.7**				**0.5**				**0.03**		
Zinc		**n.s.**				**n.s.**				**n.s.**		
Genotype x Zinc		**n.s.**				**n.s.**				**n.s.**		
CV (%)		**2.0**				**2.5**				**4.6**		
	Unextractable Glutenin (% total protein)				*Extractable Glutenin (% total protein)*				*Unextractable Glutenin (% total glutenin)*			
Frame	27.8	28.9	29.1	*28.6*	21.2	20.4	20.9	*20.8*	56.8	58.7	58.1	*57.9*
Goldmark	28.1	29.2	27.9	*28.4*	23.1	22.2	22.9	*22.7*	54.8	56.8	54.8	*55.5*
Mean	*28.0*	*29.1*	*28.5*		*22.2*	*21.3*	*21.9*		*55.8*	*57.8*	*56.9*	
LSD$_{0.05}$												
Genotype		**n.s.**				**n.s.**				**n.s.**		
Zinc		**n.s.**				**n.s.**				**n.s.**		
Genotype x Zinc		**n.s.**				**n.s.**				**n.s.**		
CV (%)		**4.8**				**2.9**				**3.1**		

n.s. = non-significant

7.3.2.4 Controlled environment, 2000

Zinc fertilisation was found to have a significant effect on the proportion of unextractable glutenin in the flour protein of grain produced under controlled environment conditions in 2000 (Table 7.13). As with the field experiments of 1998, the application of Zn in this experiment was found to reduce the proportion of unextractable glutenin in flour protein. Significant differences between cultivars were again observed for all protein fractions. Once again, cultivars with the *Glu-D1a* allele, Goldmark and Meering, were observed to have a higher proportion of glutenin in flour protein, a lower proportion of gliadin in flour protein, and a higher glutenin:gliadin ratio than those cultivars with the *Glu-D1d* allele, Frame and Halberd. The proportion of unextractable glutenins was also influenced by genotype. As observed at the Waite Institute in 1998, Meering was found to have the highest proportion of unextractable glutenins in flour protein (22.8%), followed by Goldmark (19.9%), Frame (19.8%) and Halberd (17.9%). Goldmark had the highest proportion of extractable glutenins in flour protein (30.4%), with no significant differences between the other 3 varieties. The proportion of unextractable glutenin in total glutenin was also affected by genotype, with Halberd and Goldmark having a significantly lower amount of this protein component than Frame or Meering.

There was no effect of temperature on any of the protein composition characteristics of grain produced in this experiment. This was an unexpected result, since accumulated temperatures greater than 30ºC during the first 14 days of grain filling have been found to explain a significant proportion of environmental variation for gliadin and glutenin (Panozzo and Eagles 2000). However it may be that the duration of high temperature in the present experiment was too short to affect protein composition. Graybosch *et al.* (1995) suggested that protein quality declined only after exposure to more than 90 hours of temperature greater than 32ºC during grain filling. In the present study plants experienced only 30 hours of temperatures greater than 32ºC, between 10 and 13 DAA.

Table 7.13. Effect of genotype, zinc fertilisation (mg kg^{-1} soil) and temperature treatment on the protein composition characteristics of wheat grown under controlled environment conditions in 2000.

Genotype	22/16ºC $Zn_{0.2}$	40/20ºC $Zn_{0.2}$	22/16ºC Zn_2	40/20ºC Zn_2	*Mean*	22/16ºC $Zn_{0.2}$	40/20ºC $Zn_{0.2}$	22/16ºC Zn_2	40/20ºC Zn_2	*Mean*	22/16ºC $Zn_{0.2}$	40/20ºC $Zn_{0.2}$	22/16ºC Zn_2	40/20ºC Zn_2	*Mean*
	Glutenin (% total protein)					*Gliadin (% total protein)*					*Glutenin : Gliadin ratio*				
Frame	45.7	46.6	45.9	46.6	*46.2*	49.0	48.6	47.5	47.6	*48.2*	0.93	0.96	0.97	0.98	*0.96*
Goldmark	50.1	50.6	50.4	49.9	*50.2*	44.7	43.6	44.4	43.4	*44.0*	1.12	1.16	1.14	1.15	*1.14*
Halberd	46.8	46.1	46.0	45.5	*46.1*	47.6	48.9	48.9	49.1	*48.6*	0.98	0.94	0.94	0.93	*0.95*
Meering	50.3	50.3	50.0	49.5	*50.0*	43.5	43.9	42.5	44.1	*43.5*	1.16	1.15	1.18	1.12	*1.15*
Mean	*48.2*	*48.4*	*48.1*	*47.9*		*46.2*	*46.2*	*45.8*	*46.1*		*1.05*	*1.05*	*1.06*	*1.05*	
$LSD_{0.05}$															
Genotype			**1.0**					**1.0**					**0.04**		
Zinc			**n.s.**					**n.s.**					**n.s.**		
Temperature			**n.s.**					**n.s.**					**n.s.**		
Genotype x Zinc			**n.s.**					**n.s.**					**n.s.**		
Genotype x Temp			**n.s.**					**n.s.**					**n.s.**		
Zinc x Temperature			**n.s.**					**n.s.**					**n.s.**		
Genotype x Zinc x Temp			**n.s.**					**n.s.**					**n.s.**		
CV (%)			**2.6**					**2.5**					**4.4**		

n.s. = non-significant

Table 7.13. continued

Genotype	22/16°C $Zn_{0.2}$	40/20°C $Zn_{0.2}$	22/16°C Zn_2	40/20°C Zn_2	*Mean*	22/16°C $Zn_{0.2}$	40/20°C $Zn_{0.2}$	22/16°C Zn_2	40/20°C Zn_2	*Mean*	22/16°C $Zn_{0.2}$	40/20°C $Zn_{0.2}$	22/16°C Zn_2	40/20°C Zn_2	*Mean*
	Unextractable Glutenin (% total protein)					*Extractable Glutenin (% total protein)*					*Unextractable Glutenin (% total glutenin)*				
Frame	19.0	20.4	20.1	19.5	*19.8*	26.7	26.2	25.8	27.1	*26.4*	41.6	43.8	43.8	41.9	*42.8*
Goldmark	21.1	21.2	19.3	17.8	*19.9*	29.1	29.4	31.1	32.0	*30.4*	42.1	41.9	38.3	35.8	*39.5*
Halberd	18.3	18.9	17.2	17.2	*17.9*	28.5	27.2	28.8	28.3	*28.2*	39.1	41.0	37.4	37.8	*38.8*
Meering	23.0	23.0	22.0	23.1	*22.8*	27.3	27.4	28.1	26.4	*27.3*	45.7	45.6	43.9	46.8	*45.5*
Mean	*20.3*	*20.9*	*19.7*	*19.4*		*27.9*	*27.5*	*28.4*	*28.4*		*42.1*	*43.1*	*40.9*	*40.6*	
$LSD_{0.05}$															
Genotype			**1.5**					**1.7**					**3.1**		
Zinc			**1.0**					**n.s.**					**n.s.**		
Temperature			**n.s.**					**n.s.**					**n.s.**		
Genotype x Zinc			**n.s.**					**n.s.**					**n.s.**		
Genotype x Temp			**n.s.**					**n.s.**					**n.s.**		
Zinc x Temperature			**n.s.**					**n.s.**					**n.s.**		
Genotype x Zinc x Temp			**n.s.**					**n.s.**					**n.s.**		
CV (%)			**8.8**					**7.3**					**9.0**		

n.s. = non-significant

7.3.2.5 Grain zinc concentration

When the Zn-responsive sites of 1998 were considered together, a positive correlation between the glutenin:gliadin ratio and grain Zn concentration could be clearly seen for both Frame and Goldmark (Figures 7.8a and d). It should be noted that the data from the Waite Institute have been included here, because although not Zn-responsive for protein composition, other significant effects of Zn were observed at this site, including effects on photosynthetic activity and grain growth (Chapter 5). This increase in the glutenin:gliadin ratio with an increase in grain Zn was somewhat better correlated with an increase in the percentage of glutenins in the grain protein (Figures 7.8c and f), rather than with a decrease in the percentage of gliadins (Figures 7.8b and e).

7.3.2.6 Principal component analysis

All sites

Principal component analysis was performed on data from both the 1998 and 1999 field trials and the first 3 components were found to explain 82% of the variation. The percentage variation and character loadings are shown in Table 7.14. Characters having a strong loading on PC1 were the percentage of glutenin (total and unextractable) in flour protein and the glutenin:gliadin ratio. Characters with a strong loading on PC2 were the concentrations of minerals in the grain (manganese (Mn), Cu, S, Zn) and grain protein concentration, while characters with a strong loading on PC3 were the percentage of gliadin in flour protein and grain K concentration.

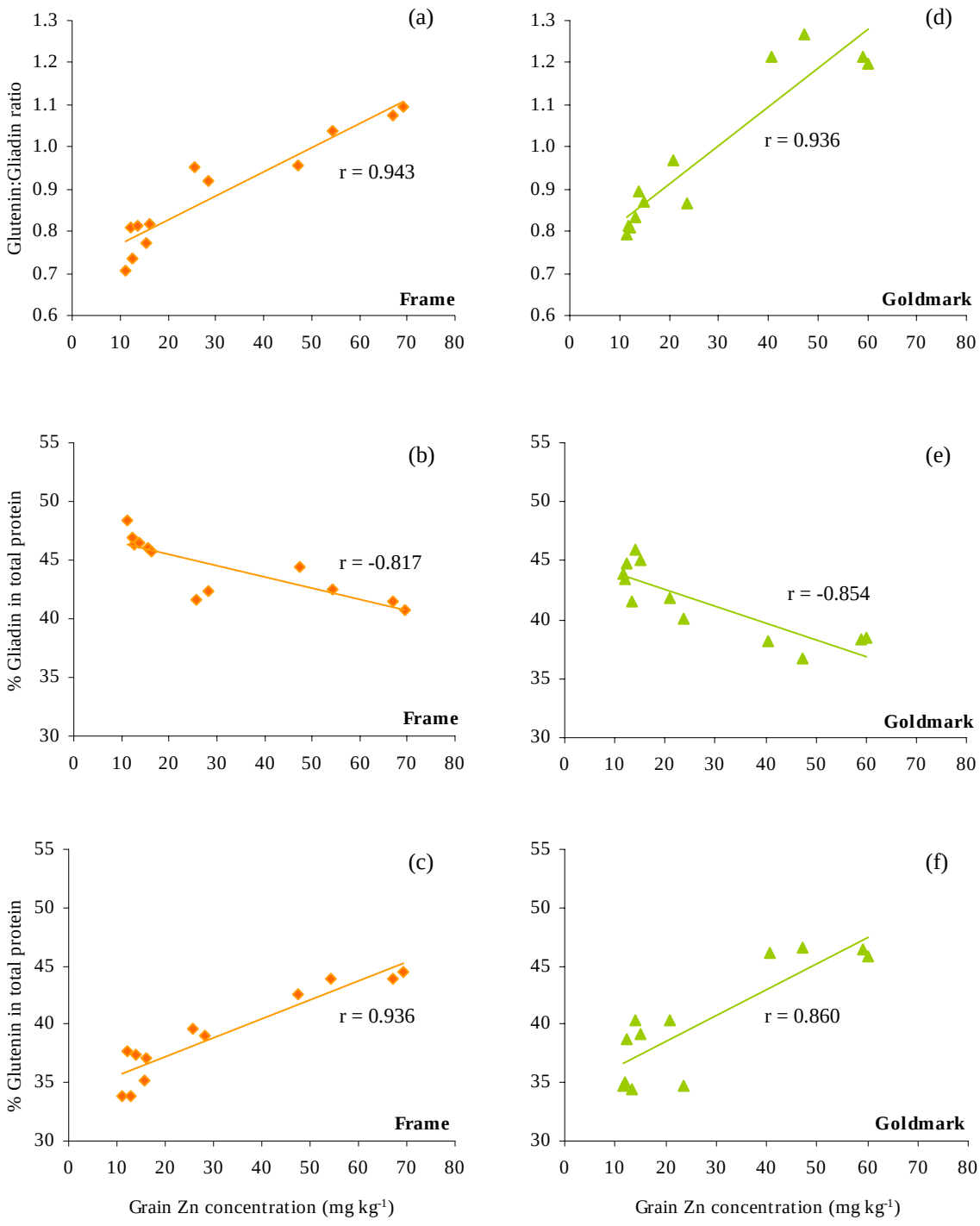


Figure 7.8 Relationship between grain zinc concentration (mg kg^{-1}) and the ratio of glutenins to gliadins (a and d), the percentage of gliadins (b and e), and the percentage of glutenins (c and f) in the grain protein of Frame (a-c) and Goldmark (d-f) wheat varieties grown at 3 field sites in 1998. The r values are significant at the 1% level. Each point is the mean of at least 3 replications. Regression equations are presented in Appendix 7.1

Table 7.14. Principal component scores 1, 2 and 3 (PC1, PC2, PC3) and percentage variation for wheat grown at six field sites in 1998 and 1999.

	PC1	PC2	PC3
Proportion of variation	0.450	0.221	0.149
Cumulative variation	0.450	0.672	0.821
Component loadings:			
yield	0.186	0.288	0.159
kernel number	0.263	0.183	0.225
kernel weight	-0.219	0.180	-0.180
grain protein	0.151	-0.357	-0.029
grain S	0.163	-0.375	0.090
grain P	0.265	0.018	0.296
grain Cu	-0.130	-0.388	-0.100
grain Mn	-0.102	-0.411	-0.152
grain Zn	-0.067	-0.345	0.311
grain Mg	0.288	-0.119	0.174
grain K	0.196	-0.115	0.349
mean temperature	0.180	-0.313	-0.162
days over 35ºC	0.266	0.007	-0.287
unextractable glutenin	-0.317	-0.032	0.023
extractable glutenin	-0.206	0.072	0.221
glutenin	-0.320	0.003	0.101
gliadin	0.173	0.054	-0.429
glutenin:gliadin ratio	-0.302	-0.029	0.224
unextractable gliadin	-0.177	-0.050	-0.261
extractable gliadin	0.274	0.084	-0.208

The scores of PC1 and PC2 are plotted in Figure 7.9 and overlayed with a bi-plot of the component scores. This shows a clear separation of field sites from each other, with the Waite Institute and Minnipa sites showing a much wider distribution than the tighter clusters of Lameroo, Tintinara, Birchip and Horsham. The bi-plot shows that the characters with the greatest influence on the 1999 sites, Horsham and Birchip, were the glutenin characteristics (unextractable, extractable, total and the glutenin:gliadin ratio), plus the percentage of unextractable gliadin in flour protein. The characters influencing the Waite site were the concentrations of Cu, Zn, Mn and S in the grain, plus the grain protein concentration, while the concentrations of the macronutrients, K and Mg, had the greatest influence on the Minnipa site. At Lameroo the gliadin characteristics (extractable and total), days above 35ºC and grain P had the greatest influence, while at Tintinara it was yield and the number of kernels per m^2.

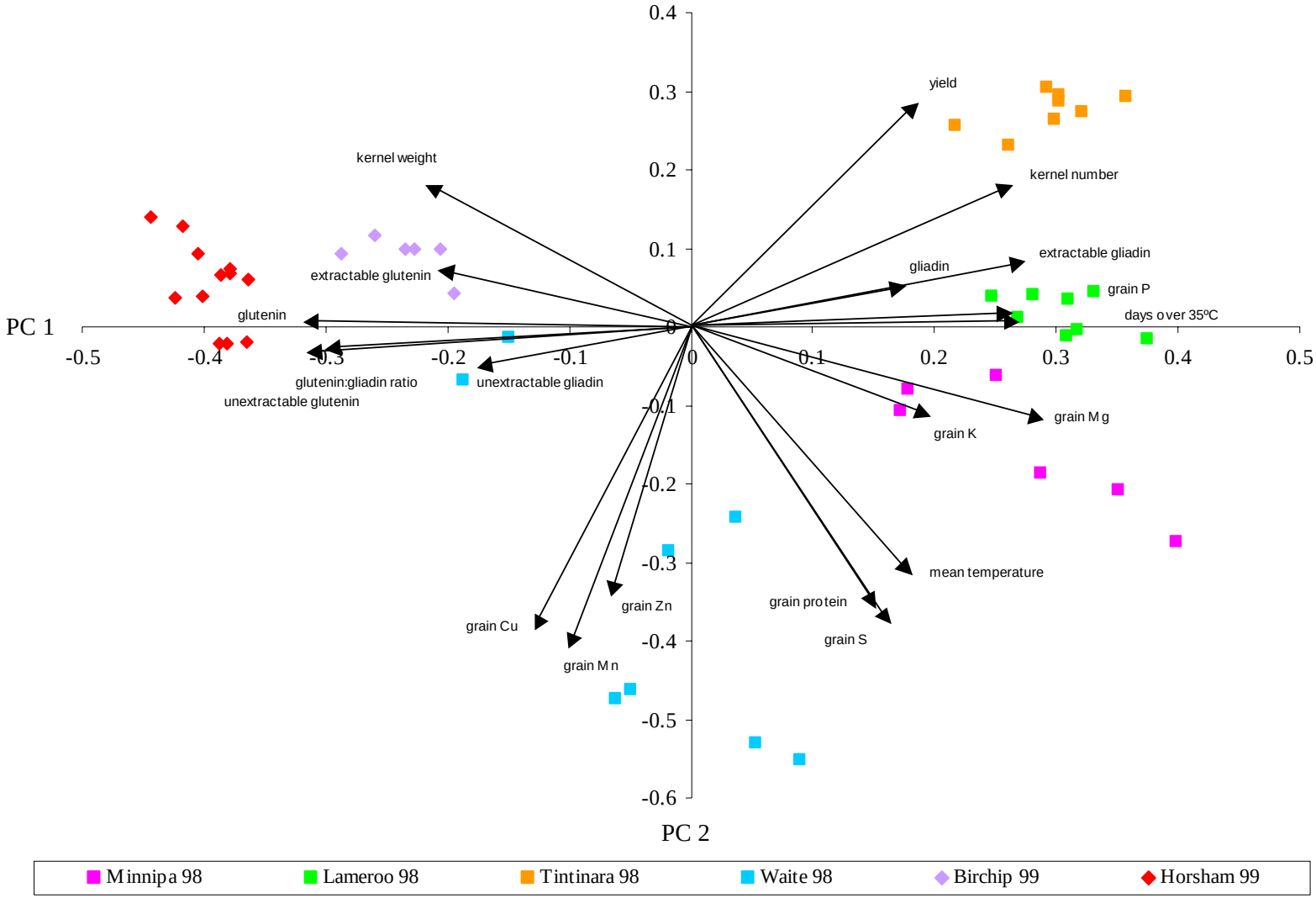


Figure 7.9. Principal component analysis of wheat grown at six field sites in 1998 and 1999. An overlay of the biplot represents the component loadings for principal components 1 and 2 for each of the qualitative characters scored.

1998 sites only

Principal component analysis was also performed on data from the 1998 field trials only, thus removing the 1999 sites where drought appears to have had a major influence on grain quality. The percentage variation and character loadings from this analysis are shown in Table 7.15. Grain Zn concentration was found to have the strongest loading on PC1, along with the glutenin characteristics (percentage of total and unextractable glutenin in flour protein). Characters with a strong loading on PC2 with this analysis were the temperature characteristics during grain filling (mean temperature and the number of days over 35ºC), grain protein concentration and the percentage of total gliadin in flour protein, while characters with the strongest loading on PC3 were the percentages of unextractable gliadin and extractable glutenin in flour protein.

The scores of PC1 and PC2 are plotted in Figure 7.10 and are overlayed with a bi-plot of the component scores. Again a clear separation of the field sites from each other can be seen. The bi-plot also shows that the characters with the greatest influence on the Waite site were the glutenin characteristics and grain Zn, while the temperature characteristics during grain filling had the greatest influence on the Minnipa site. The gliadin characteristics appear to have had the most influence at Tintinara, while at Lameroo it was the yield parameters, including the number of kernels per m^2 and kernel weight, that had the most influence.

The bi-plot illustrated in Figure 7.10 also shows that grain Zn concentration is positively associated with the glutenin characteristics (percentage of unextractable and total glutenin in flour protein and the glutenin:gliadin ratio), while negatively correlated with the number of days above 35ºC during grain filling and the gliadin characteristics of total and extractable gliadin in total protein. Other nutrients in the grain were positively associated with grain protein composition, and, to a lesser extent, with the mean temperature during grain filling. The number of kernels per m^2 was positively correlated with yield and kernel weight, which were clearly negatively associated with grain protein concentration.

Table 7.15. Principal component scores 1, 2 and 3 (PC1, PC2, PC3) and percentage variation for wheat grown at four field sites in 1998.

	PC1	PC2	PC3
Proportion of variation	0.387	0.270	0.113
Cumulative variation	0.387	0.657	0.770
Component loadings:			
yield	0.262	0.233	-0.082
kernel number	0.272	0.251	0.049
kernel weight	0.137	0.086	-0.229
grain protein	-0.200	-0.265	-0.004
grain S	-0.270	-0.150	0.072
grain P	0.102	0.278	0.101
grain Cu	-0.274	-0.217	0.097
grain Mn	-0.278	0.252	-0.031
grain Zn	-0.335	0.109	0.074
grain Mg	0.024	0.009	0.331
grain K	-0.149	0.287	-0.187
mean temperature	-0.114	-0.339	0.234
days over 35ºC	0.218	-0.312	0.062
unextractable glutenin	-0.282	0.129	-0.338
extractable glutenin	-0.086	0.195	0.462
glutenin	-0.291	0.214	-0.051
gliadin	0.203	-0.269	-0.253
glutenin:gliadin ratio	-0.279	0.244	0.071
unextractable gliadin	-0.086	-0.107	-0.545
extractable gliadin	0.263	-0.208	0.081

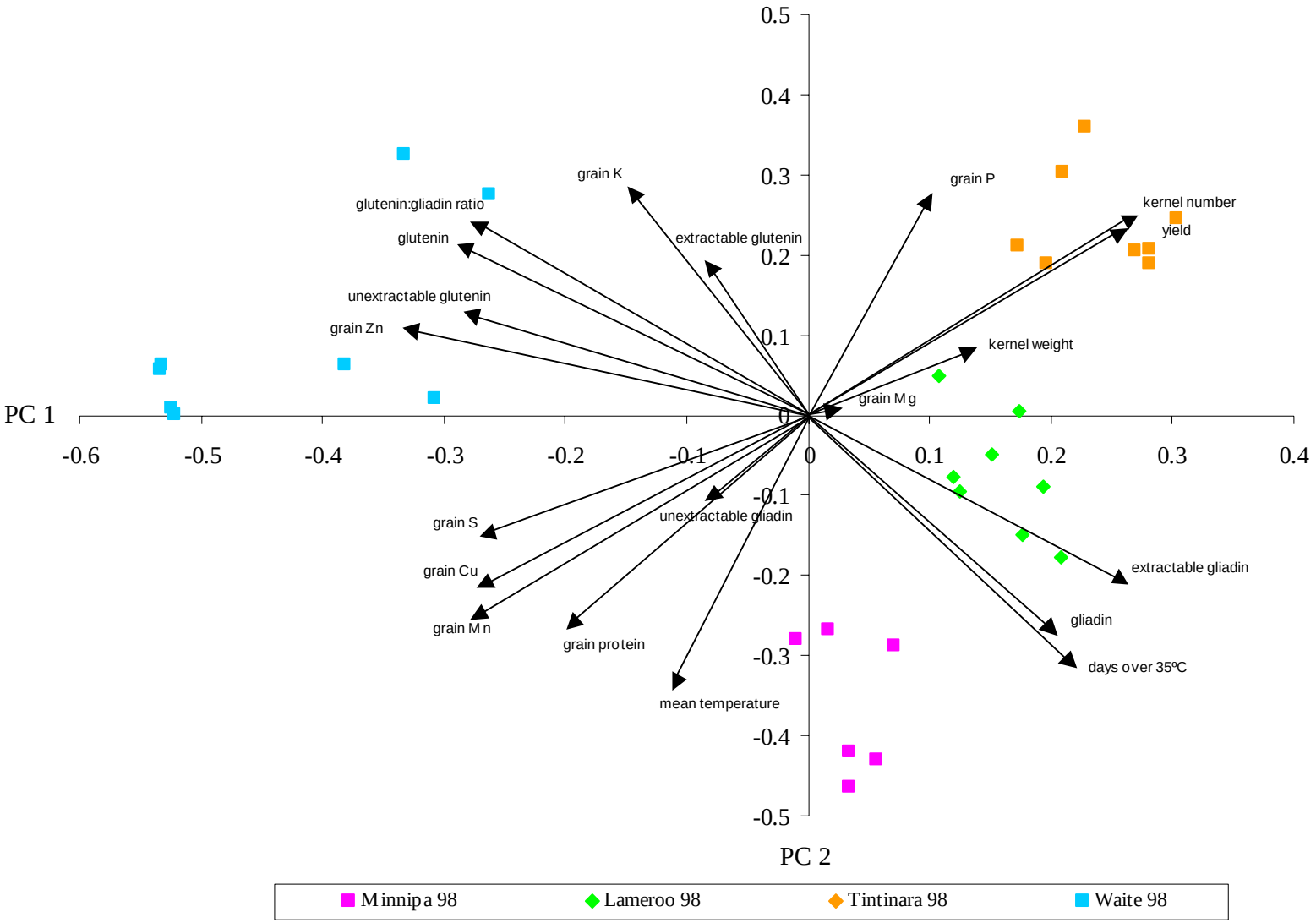


Figure 7.10. Principal component analysis of wheat grown at four field sites in 1998. An overlay of the biplot represents the component loadings for principal components 1 and 2 for each of the qualitative characters scored.

7.4 Discussion

Several studies have attempted to correlate wheat flour protein concentration with environmental factors, and it is generally accepted that as the maximum daily temperature increases during grain filling, so too does the proportion of protein relative to starch within the grain endosperm (Kolderup 1975, Randall and Moss 1990, Blumenthal *et al*. 1991b). The present results from the 1998 field trials are in agreement with this, with the flour protein concentration being highest at the warmest site, Minnipa, and lowest at the coolest site, Tintinara. Flour protein concentration also increased in two varieties at SD 2 of the Waite Institute in 1998, when the mean maximum temperature during grain filling was approximately 3ºC higher than at SD1. However this phenomenon was not observed in 1999, since the coolest site in these trials, Birchip, was found to have a higher flour protein concentration than Horsham. Rao *et al.* (1993), in their study of soft white winter wheat, also observed that some climatic variables do not have the same effect on protein content at all locations and years. These authors suggest that this is because the grain filling of wheat at a particular location in a given year is influenced by a number of variable climatic factors, which in turn influence the N availability from the soil and the soil moisture stress. Rao and co-workers explain that under drought conditions protein concentration in the grain should increase, which may help explain the higher-than-expected flour protein concentration at Birchip, perhaps a consequence of the dry spring of 1999. Both Horsham and Birchip experienced below average rainfall in 1999, with the April-October rainfall being 40 mm (16%) below average at Birchip, and 53 mm (17%) below average at Horsham (Chapter 5).

The present results showed no clear or consistent effect of Zn on flour protein concentration. This is in contrast to the results of Sadras *et al.* (2002) who examined data from 63 wheat crops in the Mallee region of south-eastern Australia over 3 growing seasons, and found a 0.26% increase in grain protein concentration with every mg Zn per kg soil. However Sadras and co-workers report a range of grain protein concentrations from 8.7% to 16.2%, with an average of 12.5%. This is much lower than the protein concentrations of the present experiment, which ranged from 12.7% to

17.8%, with an average of 14.9%. Where the present results do agree with those of Sadras *et al.*, i.e. at Tintinara in 1998 where an increase in protein concentration with Zn application was observed in the Zn-inefficient variety, was the site with the lowest flour protein concentration of any of the Zn-responsive trials.

The analysis of grain protein composition from the field experiments of 1998 and 1999 showed a greater percentage of gliadin in the flour protein of the *Glu-D1d* variety, Frame, than in the *Glu-D1a* variety, Goldmark. This was also observed in the results from the Waite Institute and growth room experiments, where the protein composition of Halberd and Meering was also analysed. The *Glu-D1d* varieties, Frame and Halberd, were found to have a higher percentage of gliadin, and lower percentage of glutenin, in the flour protein than the *Glu-D1a* varieties, Goldmark and Meering. At all sites this resulted in a significantly higher ratio of glutenin to gliadin in Goldmark and Meering. This is in agreement with other studies which have found a general tendency among Australian varieties for the 2+12 types to have a higher glutenin:gliadin ratio and stronger doughs than the 5+10 type wheats (Wrigley *et al.* 1982, Blumenthal *et al.* 1995b, Stone and Nicolas 1996b).

As a measure of protein composition the percentage of unextractable glutenin in the total amount of glutenin is important because it has been shown to be closely related to dough strength and is an important determinant of wheat quality for bread making (Preston *et al.* 1992, Gupta *et al.* 1992, 1993, 1995, Gupta and MacRitchie 1994, Jia *et al.* 1996). At the Waite Institute in 1998, the percentage of unextractable glutenin in total glutenin was reduced at SD 2, when the mean maximum temperature during grain filling was 3ºC higher than that of SD 1. Stone and Nicolas (1996a), in a study designed to determine the stage of grain growth at which fractional protein accumulation is most sensitive to heat stress, also found that the percentage of SDS-insoluble polymer in total polymer was consistently reduced by high temperature. Exposing two varieties of wheat differing in heat tolerance to 5 day periods of 40/19ºC day/night temperatures from 15 to 50 days after anthesis, these authors found that exposure to high temperature later in grain filling (30-50 DAA) had a greater effect on this protein fraction in the heat tolerant variety than high temperature applied earlier

(15-25 DAA). For the heat sensitive variety however, there was no discernible pattern to the effect of timing of high temperature on the percentage of SDS-insoluble polymer in total polymer at maturity. In the present study there was no significant genotype by sowing date interaction on the percentage of unextractable glutenin in total glutenin at maturity, however this may be because high temperatures (>35ºC) occurred at semi-regular intervals throughout the grain filling period of SD 2, and included both early and late episodes of heat stress.

The synthesis and composition of wheat seed storage proteins is affected by soil nutrient availability, particularly N and S (Wrigley *et al.* 1980, Shewry *et al.* 1995), but also P, K and Mg. Bonfil *et al.* (1997) showed that soil deficiencies of these nutrients could produce quantitative and qualitative differences in the HMW-glutenins and ω-gliadins, and they suggest that these mineral deficiencies may affect one or more aspects of seed storage protein processing, such as folding, assembly, transport or deposition. In one of the few studies regarding the effects of *micro*nutrient deficiencies in the soil on seed protein composition, Flynn *et al.* (1987) showed that flour from wheat grown under low Cu supply produced doughs that were over extensible and lacking in strength, despite having a higher grain protein content than the control plants. Although grain protein composition was not reported in this study, this suggests that an alteration to the glutenin:gliadin ratio has occurred under Cu deficiency, in favour of the gliadin fraction. Similarly in the present experiments the application of Zn in the form of Zn oxysulphate at sowing, plus a foliar spray during vegetative growth, significantly decreased the percentage of gliadins in the flour at the two most Zn-responsive sites in 1998, Lameroo and Tintinara. This in turn caused an increase in the glutenin:gliadin ratio at these sites. Since increases in the glutenin:gliadin ratio have been associated with positive effects on baking performance (Doekes and Wennekes 1982, Blumenthal *et al.* 1991a, 1993, 1994, Uthayakumaran *et al.* 1999), the results of the present investigation suggest that supplementary Zn nutrition may, under conditions of Zn deficiency, improve the baking quality of wheat flour. The glutenin:gliadin ratio increased with supplementary Zn in this study from 0.75 to 0.89 at Lameroo, and from 0.84 to 0.96 at Tintinara. Blumenthal *et al.* (1995), in a study of differences in dough quality among wheat varieties under heat stress, found highly significant weakening of dough properties with a decrease in

the glutenin:gliadin ratio from 0.76 to 0.71. This suggests that the increase in the glutenin:gliadin ratio with Zn fertilisation in the present experiments would have had an effect on baking quality, assuming that it is a linear response over the complete range. Additionally, Blumenthal *et al.* (1993) found that an increase in the reverse *gliadin:glutenin* ratio from 1.02 to 1.07 under heat stress also produced a significant dough weakening effect. In the present study the gliadin:glutenin ratio decreased with supplementary Zn fertilisation from 1.35 to 1.12 at Lameroo, and from 1.20 to 1.04 at Tintinara. Although Flynn and co-workers (1987) did not report the effect of soil Cu deficiency on grain protein composition, the present results and theirs both indicate that supplementary fertilisation of a micronutrient under deficient conditions can affect protein quality, irrespective of protein content.

A number of mechanisms by which Zn may affect the glutenin:gliadin ratio under deficient conditions can be hypothesised, since Zn is involved in many plant processes, including enzyme activity and nucleic acid synthesis and function. Flynn *et al.* (1987) proposed that low soil Cu availability may affect protein composition via an altered pattern of protein synthesis, since Cu deficiency is known to depress the activities of key enzymes. However the precise mechanism by which this may occur is not suggested. It is possible that Zn may also affect protein composition, and specifically the glutenin:gliadin ratio, through its role in enzyme activity. Under certain conditions, small amounts of gliadins can become incorporated into glutenin polymers through intermolecular disulphide bonds (Lew *et al.* 1992). It can therefore be hypothesised that this process is interrupted under Zn deficiency, since Zn is known to be involved in the activity of isomerase enzymes (Vallee and Falchuk 1993, Marschner 1995). It is possible that the activity of protein disulphide isomerase is reduced under Zn deficiency, resulting in less disulphide bond formation and an increase in gliadin percentage.

This theory does not adequately explain the increase in gliadins under Zn deficiency however, since the more general pathway of storage protein biosynthesis is an independent synthesis of gliadin and glutenin subunits (Panozzo *et al.* 2001). However another possible mechanism for the increased percentage of gliadin under Zn deficiency

may involve the role of Zn in DNA transcription and translation. It is well established that the primary control of the synthesis of storage proteins in wheat is at the level of gene transcription (Triboï *et al.* 2003). However protein synthesis is reduced under Zn deficiency because of a reduction in both DNA transcription and translation (Falchuk *et al.* 1978, Hanas *et al.* 1983). Moreover labelling studies have shown that the glutenins have a much greater level of ^{65}Zn incorporation than the other grain proteins; 47-65%, compared with 9-20% in albumins and globulins, 1-3% in gliadins and 10-28% in the remaining proteins (Starks and Johnson 1985). It appears then that glutenins may have a higher requirement for Zn, and therefore it is possible that gliadin synthesis continues at a greater rate than glutenin synthesis under conditions of Zn deficiency. It has also been shown that gliadin synthesis also continues at a greater rate than glutenin synthesis during a period of heat stress, and this is explained by the presence of heat shock elements upstream of the coding region of certain gliadin proteins (Blumenthal *et al.* 1990a, Ciaffi *et al.* 1996). It is therefore also possible that the synthesis of gliadin is more resilient than that of glutenin synthesis under other environmental stress conditions, including Zn deficiency.

The baking quality of wheat flour is not only affected by changes in the total amounts of glutenins and gliadins, but also by changes to the number and size of the subunits within the glutenin polymer (Huebner and Wall 1976). An increase in the number of HMW glutenin subunits results in a higher proportion of elastic HMW polymers, which in turn produce flours with increased dough strength (Gupta *et al.* 1993, 1995, Wrigley 1994). The results presented here from the 1998 field experiments showed a decrease in the percentage of glutenin unextractable in SDS (containing mostly HMW subunits), and a corresponding increase in the percentage of glutenin extractable in SDS (containing mostly LMW subunits), with the Zn application of soil plus foliar spray. This result was also seen in the growth room experiment of 2000, where the percentage of glutenin unextractable in SDS decreased with the application of Zn. It is worth noting here that the application of S fertiliser treatments has also been found to decrease the ratio of HMW to LMW glutenin subunits with a concomitant decrease in the percentage of glutenin unextractable in SDS (Castle and Randall 1987, MacRitchie and Gupta 1993). However this is because the LMW subunits of glutenin are relatively rich in S-

containing amino acids, whereas the HMW glutenin subunits are S-poor. Polypeptides of relatively high S content increase at the expense of the more S-poor polypeptides as the S supply is increased, resulting in a decrease in the HMW:LMW glutenin subunit ratio. The finding that the HMW:LMW ratio decreases with the application of Zn indicates that the effect of Zn nutrition on dough strength may be more complex than that suggested above. Although the glutenin:gliadin ratio may increase with supplementary Zn nutrition, suggesting an increase in dough strength, the percentage of HMW subunits within the total glutenin appears to decrease, suggesting a decrease in dough strength. Furthermore Wardlaw *et al.* (2002) showed that a decrease in the percentage of unextractable glutenins, from 44% to 36%, was sufficient to produce a dough weakening effect under heat stress. In the present study, the percentage of unextractable glutenins decreased with supplementary Zn fertilisation from 51% to 38% at Lameroo, and from 53% to 41% at Tintinara. Clearly further examination of these doughs, produced with and without supplementary Zn at Zn deficient sites, is required to improve our understanding of the overall effect of Zn nutrition on baking quality.

Another aspect of mineral nutrition and baking quality is the relationship between grain (or flour) nutrient *concentration* and protein composition. With the exception of N and S, this relationship has been little studied. Two studies have shown that grain P concentration is negatively correlated with baking quality (Bequette *et al.* 1963, Douglas and Dyson 1985), while the grain concentration of Mn (Bequette *et al.* 1963), K, Mg and Mo (Douglas and Dyson 1985) have also shown a negative relationship with baking quality. In contrast, Ca has been found to exert a positive influence on baking quality (Bequette *et al.* 1963). The present study showed a positive correlation between grain Zn concentration and the glutenin:gliadin ratio, particularly for the 1998 field sites, and this appeared to be the result of an increase in glutenin percentage, rather than a decrease in gliadin percentage. PCA analysis also showed that the concentration of Zn in the grain was positively correlated with glutenin in the grain, and negatively correlated with gliadin percentage. One anomaly with the PCA analysis was that the results showed that grain Zn concentration correlated better with unextractable glutenin, rather than extractable glutenin, which does not agree with the results of Zn *supply* on grain protein composition. Further investigation is therefore warranted in order to

clarify the effects of Zn fertilisation on the proportions of unextractable and extractable glutenins in wheat grain protein.

Although the present study found no significant interaction between Zn nutrition and high temperature during grain filling on protein concentration or composition, PCA analysis did show a negative association between grain Zn concentration and the number of days over 35ºC during grain filling. Since high temperature stress is also known to lower the glutenin:gliadin ratio, it can be postulated that this particular component of grain protein will be most affected when the two stresses of Zn deficiency and heat stress occur in combination.

7.5 Conclusion

The results of this study have shown that supplementary Zn fertilisation, under conditions of Zn deficiency, can have an effect on wheat grain protein composition, if not protein concentration. Elevated levels of Zn nutrition at Zn responsive sites were found to increase the ratio of glutenins to gliadins in the grain, and this was mainly due to a decrease in gliadin percentage. Since an increase in the glutenin:gliadin ratio is associated with an increase in baking quality, it can be hypothesised that supplementary Zn nutrition may improve the bread-making quality of wheat under conditions of Zn deficiency. However since the present study also found that the percentage of HMW glutenin subunits (also correlated with baking quality) decreased with Zn fertilisation, further tests of dough quality under Zn deficient conditions are required. The results also highlight the possibility of a negative relationship between grain Zn concentration and heat stress, suggesting that the negative effects of Zn deficiency and high temperature stress on grain protein composition will be more adverse when these stresses occur in combination.

CHAPTER 8

PHYSIOLOGICAL, BIOCHEMICAL AND STRUCTURAL RESPONSES OF WHEAT TO ZINC DEFICIENCY AND HEAT STRESS DURING VEGETATIVE GROWTH

8.1 Introduction

Chlorophyll fluorescence measurements, described in earlier chapters (Chapters 4, 5 and 6), have shown that zinc (Zn) can play a role in maintaining photosynthetic activity during a short period of heat stress, both at the vegetative stage and during grain filling. However the mechanisms by which elevated Zn nutrition may be able to provide the photosynthetic apparatus with some level of tolerance to high temperature stress remain unknown.

Inhibition of thylakoid membrane activity is the main form of high temperature injury to chloroplasts (Krause and Santarius 1975, Armond *et al.* 1980), as well as changes in the permeability of the envelope and tonoplast (Bauer and Senser 1979, McCain *et al.* 1989). Electron microscopy of chloroplast ultrastructure has revealed that high temperatures result in swollen thylakoids (Ristic and Cass 1992, Stoyanova and Yordanov 1999), progressive destacking and rearrangement of the granum stacks (Gounaris *et al.* 1984, Sayed *et al.* 1986), and loss of envelope integrity, resulting in chloroplast rupture (Bauer and Senser 1979, McCain *et al.* 1989). Examination of chloroplasts under Zn deficiency has revealed similar morphological changes, including reduced numbers of thylakoids per granum (Shrotri *et al.* 1978, Gui-chang and Zhao-ming 1984), altered orientation of the granum stacks (Thomson and Weier 1962), chloroplast swelling and rupture of the chloroplast envelope (Wang *et al.* 1993). This suggests that adequate Zn nutrition may be able to reduce the damage to chloroplast structure caused by high temperatures, through its role in maintaining the structure of biomembranes. However there have been no studies that have looked at the effects of simultaneous high temperature and Zn deficiency on chloroplast structure.

Damage to plant membranes by heat stress is largely due to increased generation of reactive oxygen species (ROS) (Kraus and Fletcher 1994, Liu and Huang 2000). To prevent accumulation of these toxic ROS, plants employ a system of physiological antioxidants, one of which is the superoxide dismutase (SOD) enzyme, which scavenges the superoxide radical molecule ($O_2 \cdot^-$) in chloroplasts (Foyer and Harbinson 1994). SODs are classified into three types according to their metal co-factor; manganese (Mn)-SOD, iron (Fe)-SOD and copper/zinc (Cu/Zn)-SOD (Bowler *et al.* 1992). While Cu/Zn-SOD and Mn-SOD are found among all plant species, Fe-SOD is more characteristic of dicotyledonous plants, and there have been no reports of the presence of Fe-SOD in wheat (Cakmak *et al.* 1997, Kaminaka *et al.* 1999, Alshcer *et al.* 2002). Zn deficiency has been found to decrease Cu/Zn-SOD activity in cereals (Cakmak *et al.* 1997, Obata *et al.* 1999) and these decreases in activity are associated with an increase in the amount of $O_2 \cdot^-$ (Cakmak and Marschner 1988a, b). Zinc is therefore an important factor in plant defence systems against destructive ROS, and it has been suggested that improvement of the Zn nutritional status of plants may reduce their sensitivity to oxidative stress factors, including heat stress (Cakmak 2000). Increased activity of Cu/Zn-SOD has been shown to enhance plant resistance to a number of oxidative stress factors, including O_3 (Pitcher and Zilinskas 1996), salinity (Van Camp *et al.* 1996), drought (Mittler and Zilinskas 1994, Yu and Rengel 1999) and low temperatures (Sen Gupta *et al.* 1993a), however the activity of Cu/Zn-SOD in plants affected by heat stress has been largely overlooked. Bakardjieva and co-workers (2000) have shown that Zn ions will increase the activity and thermostability of extracted SOD isoforms *in vitro*, but to date there have been no studies that document the sensitivity of Zn deficient plants, with reduced SOD activity, to heat stress *in vivo*.

In order to redress these gaps in our understanding of the role of Zn in providing thermotolerance to plants, the following study was conducted to investigate the effect of Zn nutrition on plant growth, photosynthetic activity, cell ultrastructure and Cu/Zn-SOD activity of wheat seedlings subjected to high temperature stress. Genotypes which differ in Zn efficiency and thermotolerance were grown at four levels of Zn supply and subjected to heat stress, with the following aims: (1) to verify that the improvement observed in the photosynthetic activity of seedlings supplied with adequate Zn under

heat stress (Chapter 4) is reproducible in other Zn-inefficient genotypes; (2) to determine whether elevated levels of Zn nutrition may reduce the damage to chloroplast structure caused by high temperatures; and (3) to assess whether an increase in activity of Cu/Zn-SOD is related to the improved photosynthetic activity of Zn sufficient plants under high temperature stress described in earlier chapters.

8.2 Materials and methods

Laffer sand was collected and prepared as described in Chapter 3 (Section 3.3), and 1.4 kg was weighed into polythene-lined plastic pots (140 mm x 110 mm x 110 mm) of 1.2 L volume. Basal nutrients were applied as described in Chapter 3, together with four Zn treatments; 0, 0.2, 2 and 20 mg Zn kg^{-1} soil, applied as $ZnSO_4.7H_20$. Designated as Zn_0, $Zn_{0.2}$, Zn_2 and Zn_{20}, these four Zn treatments were expected to provide deficient, marginal, adequate and luxurious levels of Zn fertilisation, respectively. Nanopure water was added to each pot to bring the soil water content to field capacity (12% w/w), and this was maintained by daily watering to weight. A solution of NH_4NO_3 (120 mg N kg^{-1} dry soil) was applied to each pot at 22 days after sowing (DAS).

Four wheat genotypes differing in Zn efficiency and heat tolerance were used in this experiment. These cultivars were Meering (Zn efficient and thermosensitive), Halberd (Zn efficient and thermotolerant), Frame (moderately Zn efficient and thermotolerant with respect to photosynthetic activity) and Goldmark (Zn inefficient and moderately thermosensitive) (Wrigley *et al.* 1994, Stone and Nicolas 1995a, McDonald *et al.* 2001 and Chapters 5 and 6). Seed was obtained from the field experiment at Tintinara (Chapter 5) and all genotypes had similar seed Zn contents: 500, 550, 540 and 530 ng/seed for Meering, Halberd, Frame and Goldmark, respectively. Seeds were prepared and germinated as described in Chapter 3 (Section 3.4). Fourteen uniformly germinated seeds with radicle emerged were sown into each pot, and following emergence, plants were thinned to give a final population of twelve uniform plants per pot. A layer of acid washed black polypropylene beads was placed over the soil surface of each pot to a

depth of approximately 10 mm to prevent excess soil moisture loss by evaporation.

Plants were grown in a growth room at 22/16ºC day/night temperature, 14 h photoperiod and 725 µmol s^{-1} m^{-2} light intensity at the plant canopy level. Heat treatments were applied for a duration of 6 days starting from 30 DAS. Heat-treated plants were moved during the night cycle to a second growth room in which the night temperature of 20ºC was maintained for 10 h and the peak of 40ºC day temperature was kept for 8 h. The rate of heating to, and cooling from, the day temperature maximum was 7˚C h^{-1}. The light intensity in this growth room was 715 µmol s^{-1} m^{-2} at the plant canopy level. Heat-treated plants were watered to weight four times daily to ensure an adequate level of soil moisture was maintained. No visible symptoms of plant water deficit were evident during the heat treatment period.

Leaf chlorophyll content was measured on the youngest emerged leaf blades (YEBs) of 2 plants per pot at 39 DAS, as described in Chapter 3 (Section 3.5.2). Chlorophyll fluorescence measurements were made on YEBs of 2 plants per pot at regular intervals from 25 DAS until the end of the experiment, as described earlier (Chapter 3, Section 3.5.1). Each measurement was recorded at the same time of day for each genotype, at 4-5 h into the light period.

The YEBs of two plants per pot of the varieties Frame and Goldmark were harvested at 28, 31 and 39 DAS, 7 h into the light period, and placed immediately in liquid N before being stored at –80ºC for SOD assays. In addition, leaf pieces of approximately 1 cm^2, excluding necrotic areas, were harvested 2 cm from the tip of the YEBs of Frame and Goldmark at 33 DAS, also 7 h into the light period. These samples were placed immediately in 2.9 mL of EM fixative (1.25% glutaraldehyde, 4% sucrose, 4% paraformaldehyde in phosphate buffered saline, pH 7.2) and stored at 4ºC for examination by transmission electron microscopy.

Measurements of leaf length were taken prior to the heat stress treatment (28 DAS), and every alternate day from then on until the end of the experiment. Two plants from each pot were harvested prior to the heat stress treatment (28 DAS), during the heat stress

treatment (34 DAS) and again at the end of the experiment (39 DAS). Shoots were dried at 80˚C for 48 h and weighed to determine dry weight. The relative growth rate (RGR) was calculated by the equation

$$RGR = (\ln W_2 - \ln W_1)/(t_2 - t_1)$$

where W_2 = the final shoot weight; W_1 = the initial shoot weight; t_2 = time of final harvest; and t_1 = time of initial harvest. The YEBs from two plants of each replicate were sub-sampled at 39 DAS, and analysed for Zn and other nutrient concentrations by ICP spectrometry, as described in Chapter 3 (Section 3.6).

The experiment was set up as a factorial, completely randomised block design (4 genotypes x 4 Zn levels x 2 temperature treatments) with four replicates. The sowing of each replicate was staggered, seven days apart, in order to allow sufficient time for all measurements to be taken. Results were statistically analysed as explained in Chapter 3 (Section 3.7).

The activity of SOD (EC 1.15.1.1) was assayed by monitoring the inhibition of photochemical reduction of nitro blue tetrazolium (NBT) according to the method of Giannopolitis and Ries (1977) as modified by Dhindsa *et al.* (1981). Seven hundred milligrams of leaf tissue (without necrotic areas) was homogenised on ice for 2 min in a mortar and pestle containing 7 mL of 50 mM Hepes buffer and 0.1 mM Na_2EDTA at pH 7.6. The homogenate was centrifuged twice at 13 200 *g* for 15 min at 4ºC and the supernatant was used for assay of the enzymes. The reaction mixture (5 mL) contained 50 mM Hepes (pH 7.6), 0.1 mM EDTA (pH 8.0), 50 mM Na_2CO_3 (pH 10.4), 13 mM methionine, 0.025% (w/v) Triton X-100, 75 µM NBT, 2 µM riboflavin and the enzyme aliquot (0 to 500 µL). Riboflavin was added last and the tubes were shaken before being placed in front of a light bank consisting of two 15 W fluorescent lamps. The reaction was started by switching on the light and the reaction mixtures were illuminated for 10 min at a light intensity of 270 µM m^{-2} s^{-1}. The reaction was stopped by switching off the light and the tubes were covered with a black cloth. One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the rate of NBT reduction measured at 560 nm using a Sequoia Turner spectrophotometer (Sequoia Turner Corporation, Mountain View, CA, USA). Identical

reaction mixtures that had not been illuminated were used to correct for background absorbance. Activities of the three different forms of SOD have been distinguished from each other on the basis of their sensitivities to cyanide and H_2O_2. Activity of Cu/Zn-SOD is inhibited by cyanide, while Fe-SOD is not sensitive to cyanide but is inhibited by H_2O_2. Mn-SOD is unaffected by both H_2O_2 and cyanide (Scandalios 1993). Activity of Mn-SOD was measured after addition of potassium cyanide (KCN) and H_2O_2 to the assay solution at a final concentration of 3 mM and 5 mM, respectively. Preliminary studies of Fe-SOD activity, obtained by subtracting Mn-SOD activity from the activity yielded in the presence of 3 mM KCN only, demonstrated that the activity of Fe-SOD was not measurable. Activity of Cu/Zn-SOD was therefore determined by subtracting Mn-SOD activity from the total SOD activity in assay solutions containing no KCN or H_2O_2.

Leaf segments for transmission electron microscopy were recut to 1 mm^2 under glutaraldehyde and washed twice in phosphate buffered saline plus 4% sucrose, for 10 min each time. Samples were post-fixed in 1% osmium tetroxide for 2 h and dehydrated in a graded series of acetone (70%, 90%, 95%, 100%). Samples were then infiltrated overnight with a mixture of epoxy resin and 100% acetone (1:1), followed by three changes of 100% epoxy resin of 8 h each, and embedded in fresh resin. Polymerisation was completed in 24 h at 70ºC. Ultra thin sections of 70 nm were cut on a diamond knife (Diatome, Fort Washington, PA, USA) using a Reichert Ultracut E Ultramicrotome (Leica-Reichert, Vienna, Austria), and collected from the knife's water bath on 200 mesh Cu/Pd grids. The sections were stained for 20 min with 5% uranyl acetate in 70% ethanol, followed by 20 min with 0.4% lead citrate (Reynolds 1963). Sections were examined with a Philips CM100 Transmission Electron Microscope (Philips Electron Optics, Eindhoven, The Netherlands) at an accelerating voltage of 80 kV, and photographed with an analySIS® Mega-View II digital image capture system. Qualitative observations were made of approximately 120-150 randomly chosen mesophyll cells in each plant, photographs were taken of at least three random sites in three sections, and representative pictures are presented.

8.3 Results

8.3.1 Leaf length

Both Zn deficiency and high temperature stress reduced leaf length in this experiment, but there was no interaction between the two stresses. Supplementary Zn fertilisation increased the length of leaf 5 in all varieties, by 4%, 5% and 7% for the Zn treatments of $Zn_{0.2}$, Zn_2 and Zn_{20}, respectively (Table 8.1). All three supplementary Zn treatments also increased the length of leaf 6, with the exception of the $Zn_{0.2}$ treatment in Meering, which did not differ significantly from the length of leaves receiving no additional Zn. In the other three varieties, Frame, Goldmark and Halberd, there was no significant difference between the three supplementary Zn treatments on the length of leaf 6. The length of leaf 7 varied to a greater extent between genotypes. In the Zn-inefficient variety, Goldmark, each of the treatments with added Zn increased the length of leaf 7 relative to the Zn_0 treatment by an average 29%, with no significant difference among the three. A similar result was found in Meering, which produced leaves at a faster rate than the other three varieties. All three supplementary Zn treatments increased the length of leaf 7 by an average 12% in Meering, again with no significant difference among the three. In the Zn-efficient varieties, Halberd and Frame, the length of leaf 7 was largely unaffected by Zn fertilisation, with the exception of Zn_{20} in Frame, which produced leaves that were 19% shorter than the other treatments of Zn_0, $Zn_{0.2}$ and Zn_2.

The high temperature treatment of 40/20ºC for 6 days from 30 DAS significantly reduced the length of the actively elongating leaf, leaf 7, in three of the four varieties, Frame, Goldmark and Halberd (Table 8.2). The length of this leaf was reduced by the heat stress by between 24% and 32% in these varieties, relative to the control plants grown at 22/16ºC throughout the experiment. In Meering, however, the length of leaf 7 was unaffected by the high temperature treatment. This cultivar was observed to produce leaves at a faster rate than the other three varieties, with leaf 7 elongating between 29 and 36 DAS, whereas leaf 7 of Frame, Goldmark and Halberd elongated between 31 and 38 DAS. The length of earlier produced leaves, 5 and 6, was unaffected by the high temperature treatment in all varieties.

Table 8.1. Effects of zinc fertilisation (mg kg^{-1} soil) on the leaf length (mm) of wheat genotypes at 39 DAS.

Genotype	Leaf 5					Leaf 6					Leaf 7				
	Zn 0	**Zn 0.2**	**Zn 2**	**Zn 20**	***Mean***	**Zn 0**	**Zn 0.2**	**Zn 2**	**Zn 20**	***Mean***	**Zn 0**	**Zn 0.2**	**Zn 2**	**Zn 20**	***Mean***
Frame	295	293	310	321	*305*	226	244	258	245	*243*	169	166	155	137	*157*
Goldmark	289	303	304	311	*302*	205	256	253	263	*245*	133	176	165	174	*162*
Halberd	337	358	356	361	*353*	213	263	258	284	*255*	169	173	169	169	*170*
Meering	284	299	298	298	*295*	224	236	242	250	*238*	181	206	200	202	*197*
Mean	*301*	*313*	*317*	*323*		*217*	*250*	*253*	*261*		*163*	*180*	*172*	*171*	
LSD$_{0.05}$															
Genotype			**7**					**9**					**7**		
Zinc			**7**					**9**					**7**		
Genotype x Zinc			**n.s.**					**18**					**15**		
CV(%)			**4.2**					**7.3**					**8.8**		

n.s. = non-significant

Table 8.2. Effects of temperature treatment on the leaf length (mm) of wheat genotypes at 39 DAS. Leaf 7 was the actively expanding leaf during the heat treatment in three of the four varieties, Frame, Goldmark and Halberd, while Leaf 8 was the actively expanding leaf during the high temperature treatment in Meering.

Genotype	Leaf 5			Leaf 6			Leaf 7		
	22/16ºC	40/20ºC	*Mean*	22/16ºC	40/20ºC	*Mean*	22/16ºC	40/20ºC	*Mean*
Frame	305	305	*305*	250	237	*243*	178	134	*157*
Goldmark	300	304	*302*	248	241	*245*	181	143	*162*
Halberd	352	354	*353*	256	253	*255*	188	152	*170*
Meering	295	294	*295*	241	235	*238*	201	193	*197*
Mean	*313*	*314*		*249*	*242*		*187*	*156*	
$LSD_{0.05}$									
Temperature		**n.s.**			**n.s.**			**23**	
Genotype		**7**			**9**			**7**	
Temperature x Genotype		**n.s.**			**n.s.**				
(a)								**11**	
(b)								**23**	
CV(%)		**4.2**			**7.3**			**8.8**	

(a) For comparisons within the same genotype

(b) For other comparisons

n.s. = non-significant

8.3.2 Chlorophyll content

Both high temperature and Zn treatment significantly affected the chlorophyll content of plants in this experiment, but there was no significant interaction between temperature treatment and Zn fertilisation. High temperature significantly reduced the chlorophyll content in the YEBs of two of the four varieties, Goldmark (20%) and Meering (10%) (Figure 8.1). Zn fertilisation also affected the chlorophyll content in the YEBs, with no significant differences between genotypes. The $Zn_{0.2}$ treatment were found to have a significantly lower chlorophyll content than either the Zn_0 or Zn_2 plants, while the Zn_{20} treatment was significantly lower again, containing 6% less chlorophyll than the $Zn_{0.2}$ treatment, and 13% less chlorophyll than the Zn_0 and Zn_2 treatments (Figure 8.2).

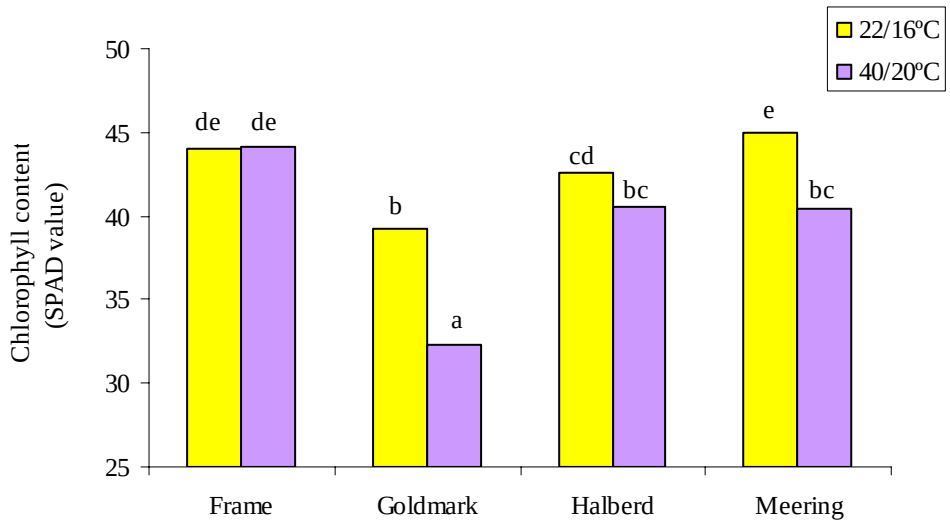


Figure 8.1. Effect of temperature treatment on the chlorophyll content of the YEBs of wheat seedlings at 39 DAS. Data are the mean of all Zn treatments. Means sharing a common letter are not significantly different from each other at the *P*=0.05 level.

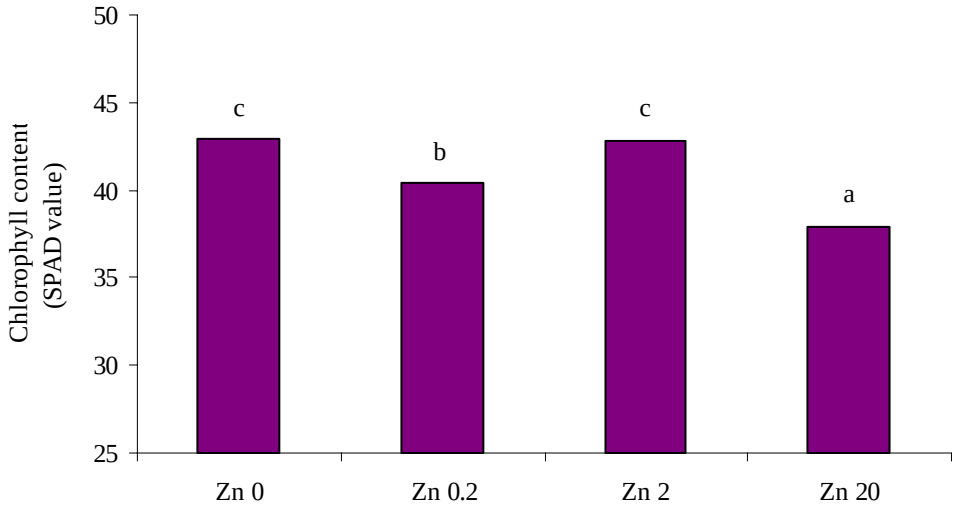


Figure 8.2. Effect of Zn fertilisation (mg kg^{-1} soil) on the chlorophyll content of the YEBs of wheat seedlings at 39 DAS. Data are the mean of both genotypes and both temperature treatments. Means sharing a common letter are not significantly different from each other at the *P*=0.05 level.

8.3.3 Chlorophyll fluorescence

Plants subjected to the six-day period of high temperature stress showed a significantly lower chlorophyll fluorescence ratio (Fv/Fm) than the control plants throughout the heat stress period (Figure 8.3a), with no significant difference between varieties. The Fv/Fm was found to be an average 15% lower in the 40/20ºC treatment on the first day of heat stress, after which plants recovered to some extent, and remained 7-9% lower for the rest of the heat stress period. Upon return to 22/16ºC conditions at 36 DAS, however, the Fv/Fm recovered rapidly, and no difference between control and heat stressed plants was found 15 h after removal from the heat stress.

Supplementary Zn fertilisation affected the Fv/Fm ratio of the heat-treated plants during the first four days of the heat stress period, with no difference between varieties. At 30 DAS, the first day of high temperature stress, the Fv/Fm ratio of the Zn_2 plants was significantly higher than the other Zn treatments, with an average value of 0.749. The Zn_0 treatment showed the next highest Fv/Fm ratio, with an average value of 0.730,

significantly higher than the $Zn_{0.2}$ and Zn_{20} treatments, each with an average Fv/Fm value of 0.692 and 0.695, respectively. The average Fv/Fm value of the control plants, maintained at 22/16ºC, was 0.826 at 30 DAS, with no significant difference between Zn treatments. At 31 DAS, 39 h into the high temperature treatment, the Fv/Fm ratio of all heat stressed plants showed some level of recovery. The Zn_0 plants at 31 DAS were not significantly different to the Zn_2 plants, with Fv/Fm ratios of 0.762 and 0.771, respectively. The $Zn_{0.2}$ and Zn_{20} plants remained significantly lower, 0.743 and 0.746 respectively, while the control plants maintained at 22/16ºC were again showing an Fv/Fm value of 0.826. This trend continued for the subsequent two days of heat stress, with the Zn_0 and Zn_2 treatments remaining significantly higher than the $Zn_{0.2}$ and Zn_{20} treatments. By 34 DAS however (the 5th day of 40/20ºC) there was no significant difference between the Fv/Fm ratios of any of the Zn treatments subjected to high temperature stress, with an average value of 0.772, compared with the 22/16ºC control plants' Fv/Fm of 0.822.

The differences in Fv/Fm observed between Zn treatments under high temperature stress were largely due to the effects on chlorophyll initial fluorescence (Fo) (Figure 8.3b), rather than effects on chlorophyll maximum fluorescence (Fm) (Figure 8.4a). At 30 DAS Fo was a significant 6% higher in the Zn_0 plants than the Zn_2 plants at 40/20ºC, and 8% higher again in the $Zn_{0.2}$ and Zn_{20} plants, with no significant difference between the latter two treatments. Conversely there was no difference in Fm between the Zn_2 and Zn_0 treatments, and only a 6% difference between these two treatments and the $Zn_{0.2}$ and Zn_{20} treatments. The observed values for chlorophyll variable fluorescence (Fv) were similar to those observed for Fm (Figure 8.4b). Following this first day of heat stress, the Fm of plants subjected to high temperature stress varied little between Zn treatments, whereas the Fo remained significantly lower in the Zn_2 plants at 31 DAS, with no difference between this treatment and those plants maintained at 22/16ºC. The next treatment to recover to that of the control plants was Zn_0, at 32 DAS, followed by Zn_{20} at 33 DAS, and $Zn_{0.2}$ at 34 DAS. This also shows that the Fo component of chlorophyll fluorescence is responsible for the partial recovery of Fv/Fm during the heat stress period, since the Fm component was found to recover slightly, remaining at 28–34% below that of the control plants throughout the six days of high temperature.

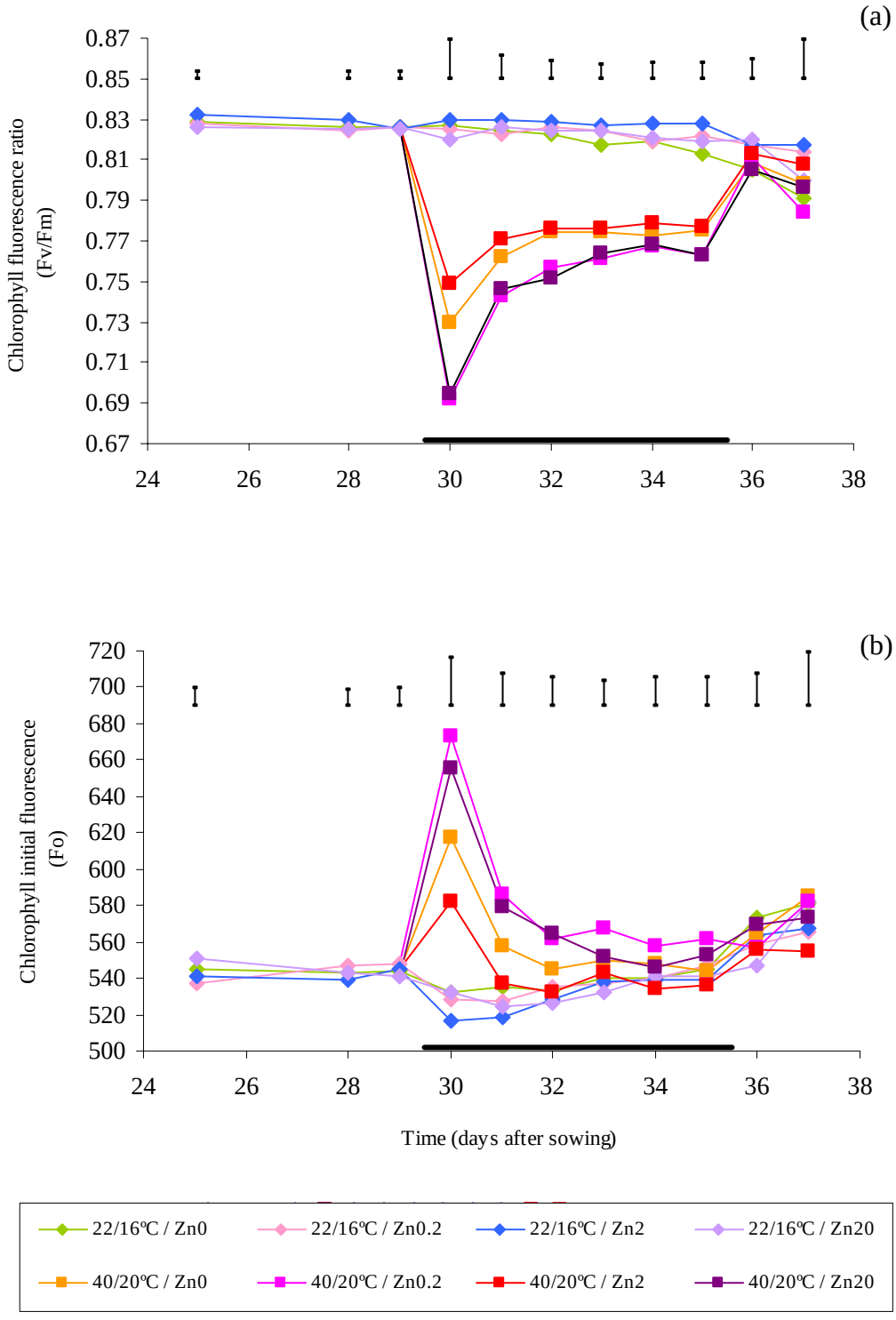


Figure 8.3. Effects of Zn supply (mg kg^{-1} soil) and temperature treatment on the chlorophyll fluorescence ratio (a) and chlorophyll initial fluorescence (b) of wheat YEBs from 25 DAS. Vertical bars represent the $LSD_{0.05}$ for the interaction of Zinc x Temperature. Horizontal bars on x-axes represent the period of high temperature treatment.

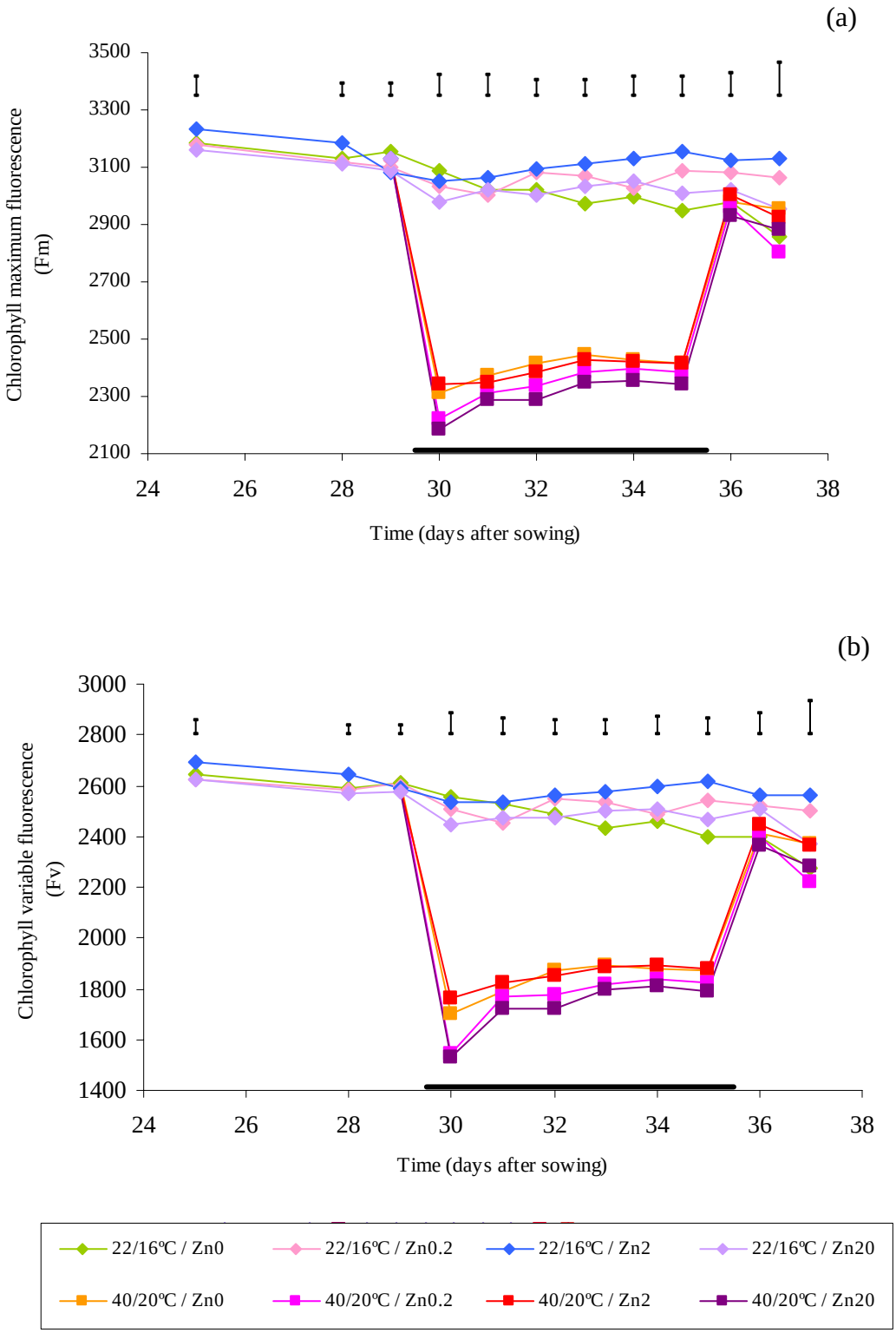


Figure 8.4. Effects of Zn supply (mg kg^{-1} soil) and temperature treatment on chlorophyll maximum fluorescence (a) and chlorophyll variable fluorescence (b) of wheat YEBs from 25 DAS. Vertical bars represent the $LSD_{0.05}$ for the interaction of Zinc x Temperature. Horizontal bars on x-axes represent the period of high temperature treatment.

8.3.4 Shoot dry matter production

Shoot dry matter was significantly affected by Zn fertilisation at all harvests, with no significant differences between varieties (Figure 8.5). At the first harvest, 28 DAS, shoot growth was depressed in the Zn_0 and Zn_{20} treatments, which were an average of 9% lighter than the $Zn_{0.2}$ and Zn_2 treatments. At the second harvest, 34 DAS, only the treatment with no supplementary Zn, Zn_0, showed a significantly lower shoot dry weight, an average 15% lower than the other three Zn treatments. By the third harvest at 39 DAS however, the shoot dry weights of the Zn_{20} treatment were once again significantly lower than the $Zn_{0.2}$ or Zn_2 treatments (7%). Shoot growth in the treatment without any supplementary Zn, Zn_0, was further depressed at 39 DAS, being 15% lower than the Zn_{20} treatment and 20% lower than the $Zn_{0.2}$ and Zn_2 treatments.

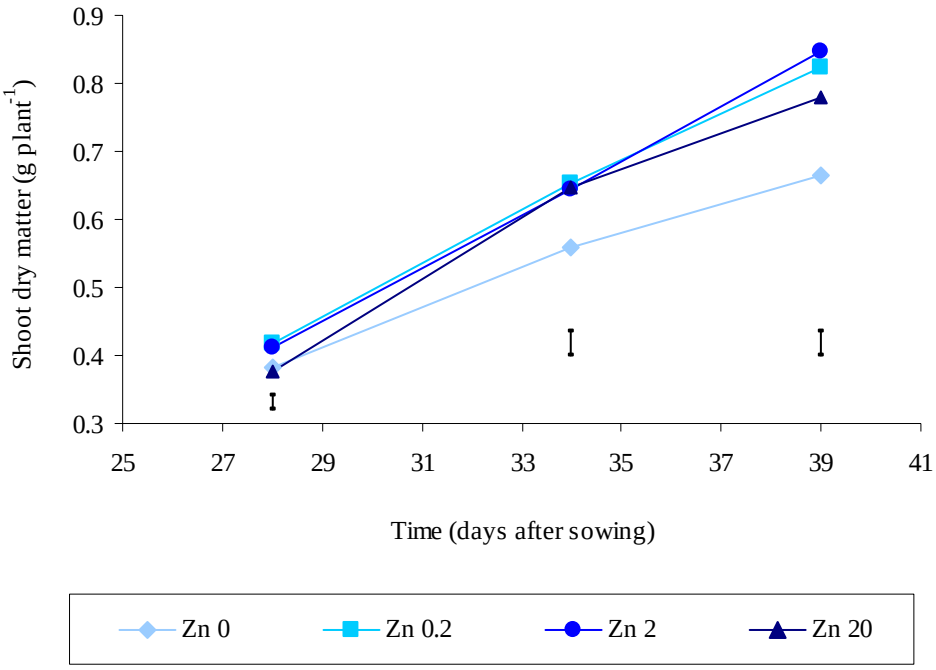


Figure 8.5. Effect of Zn fertilisation (mg kg^{-1} soil) on the accumulation of above ground biomass by wheat seedlings from 28 DAS. Each data point is the mean of all genotypes and both temperature treatments. Vertical bars represent the $LSD_{0.05}$ for the main effect of Zinc.

The high temperature treatment of 40/20ºC affected shoot dry matter production at the second harvest (5 days after the beginning of the heat stress period) and at the third harvest (4 days after the end of the heat stress period) with a significant temperature by genotype interaction (Figure 8.6). The shoot dry weight of the thermotolerant variety, Frame, was unaffected by the high temperature treatment, as was the dry weight of Goldmark (moderately thermosensitive). In contrast the shoot dry weight of the thermosensitive wheat variety, Meering, was reduced by 11% at harvest 2, while that of Halberd was reduced both at harvest 2 (9%) and at harvest 3 (17%). There was no significant interaction between Zn fertilisation and temperature treatment on shoot dry matter production.

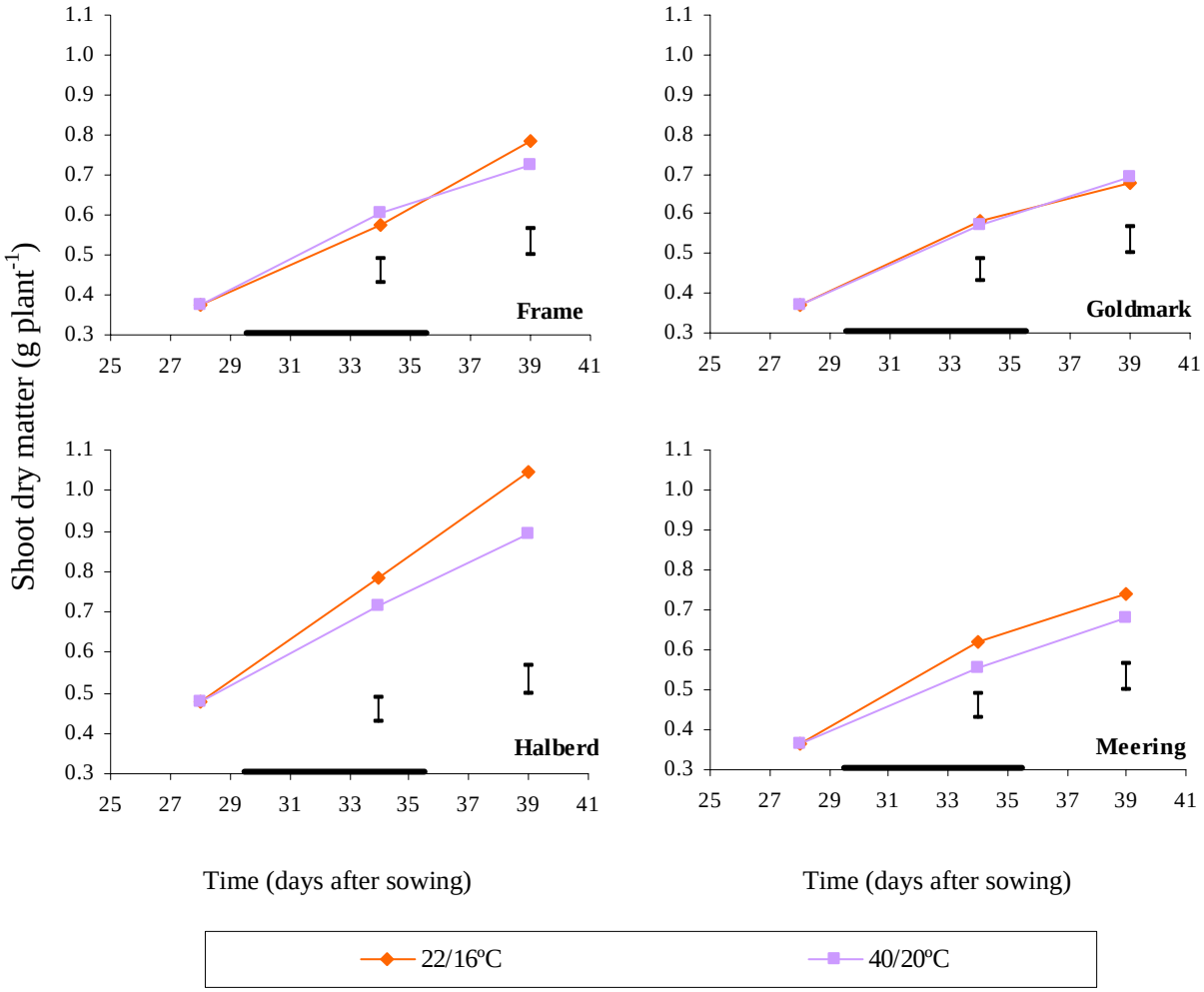


Figure 8.6. Effect of temperature treatment on the accumulation of above ground biomass by wheat genotypes from 28 DAS. Vertical bars represent the $LSD_{0.05}$ for the Genotype x Temperature interaction. Horizontal bar on the x-axis represents the period of high temperature treatment.

The relative growth rate of wheat seedlings in this experiment differed between harvests and Zn fertilisation treatments (Figure 8.7). Between 28 and 34 DAS the relative growth rate of seedlings increased with increasing Zn fertilisation, with the rate of growth of the Zn_{20} treatment being significantly greater than that of the Zn_0 treatment. From 34 to 39 DAS however, which encompassed the final two days of high temperature stress, the relative growth rate of the adequate Zn treatment, Zn_2, was significantly higher than both that of the treatment with no supplementary Zn, Zn_0, and that of the treatment with very high Zn fertilisation, Zn_{20}. There was no effect of temperature on these relative growth rates.

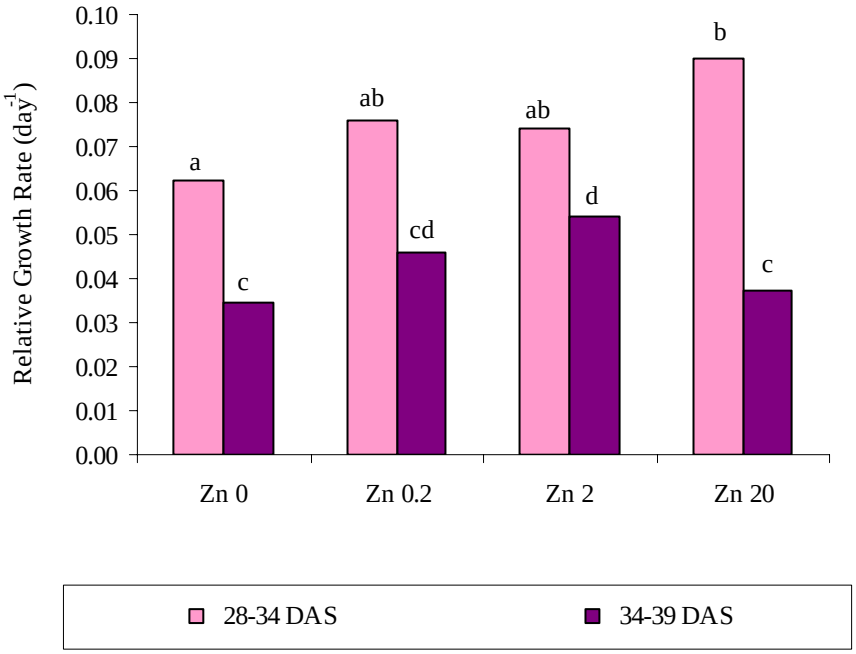


Figure 8.12. Effects of Zn fertilisation (mg kg^{-1} soil) on the relative growth rate of wheat seedlings from 28-34 DAS and from 34-39 DAS. Means sharing a common letter are not significantly different from each other at the *P*=0.05 level.

8.3.5 Superoxide dismutase activity

An increase in Zn fertilisation generally increased the total activity of SOD in the YEBs of wheat seedlings, with the exception of Goldmark at the third harvest (39 DAS). At the first harvest, prior to the imposition of the heat stress, total SOD activity in the YEBs was significantly greater in the Zn_2 and Zn_{20} treatments than in the Zn_0 and $Zn_{0.2}$ treatments (Figure 8.8a), with no difference between varieties. This increase in total SOD activity with Zn fertilisation was due to a greater Cu/Zn-SOD activity in the Zn_2 and Zn_{20} treatments (Figure 8.8b), since Mn-SOD activity was not affected by Zn fertilisation.

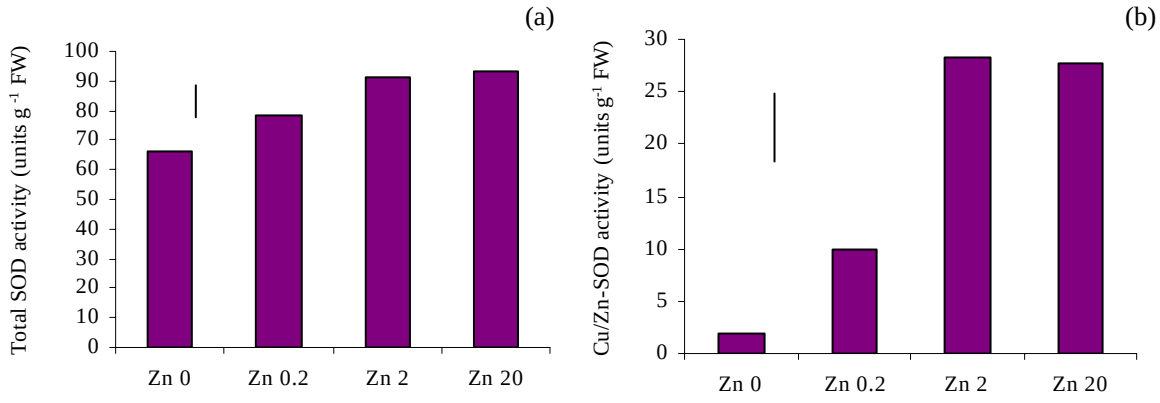


Figure 8.8. Effects of Zn supply (mg kg^{-1} soil) on the total SOD activity (a) and Cu/Zn-SOD activity (b) of wheat YEBs at 28 DAS. Data are the mean of both genotypes; vertical bars represent the $LSD_{0.05}$ for the main effect of Zinc.

At harvest 2, performed at 31 DAS, 42 h into the high temperature treatment, the total SOD activity of the Zn_0 treatment was still significantly lower than that of the other three Zn treatments (Figure 8.9a). The activity of Cu/Zn-SOD differed between varieties this harvest (Figure 8.9b). While the Cu/Zn-SOD activity of Frame was lower in the Zn_0 treatment, the Cu/Zn-SOD activity of Goldmark was not significantly affected by Zn fertilisation at this harvest.

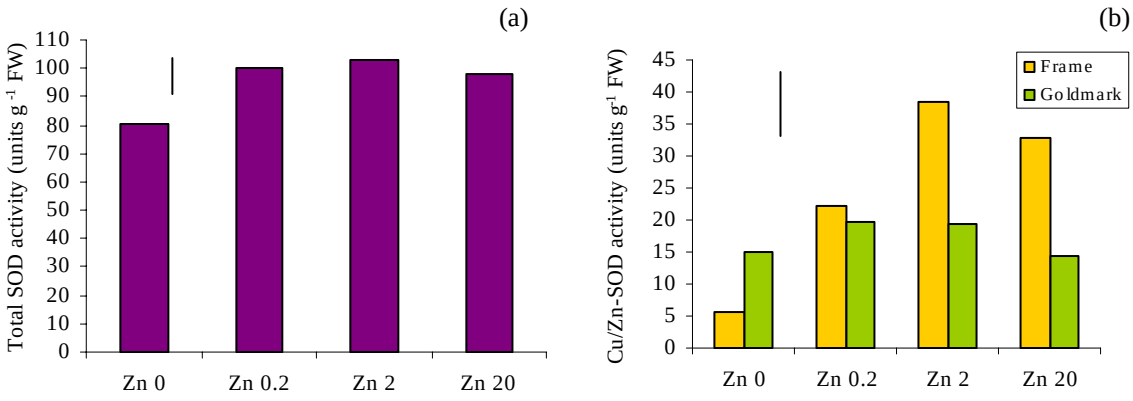


Figure 8.9. Effects of Zn supply (mg kg^{-1} soil) on the total SOD activity (a) and Cu/Zn-SOD activity (b) of wheat YEBs at 31 DAS. Data are the mean of both temperature treatments and genotypes (a), and both temperature treatments (b), vertical bars represent the $LSD_{0.05}$ for (a) the main effect of Zinc; and (b) the interaction of Variety by Zinc.

The high temperature treatment had no significant effect on total SOD activity at 31 DAS, but significantly increased the activity of Cu/Zn-SOD at all levels of Zn fertilisation, with no interaction between the two stresses (Figure 8.10).

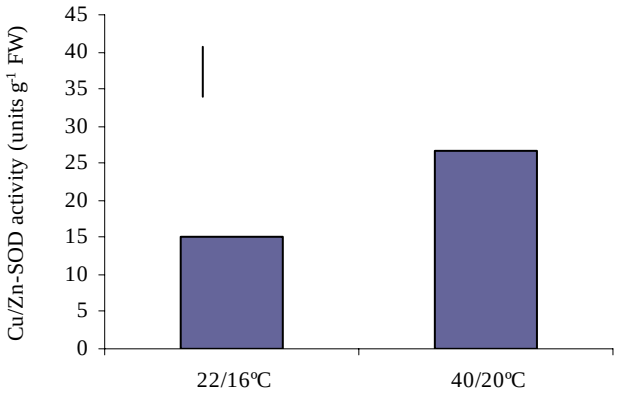


Figure 8.10. Effects of temperature treatment on the Cu/Zn-SOD activity of wheat YEBs at 31 DAS. Data are the mean of both genotypes and all Zn treatments; vertical bar represents the $LSD_{0.05}$ for the main effect of Temperature.

At 39 DAS the total SOD activity in these same leaves (now YEB +1) was again significantly lower in the Zn_0 treatment of Frame, but varied in Goldmark, depending on the temperature treatment (Figure 8.11). There was no significant difference in the total SOD activity of Goldmark maintained at 22/16ºC, with the exception of the $Zn_{0.2}$ treatment, which was significantly lower than the Zn_0 treatment. In the heat stressed plants however, the total SOD activity was significantly higher in the Zn_{20} treatment of Goldmark. At this harvest there was a small increase in Mn-SOD activity with Zn fertilisation (Figure 8.12) but the main influence on total SOD activity was again the activity of the Cu/Zn-SOD enzyme (Figure 8.13a).

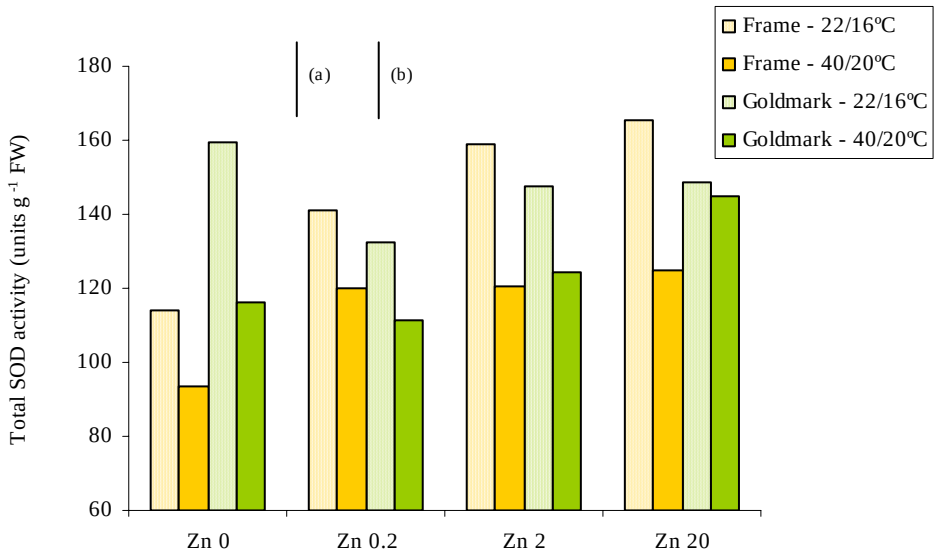


Figure 8.11. Effects of variety, Zn supply (mg kg^{-1} soil) and temperature treatments on the total SOD activity of wheat YEBs + 1 at 39 DAS.
Vertical bars represent the $LSD_{0.05}$ for the interaction of Temperature x Variety x Zinc; (a) for comparisons within the same level of Temperature, (b) for other comparisons.

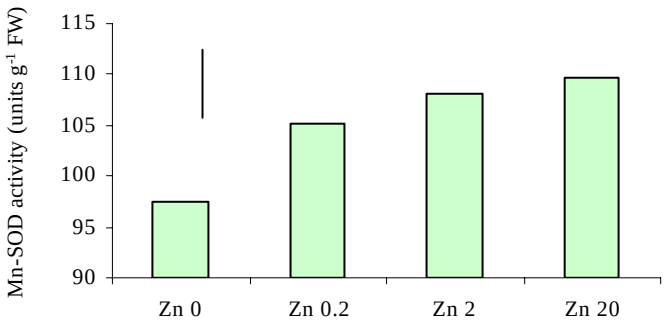


Figure 8.12. Effect of Zn supply (mg kg^{-1} soil) on the Mn-SOD activity of wheat YEBs + 1 at 39 DAS. Data are the mean of both temperature treatments and genotypes; vertical bar represents the $LSD_{0.05}$ for the main effect of Zinc.

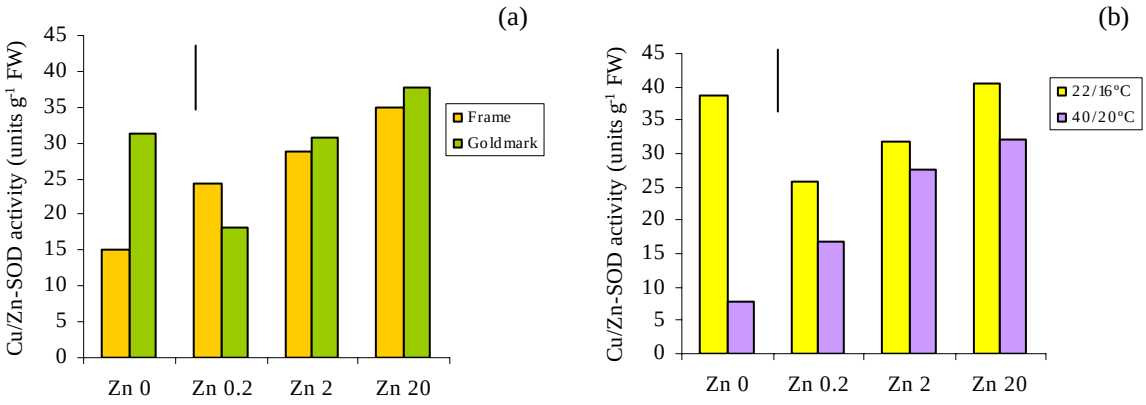


Figure 8.13. Effects of variety and Zn supply (mg kg^{-1} soil) (a) and variety and temperature treatment (b) on theCu/Zn-SOD activity of wheat YEBs + 1 at 39 DAS. Data are the mean of both temperature treatments (a) and both genotypes (b), vertical bars represent the $LSD_{0.05}$ for the interaction of Variety x Zinc (a) and Temperature x Zinc (b).

At 39 DAS, four days after removal from the heat stress, the SOD activities of the control and heat stressed plants had reversed compared to 31 DAS, with the 22/16ºC plants now having a significantly higher total SOD activity than the 40/20ºC plants in all Zn treatments apart from the Zn_{20} treatment of Goldmark (Figure 8.11). The activity of the Cu/Zn-SOD enzyme also showed this trend; with the 40/20ºC plants significantly lower than the 22/16ºC plants in the Zn_0 and $Zn_{0.2}$ treatments (Figure 8.13b). The

interaction effects of Variety x Zinc and Temperature x Zinc were both significant for Cu/Zn-SOD activity, but the three way interaction of Temperature x Variety x Zinc was not significant.

8.3.6 Chloroplast ultrastructure

Mesophyll cell chloroplasts of plants grown with adequate Zn fertilisation at 22/16ºC were well developed, with distinct chloroplast envelope membranes (Plate 8.1a and b). Numerous grana and stromal thylakoid membranes were distributed evenly throughout the granular stromal matrix, and starch grains were also obvious within the stroma. Thylakoid granum stacks lay parallel to the longitudinal axis of these chloroplasts and small, electron-dense, plastoglobuli were apparent within the stroma (Plate 8.3a and b). These osmiophilic, spherical bodies are composed of triacylglycerols and lipophilic prenyl quinones, and serve as a repository for degraded thylakoid constituents, especially lipids, during leaf senescence (Steinmüller and Tevini 1985, Tevini and Steinmüller 1985, Xu *et al.* 1995).

The ultrastructure of wheat leaf chloroplasts from plants grown with no supplementary Zn fertilisation (Zn_0) was altered considerably (Plates 8.1c and d, 8.3c and d). These chloroplasts were swollen and in many cases the envelope had ruptured completely, allowing leakage of the stroma contents into the cytoplasm. There was an increase in the number of plastoglobuli within the stroma of these chloroplasts from plants grown under Zn deficiency, as well as some evidence of a reduced number of thylakoids per granum stack. Furthermore many grana had undergone a change in spatial orientation, becoming perpendicular or at an angle to the longitudinal axis of the chloroplast. Genotypic differences in tolerance to Zn deficiency were evident, with the Zn-inefficient variety, Goldmark, having a greater number of ruptured chloroplasts than the Zn-efficient variety, Frame. There was also evidence of swelling of the thylakoid membranes within the chloroplasts of Goldmark, resulting in an increase in the volume of the intrathylakoid space.

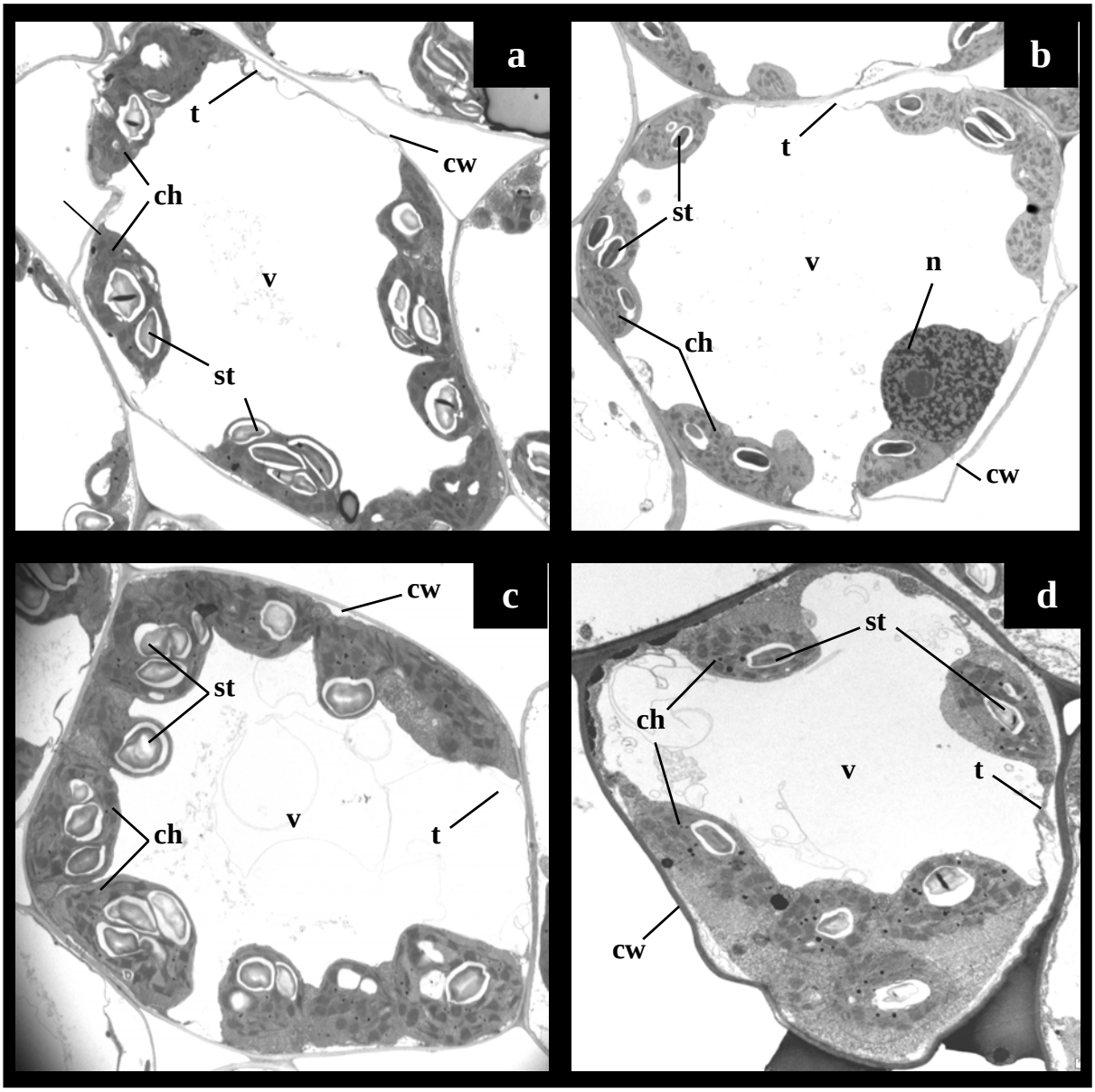


Plate 8.1. Transmission electron micrographs of wheat mesophyll cells at 33 DAS, from two varieties grown at 22/16ºC (x3400).

(a) Mesophyll cell of the Zn-efficient variety, Frame, supplied with adequate Zn (2 mg kg^{-1} soil).

(b) Mesophyll cell of the Zn-inefficient variety, Goldmark, supplied with adequate Zn (2 mg kg^{-1} soil).

(c) Mesophyll cell of Frame grown with no supplementary Zn (0 mg kg^{-1} soil).

(d) Mesophyll cell of Goldmark grown with no supplementary Zn (0 mg kg^{-1} soil).

cw = cell wall, ch=chloroplasts, st = starch grains, t = tonoplast, v = vacuole, n = nucleus

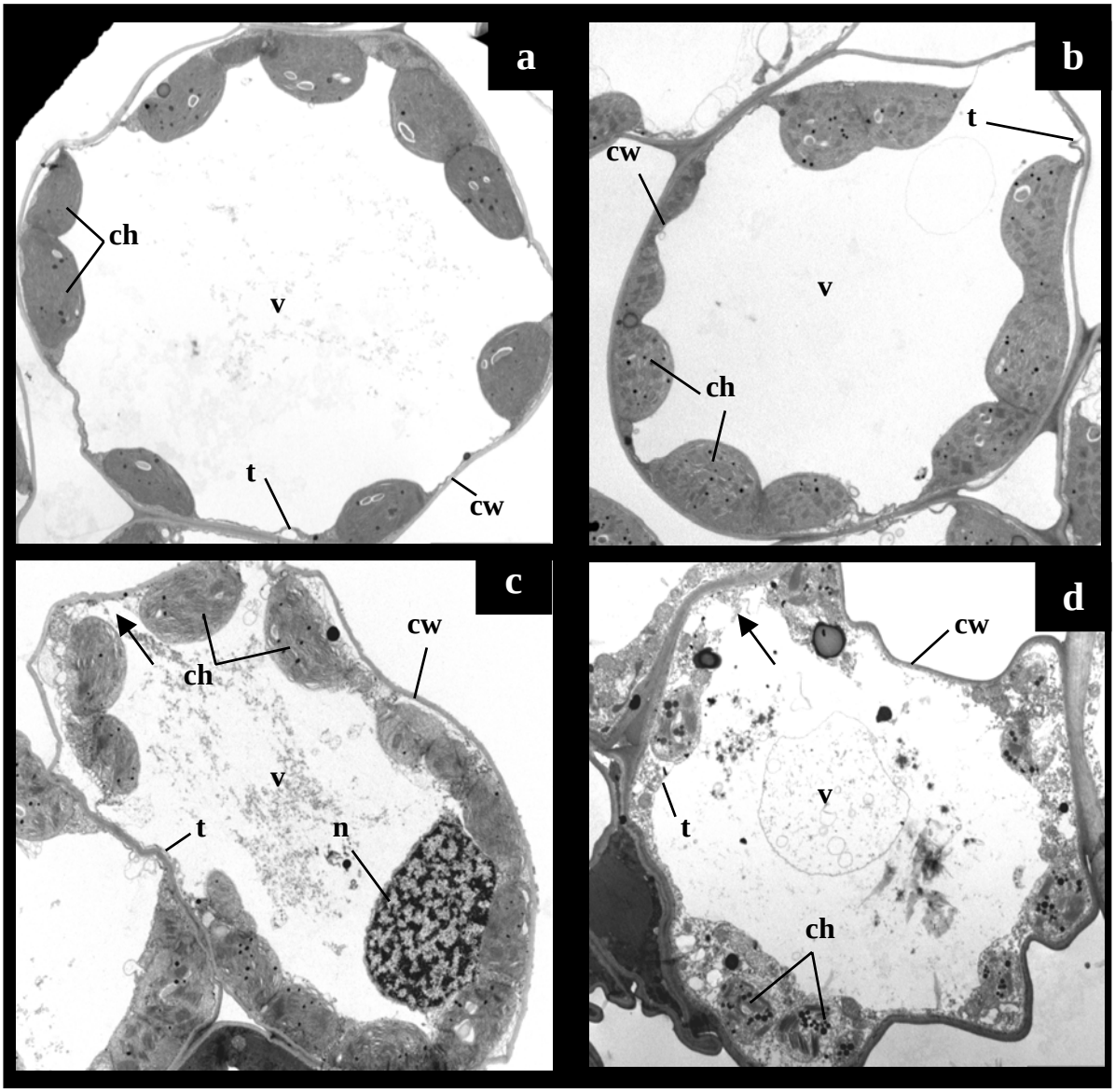


Plate 8.2. Transmission electron micrographs of wheat mesophyll cells at 33 DAS, from two varieties subjected to a high temperature treatment of 40/22ºC from 30 DAS (x3400).

(a) Mesophyll cell of the Zn-efficient variety, Frame, supplied with adequate Zn (2 mg kg^{-1} soil).

(b) Mesophyll cell of the Zn-inefficient variety, Goldmark, supplied with adequate Zn (2 mg kg^{-1} soil).

(c) Mesophyll cell of Frame grown with no supplementary Zn (0 mg kg^{-1} soil).

(d) Mesophyll cell of Goldmark grown with no supplementary Zn (0 mg kg^{-1} soil).

cw = cell wall, ch=chloroplasts, st = starch grains, t = tonoplast, v = vacuole, n = nucleus
arrow = ruptured tonoplast

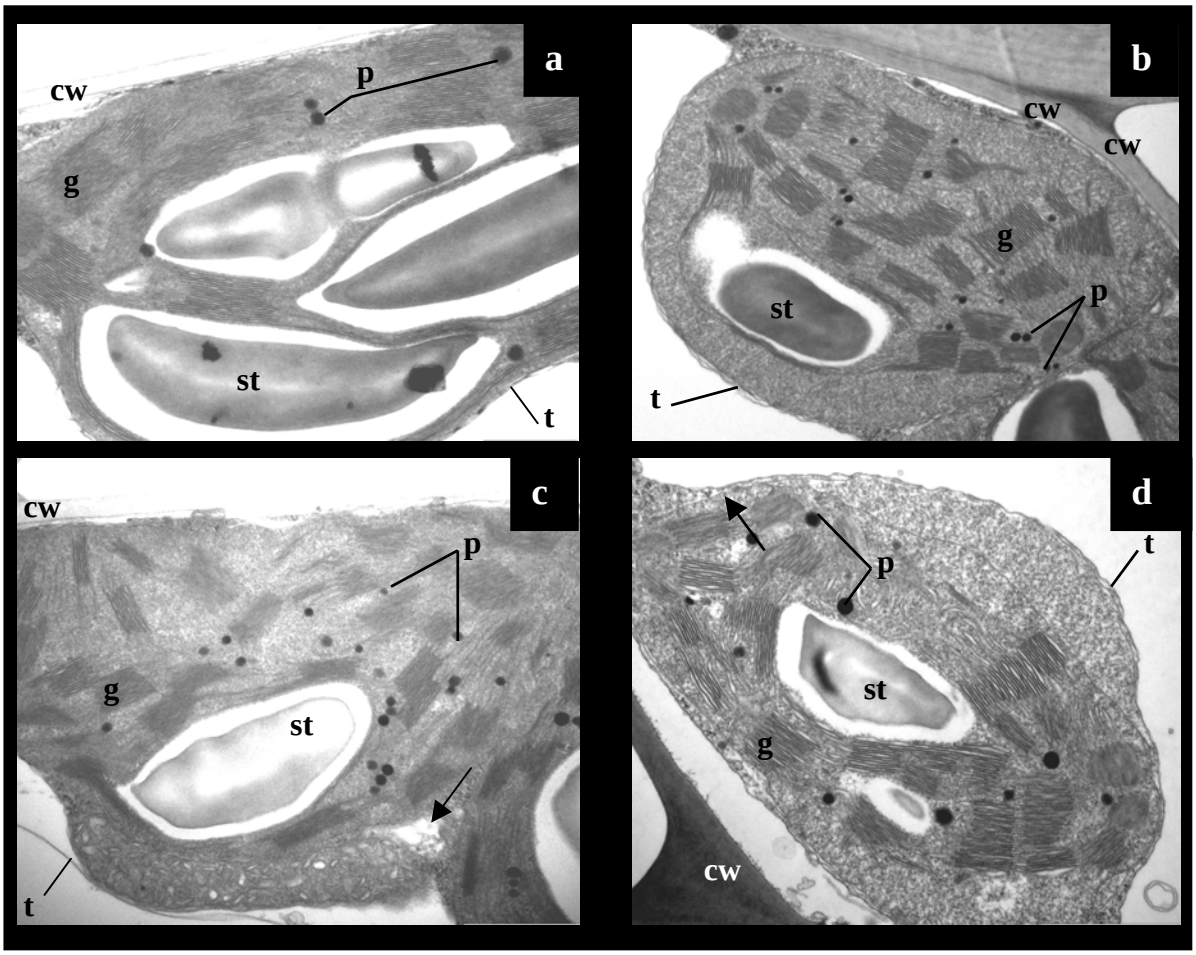


Plate 8.3. Transmission electron micrographs of wheat mesophyll chloroplasts at 33 DAS, from two varieties grown at 22/16ºC (x19000).

(a) Mesophyll chloroplast of the Zn-efficient variety, Frame, supplied with adequate Zn (2 mg kg^{-1} soil).

(b) Mesophyll chloroplast of the Zn-inefficient variety, Goldmark, supplied with adequate Zn (2 mg kg^{-1} soil).

(c) Mesophyll chloroplast of Frame grown with no supplementary Zn (0 mg kg^{-1} soil).

(d) Mesophyll chloroplast of Goldmark grown with no supplementary Zn (0 mg kg^{-1} soil).

cw = cell wall, st = starch grains, g = grana stacks t = tonoplast, p = plastoglobuli, arrow = ruptured chloroplast envelope

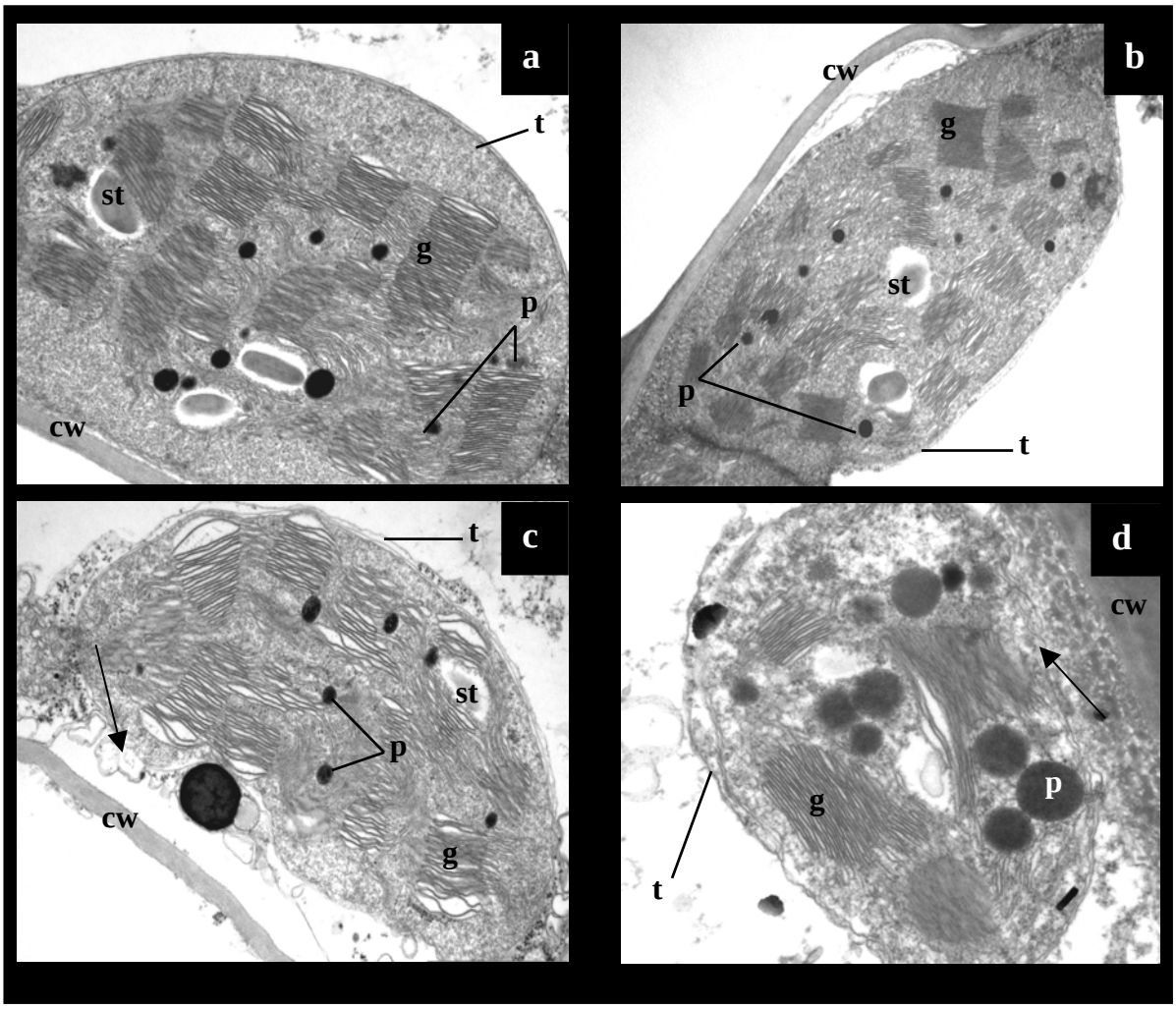


Plate 8.4. Transmission electron micrographs of wheat mesophyll chloroplasts at 33 DAS, from two varieties subjected to a high temperature treatment of 40/22°C from 30 DAS (x19000).

(a) Mesophyll chloroplast of the Zn-efficient variety, Frame, supplied with adequate Zn (2 mg kg^{-1} soil).

(c) Mesophyll chloroplast of the Zn-inefficient variety, Goldmark, supplied with adequate Zn (2 mg kg^{-1} soil).

(c) Mesophyll chloroplast of Frame grown with no supplementary Zn (0 mg kg^{-1} soil).

(d) Mesophyll chloroplast of Goldmark grown with no supplementary Zn (0 mg kg^{-1} soil).

cw = cell wall, st = starch grains, g = grana stacks t = tonoplast, p = plastoglobuli, arrow = ruptured chloroplast envelope

Damage to the chloroplast ultrastructure of plants subjected to the high temperature treatment of 40/20ºC from 30 DAS was also evident (Plates 8.2a and b, 8.4a and b). The thylakoid membranes of both varieties were swollen in plants subjected to heat stress, and therefore the intrathylakoid spaces had increased. Large plastoglobuli were present within the stroma, and the starch grains within the chloroplasts were smaller and fewer compared with those maintained at 22/16ºC. The number of thylakoids per granum stack had increased, and many grana were irregular in shape. This structural damage to chloroplasts caused by exposure to high temperature appeared to differ little between the two varieties examined, with both Frame and Goldmark exhibiting similar abnormalities in response to heat stress.

Mesophyll chloroplasts from wheat plants grown under both Zn deficiency and high temperature stress appeared to suffer a much greater level of damage than those subjected to either stress independently (Plates 8.2c and d, 8.4c and d). These chloroplasts were both swollen and ruptured, as were those under Zn deficiency alone, and were virtually devoid of starch grains. The combined stresses resulted in thylakoids that were extremely swollen. The number of thylakoids per granum stack was increased, and these grana were highly irregular in shape and orientation. Very large, densely stained, plastoglobuli were evident throughout the stroma. The simultaneous stresses also caused some damage to other parts of the cell that were not obvious when each stress occurred alone. This damage included distortion of the cell wall (observed predominantly in the Zn-inefficient variety, Goldmark) and rupture of the tonoplast, which resulted in the cytoplasmic contents leaking into the vacuole. Again genotypic differences between the two varieties were observed, with Goldmark exhibiting a greater degree of structural injury than Frame when subjected to both Zn deficiency and high temperature stress. A summary of the ultrastructural damage to wheat leaf mesophyll cells and their chloroplasts under Zn deficiency and heat stress is presented in Table 8.3.

Table 8.3. Summary of ultrastructural damage to wheat leaf mesophyll cells and their chloroplasts in response zinc deficiency and heat stress conditions.

Ultrastructural damage	Zinc deficiency ONLY (Low temperature)	Zinc deficiency AND Heat stress	Heat stress ONLY (Adequate zinc)
Injuries to membranes			
Ruptured tonoplast		++	
Swollen chloroplasts	++	+	
Ruptured chloroplasts	++	+++	
Swollen thylakoids	+	+++	++
Other injuries			
Distorted cell wall		+	
Absence of starch grains		++	+
Increased size of granum stacks		+	+
Decreased size of granum stacks	+		
Altered orientation of granum stacks	+	++	+
Increased number of plastoglobuli	+		
Large plastoglobuli		+	+

NB: The number of crosses is indicative of the magnitude of the response.

8.3.7 Nutrient concentrations in the youngest emerged leaf blades

8.3.7.1 Zinc

Zinc concentration in the YEBs harvested at 39 DAS increased significantly with increasing Zn fertilisation, and ranged from 8.6 mg Zn kg^{-1}, for the Zn_0 treatment, up to 54 mg Zn kg^{-1}, for the Zn_{20} treatment. Significant differences in leaf Zn concentration were also evident between varieties and depended on temperature treatment (Figure 8.14). For example, the $Zn_{0.2}$ treatment of Goldmark subjected to high temperature stress did not differ significantly from the Zn_0 treatment. Almost all of the Zn concentrations in the YEBs of the $Zn_{0.2}$ treatment were within or lower than the reported critical deficiency range of 14-19 mg Zn kg^{-1} for YEBs of wheat seedlings (Bansal *et al.* 1990, Riley *et al.* 1992, Wilhelm *et al.* 1993, Brennan and Bolland 2002), with the exception of Halberd maintained at 22/16ºC (19.8 mg Zn kg^{-1}).

The Zn concentration in the YEBs of Goldmark for the Zn_2 treatment was also significantly lower than the other three varieties; 26.1 mg Zn kg^{-1}, compared with an average of 36.0 mg Zn kg^{-1} for Frame, Halberd and Meering. Furthermore, the leaf Zn concentrations of Frame, Halberd and Meering for the Zn_2 and Zn_{20} treatments subjected to high temperature were significantly higher than those maintained at 22/16ºC throughout the experiment. For the Zn_{20} treatment, Zn concentrations in the YEBs of 22/16ºC plants of Frame, Halberd and Meering were 50.2, 43.6 and 43.3 mg Zn kg^{-1}, respectively, while the corresponding Zn concentrations in the YEBs of plants subjected to 40/20ºC were 85.1, 52.7 and 53.3 mg Zn kg^{-1}, respectively.

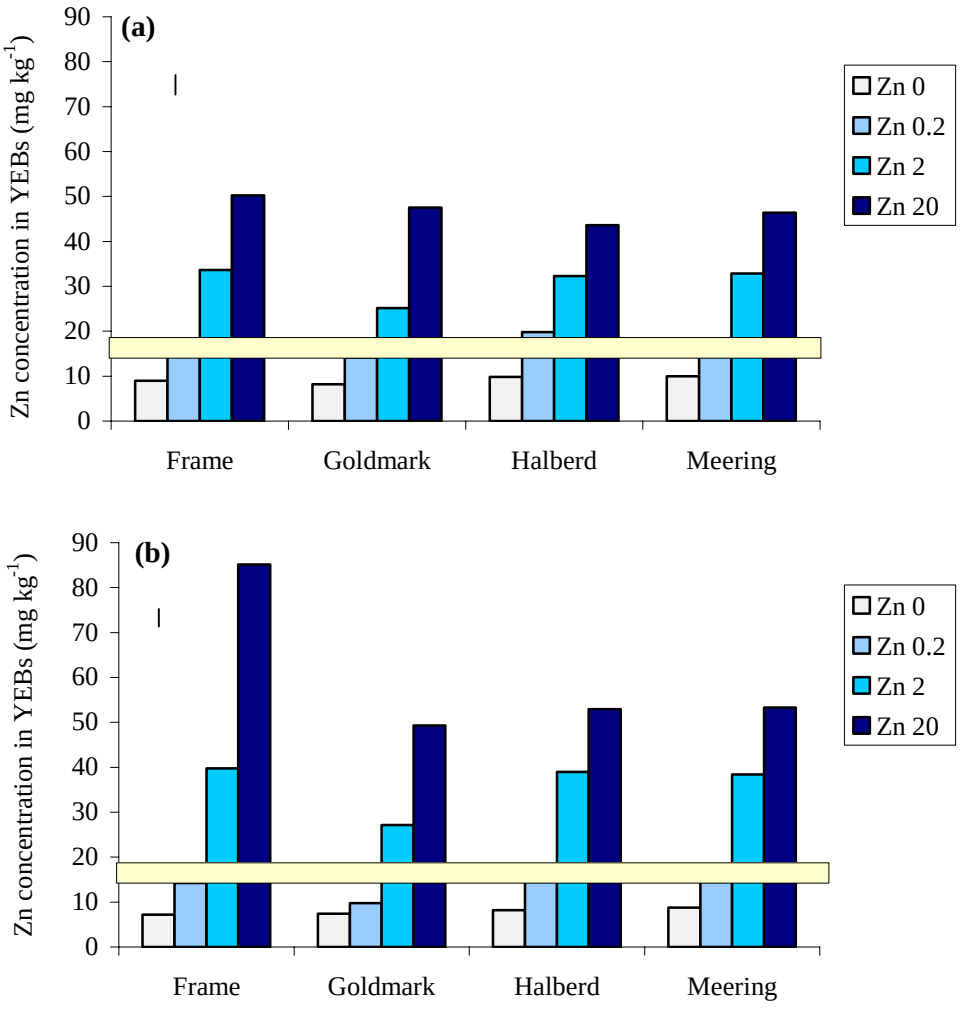


Figure 8.14. Effects of Zn fertilisation (mg kg^{-1} soil) and temperature treatment [(a) 22/16ºC, (b) 40/20ºC] on the zinc concentration in the YEBs of wheat genotypes at 39 DAS.
The shaded horizontal area represents the critical level for Zn deficiency in the YEBs of wheat seedlings.
The vertical bar represents the $LSD_{0.05}$ for the Temperature x Genotype x Zinc interaction.

8.3.7.2 Other nutrients

The concentration of phosphorus (P), sulphur (S), magnesium (Mg) and iron (Fe) in the YEBs generally decreased with Zn application. The concentration of both Fe (Table 8.4) and S (Table 8.5) was significantly higher in the Zn_0 treatment than in the other three Zn treatments, and significantly lower in the Zn_{20} treatment. The concentration of Mg in the YEBs was significantly higher in the Zn_0 treatment of three varieties, Frame, Goldmark and Meering, than in the other Zn treatments, and significantly lower in the Zn_{20} treatment of Goldmark, Halberd and Meering (Table 8.4). Phosphorus concentration was higher in the Zn_0 treatment of Goldmark and Meering than in the other Zn treatments, and lower in the Zn_{20} treatment of Frame and Halberd (Table 8.5).

While the range of P concentrations in the YEBs was generally above the reported critical deficiency level of 2700 mg kg^{-1} for P in the YEBs of wheat plants at this stage (Elliott *et al.* 1997), the concentrations of Mg, Fe and S in the Zn_{20} treatment were approaching the deficiency level. The critical S concentration for the YEBs of wheat seedlings has been estimated to be 2800 mg kg^{-1} (Robson *et al.* 1995, Reuter *et al.* 1997a). In the present experiment S concentrations in the YEBs of the $Zn_{0.2}$, Zn_2 and Zn_{20} treatments were under this critical level. Similarly a range of 500-1500 mg kg^{-1} for Mg in the YEBs of wheat seedlings has been reported as marginal (Reuter *et al.* 1997a). The Mg concentrations in the YEBs of the $Zn_{0.2}$, Zn_2 and Zn_{20} treatments of Frame, Goldmark and Halberd in the present experiment fell within this marginal range. Little information is available regarding the critical concentration of Fe in the YEBs of young wheat plants, however experiments with oats (*Avena byzantina* K. Koch) showed that Fe concentrations of less than 50 mg kg^{-1} in the shoots at 26 DAS can be considered deficient (Brown 1979). In the present experiment the concentration of Fe in the YEBs of the Zn_{20} treatment was 44 mg kg^{-1}, significantly lower than the other Zn treatments.

The concentration of other nutrients in the YEBs of wheat seedlings in the present experiment, including manganese, copper, calcium and potassium, were found to vary with genotype, Zn treatment and temperature treatment (Tables 8.6 and 8.7). However the concentrations of these and all other nutrients were within the reported adequate range for the YEBs of wheat seedlings (Reuter *et al.* 1997a).

Table 8.4. Effects of Zn fertilisation (mg kg^{-1} soil) and temperature treatment on the concentration of iron and magnesium (mg kg^{-1} D.W.) in the YEBs of wheat genotypes at 39 DAS.

Temperature	Genotype	Iron concentration				
		Zn 0	**Zn 0.2**	**Zn 2**	**Zn 20**	***Mean***
22/16ºC	**Frame**	89.1	64.1	64.7	47.6	*66.4*
	Goldmark	82.9	64.0	54.0	44.1	*61.3*
	Halberd	99.6	75.9	56.8	45.4	*69.4*
	Meering	101.8	75.5	72.7	59.7	*77.4*
40/20ºC	**Frame**	76.3	59.0	51.8	42.5	*57.4*
	Goldmark	67.6	41.1	52.3	30.8	*48.0*
	Halberd	69.9	68.4	56.3	35.7	*57.6*
	Meering	87.8	71.6	60.9	44.9	*66.3*
Mean		*84.4*	*64.9*	*58.7*	*43.8*	
$LSD_{0.05}$						
Genotype				**5.8**		
Zinc				**5.8**		
Temperature				**2.4**		
Genotype x Zinc				**n.s.**		
Genotype x Temperature				**n.s.**		
Zinc x Temperature				**n.s.**		
Genotype x Zinc x Temperature				**n.s.**		

Temperature	Genotype	Magnesium concentration				
		Zn 0	**Zn 0.2**	**Zn 2**	**Zn 20**	***Mean***
22/16ºC	**Frame**	1932	1210	1188	978	*1327*
	Goldmark	2178	1478	1410	1265	*1583*
	Halberd	1550	1320	1192	923	*1246*
	Meering	2550	1788	1918	1440	*1924*
40/20ºC	**Frame**	2135	1273	1383	1288	*1519*
	Goldmark	1860	1178	1270	1113	*1355*
	Halberd	1515	1478	1468	1233	*1423*
	Meering	2669	2130	2113	1913	*2206*
Mean		*2049*	*1482*	*1493*	*1269*	
$LSD_{0.05}$						
Genotype				**103**		
Zinc				**103**		
Temperature				**n.s.**		
Genotype x Zinc				**205**		
Genotype x Temperature						
(a)				**145**		
(b)				**266**		
Zinc x Temperature				**n.s.**		
Genotype x Zinc x Temperature				**n.s.**		

(a) For comparisons within the same temperature treatment

(b) For other comparisons

n.s. = non-significant

Table 8.5. Effects of Zn fertilisation (mg kg^{-1} soil) and temperature treatment on the concentration of phosphorus and sulphur (mg kg^{-1} D.W.) in the YEBs of wheat genotypes at 39 DAS.

Temperature	Genotype	Phosphorus concentration				
		Zn 0	**Zn 0.2**	**Zn 2**	**Zn 20**	***Mean***
22/16ºC	**Frame**	3400	3125	3300	2952	*3194*
	Goldmark	4025	3125	2925	2675	*3188*
	Halberd	3675	3625	3600	2900	*3450*
	Meering	4500	3450	3500	3200	*3662*
40/20ºC	**Frame**	3975	3475	3675	2825	*3488*
	Goldmark	3650	2317	2875	2438	*2820*
	Halberd	4725	4500	4700	4150	*4519*
	Meering	4949	3700	3875	3600	*4031*
Mean		*4112*	*3415*	*3556*	*3092*	
$LSD_{0.05}$						
Genotype				**238**		
Zinc				**238**		
Temperature				**n.s.**		
Genotype x Zinc				**477**		
Genotype x Temperature						
(a)				**337**		
(b)				**473**		
Zinc x Temperature				**n.s.**		
Genotype x Zinc x Temperature				**n.s.**		

Temperature	Genotype	Sulphur concentration				
		Zn 0	**Zn 0.2**	**Zn 2**	**Zn 20**	***Mean***
22/16ºC	**Frame**	2825	2375	2375	2192	*2442*
	Goldmark	3125	2275	2275	2250	*2481*
	Halberd	2875	2550	2600	2368	*2598*
	Meering	3650	2975	3050	2975	*3162*
40/20ºC	**Frame**	3000	2250	2400	2295	*2486*
	Goldmark	2650	1738	1928	1763	*2019*
	Halberd	2775	2625	2800	2400	*2650*
	Meering	3458	3175	3025	2875	*3133*
Mean		*3045*	*2495*	*2557*	*2390*	
$LSD_{0.05}$						
Genotype				**133**		
Zinc				**133**		
Temperature				**n.s.**		
Genotype x Zinc				**n.s.**		
Genotype x Temperature						
(a)				**188**		
(b)				**314**		
Zinc x Temperature				**n.s.**		
Genotype x Zinc x Temperature				**n.s**		

(a) For comparisons within the same temperature treatment

(b) For other comparisons

n.s. = non-significant

Table 8.6. Effects of Zn fertilisation (mg kg^{-1} soil) and temperature treatment on the concentration of manganese and copper (mg kg^{-1} D.W.) in the YEBs of wheat genotypes at 39 DAS.

Temperature	Genotype	Manganese concentration				
		Zn 0	**Zn 0.2**	**Zn 2**	**Zn 20**	***Mean***
22/16ºC	**Frame**	57.9	34.5	36.8	33.2	*40.6*
	Goldmark	82.0	50.9	51.8	52.0	*59.2*
	Halberd	52.3	41.4	37.0	34.1	*41.2*
	Meering	101.5	59.1	61.2	62.5	*71.1*
40/20ºC	**Frame**	60.0	43.2	45.2	54.1	*50.6*
	Goldmark	61.8	37.8	40.4	41.0	*45.3*
	Halberd	53.4	57.8	56.1	52.9	*55.1*
	Meering	73.6	69.4	60.1	73.9	*71.2*
Mean		*67.8*	*49.3*	*49.6*	*50.5*	
$LSD_{0.05}$						
Genotype				**3.9**		
Zinc				**3.9**		
Temperature				**n.s.**		
Genotype x Zinc				**7.8**		
Genotype x Temperature						
(a)				**5.5**		
(b)				**9.9**		
Zinc x Temperature						
(a)				**5.5**		
(b)				**9.9**		
Genotype x Zinc x Temperature				**n.s.**		

Temperature	Genotype	Copper concentration				
		Zn 0	**Zn 0.2**	**Zn 2**	**Zn 20**	***Mean***
22/16ºC	**Frame**	10.5	9.2	9.7	9.1	*9.7*
	Goldmark	11.4	9.2	10.0	9.0	*9.9*
	Halberd	10.2	9.2	10.0	9.7	*9.8*
	Meering	11.5	10.1	10.3	11.0	*10.7*
40/20ºC	**Frame**	10.7	9.1	10.2	9.7	*9.9*
	Goldmark	10.1	7.5	7.9	7.1	*8.2*
	Halberd	8.3	9.2	10.7	11.2	*9.9*
	Meering	10.8	10.2	10.2	10.4	*10.4*
Mean		*10.4*	*9.2*	*9.9*	*9.7*	
$LSD_{0.05}$						
Genotype				**0.5**		
Zinc				**0.5**		
Temperature				**n.s.**		
Genotype x Zinc				**1.0**		
Genotype x Temperature						
(a)				**0.7**		
(b)				**0.8**		
Zinc x Temperature				**n.s.**		
Genotype x Zinc x Temperature				**n.s.**		

(a) For comparisons within the same temperature treatment

(b) For other comparisons

n.s. = non-significant

Table 8.7. Effects of Zn fertilisation (mg kg^{-1} soil) and temperature treatment on the concentration of calcium and potassium (mg kg^{-1} D.W.) in the YEBs of wheat genotypes at 39 DAS.

Temperature	Genotype	Calcium concentration				
		Zn 0	**Zn 0.2**	**Zn 2**	**Zn 20**	***Mean***
22/16ºC	**Frame**	4675	3000	2875	2775	*3331*
	Goldmark	6150	4125	3950	4325	*4637*
	Halberd	4650	3525	3200	3500	*3719*
	Meering	6325	4325	4275	4725	*4912*
40/20ºC	**Frame**	6225	4600	5200	6450	*5619*
	Goldmark	5400	4075	4100	4350	*4481*
	Halberd	5425	5425	5375	5525	*5437*
	Meering	7117	6800	6450	6925	*6823*
Mean		*5746*	*4484*	*4428*	*4822*	
$LSD_{0.05}$						
Genotype				**355**		
Zinc				**355**		
Temperature				**803**		
Genotype x Zinc				**n.s.**		
Genotype x Temperature						
(a)				**502**		
(b)				**857**		
Zinc x Temperature						
(a)				**502**		
(b)				**857**		
Genotype x Zinc x Temperature				**n.s.**		

Temperature	Genotype	Potassium concentration				
		Zn 0	**Zn 0.2**	**Zn 2**	**Zn 20**	***Mean***
22/16ºC	**Frame**	15100	16700	18050	17550	*16850*
	Goldmark	13725	14650	14150	13800	*14081*
	Halberd	14125	18100	18800	16975	*17000*
	Meering	17275	19275	19275	15825	*17912*
40/20ºC	**Frame**	17575	18450	18900	12775	*16925*
	Goldmark	13100	10375	15825	11625	*12731*
	Halberd	20075	25500	25500	22550	*23331*
	Meering	24177	18525	21150	20325	*21044*
Mean		*16894*	*17697*	*18956*	*16391*	
$LSD_{0.05}$						
Genotype				**1646**		
Zinc				**1646**		
Temperature				**1906**		
Genotype x Zinc				**n.s.**		
Genotype x Temperature						
(a)				**2327**		
(b)				**2593**		
Zinc x Temperature				**n.s.**		
Genotype x Zinc x Temperature				**n.s.**		

(a) For comparisons within the same temperature treatment

(b) For other comparisons

n.s. = non-significant

8.4 Discussion

The results of this experiment have again shown that the twin stresses of high temperature and inadequate Zn fertilisation significantly suppress the growth and physiology of wheat seedlings, and that certain aspects of plant anatomy and physiology will be most affected when these stresses occur simultaneously. Exposing plants to heat stress significantly reduced shoot biomass production, chlorophyll content and photosynthetic activity, transiently increased SOD activity and also caused extensive damage to chloroplast structure. Low levels of soil available Zn also reduced shoot biomass production, reduced SOD activity and resulted in injury to the structure of the chloroplasts. However damage to chloroplast structure (and chloroplastic membranes in particular) was greatest when the two stresses occurred in combination, while the decline in chlorophyll fluorescence caused by high temperature stress was lessened with the application of adequate Zn fertilisation. These results are consistent with previous findings of the improvement in chlorophyll fluorescence under heat stress when adequate Zn fertilisation is provided (Chapters 4, 5 and 6).

Several results of the present experiment have shown that the highest level of Zn fertilisation, Zn_{20}, was injurious to plant growth and function. While reports of toxic Zn concentrations in the YEBs of wheat seedlings are scarce, other results would suggest that the Zn concentrations of up to 68 mg kg^{-1} found in the YEBs of the Zn_{20} plants in the present experiment were well within the adequate range for wheat seedlings at this growth stage (Wilhelm *et al.* 1993). Nevertheless shoot dry matter production, relative growth rate from 34-39 DAS, leaf chlorophyll content, chlorophyll fluorescence under heat stress, and the length of leaf 7 in Frame, were all reduced by Zn_{20} fertilisation relative to the adequate Zn fertilisation level of Zn_2. Furthermore in some instances (chlorophyll content and chlorophyll fluorescence) Zn_{20} was more harmful to plant physiology than the treatment with no supplementary Zn, Zn_0. High levels of Zn are known to induce deficiency of other nutrients in plant tissues, particularly Mg and Fe, which often leads to chlorosis of young leaves (Marschner 1995). This is thought to be due to the similar radius of Zn^{2+} to Mg^{2+} and Fe^{2+} (Woolhouse 1983, Boardman and McGuire 1990). In the present experiment the concentration of Fe in the YEBs was found to be significantly lower in the Zn_{20} treatment than in the other Zn treatments, and

less than the critical concentration of 50 mg kg^{-1} reported for the whole shoots of oats at this stage of growth (Brown 1979). Furthermore the Mg concentration in the Zn_{20} treatment of three varieties was within the marginal range of 500-1500 mg kg^{-1} for Mg in the YEBs of wheat seedlings at this growth stage (Reuter *et al.* 1997a). Sulphur concentrations in the YEBs of the Zn_{20} treatment were also under the critical level for optimal plant growth (Robson *et al.* 1995).

High tissue concentrations of Zn have been found to inhibit photosynthesis at various steps and through various mechanisms. The competition of Zn with Fe for an iron-requiring step in the biosynthesis of chlorophyll has been identified in maize (*Zea mays* L.) (Woolhouse 1983). The depression of Rubisco carboxylase activity is thought to be caused by competition with Mg (Van Assche and Clijsters 1986a) while the inhibition of photosystem II (PSII) activity is caused by the replacement of manganese (Mn) by Zn in the thylakoid membranes (Van Assche and Clijsters 1986b). Under normal circumstances approximately six atoms of both Mn and Zn are bound per 400 chlorophyll molecules, however under Zn toxicity this proportion shifts to two Mn and 30 Zn atoms (Van Assche and Clijsters 1986b). While the level of Zn toxicity in the present experiment was not high enough to affect chlorophyll fluorescence under low temperatures, the chlorophyll maximum fluorescence (Fm) was significantly reduced and chlorophyll initial fluorescence (Fo) increased by the Zn_{20} treatment (relative to the Zn_2 and Zn_0 treatments) when combined with the high temperature stress. Several studies have demonstrated that the heat inactivation of the PSII complex is initiated by the destruction of the Mn cluster that catalyses the oxidation of water to molecular oxygen (Nash *et al.* 1985, Thompson *et al.* 1989, Mamedov *et al.* 1993), and it is therefore feasible that the reduction in Fv/Fm under heat stress and high Zn is due to the reduced proportion of Mn ions on the thylakoid membranes.

It was expected that the $Zn_{0.2}$ treatment would produce better plant growth and health than those that received no supplementary Zn fertilisation, the Zn_0 treatment. In reality however, although the $Zn_{0.2}$ treatment did produce better shoot growth than the Zn_0 treatment, contrary to expectations the chlorophyll content, SOD activity and chlorophyll fluorescence parameters of this treatment were actually reduced in

comparison to the Zn_0 treatment. The chlorophyll content of the $Zn_{0.2}$ plants was significantly lower than either the Zn_0 or Zn_2 treatment, when measured on the YEBs at 39 DAS. At the same harvest, 39 DAS, total SOD and Cu/Zn-SOD activities were significantly lower in the $Zn_{0.2}$ treatment of Goldmark, than in either the Zn_0 or Zn_2 treatments. In addition the chlorophyll fluorescence ratio of heat-treated plants fertilised with 0.2 mg Zn kg^{-1} soil was significantly lower than that of the Zn_0 or Zn_2 plants for the first four days of high temperature. It is possible that these results are again due to a nutrient imbalance effect, in a similar fashion to the effects of the Zn_{20} treatment, described above. Zinc concentrations in the YEBs of the $Zn_{0.2}$ treatment were within the critical deficiency range of 14-19 mg kg^{-1} for YEBs of wheat seedlings (Bansal *et al.* 1990, Riley *et al.* 1992, Wilhelm *et al.* 1993, Brennan and Bolland 2002). In addition the concentrations of Fe and S, and Mg and P in all varieties except Halberd, were significantly lower in the $Zn_{0.2}$ treatment than in the Zn_0 treatment. Furthermore, in the case of Mg and S, these concentrations were either marginal or below the critical level for the YEBs of wheat seedlings (Robson *et al.* 1995, Reuter *et al.* 1997a). It is therefore likely that the combined deficiencies of Zn, S and Mg produced the deleterious effects on plant physiology observed in the $Zn_{0.2}$ treatment in the present experiment.

The total activity of SOD decreased in the present experiment as Zn fertilisation decreased, in agreement with a number of other reports, both in wheat (Cakmak *et al.* 1997, 1998a) and other crop species (Cakmak and Marschner 1993, Yu *et al.* 1998, Yu and Rengel 1999). This decrease occurred mainly at the expense of Cu/Zn-SOD activity, as previously reported in wheat (Cakmak *et al.* 1997), since Mn-SOD activity was not affected by Zn deficiency. In contrast to other reports however (Cakmak *et al.* 1997, 1998a, Hacisalihoglu *et al.* 2003), the present results did not show any genotypic differences in Cu/Zn-SOD activity at low Zn fertilisation between varieties differing in Zn efficiency. These authors have suggested that the activity of Cu/Zn-SOD is a good indicator of the Zn nutritional status of plants, and that Zn-efficient genotypes may be able to maintain the functioning of Zn-requiring enzymes under low Zn conditions. In our study however, the Cu/Zn-SOD activity did not differ between genotypes at 28 DAS, while at 31 DAS the activity of this enzyme was greater in the Zn-efficient

variety, but only at the higher Zn fertilisation treatments of Zn_2 and Zn_{20}. At 39 DAS the Cu/Zn-SOD activity of Zn-inefficient Goldmark was much higher than the more Zn-efficient Frame in the Zn_0 treatment, which was an unexpected result. The Cu/Zn-SOD activity of the other Zn treatments did not differ between the genotypes at 39 DAS. This discrepancy between the present findings and those of others may be related to the differences in the nature of the Zn-efficiency of the genotypes studied; Frame and Goldmark in the present experiment may not differ as widely in their Zn efficiency as that of the other genotypes studied. Cakmak *et al.* (1997), for example, found larger differences in Cu/Zn-SOD activity between cereal species than within species in response to Zn deficiency, and furthermore, the decreases in Cu/Zn-SOD activity due to Zn deficiency were more pronounced between the durum wheat cultivars rather than between the bread wheat varieties. Moreover, the measurements of Cakmak and co-workers (1997) were performed at an earlier growth stage (21 DAS) than the current experiment, and under a more severe Zn deficiency regime, which may be required to detect differences in Cu/Zn-SOD activity between bread wheat genotypes.

High temperature stress has been found to enhance the activity of SOD in plants (Kraus and Fletcher 1994, Jagtap and Bhargava 1995, Sairam *et al.* 2000). The activity of the Cu/Zn-SOD enzyme in the present experiments was 76% higher in the heat-stressed plants during the period of high temperature than it was in the control plants, but then declined to the level of the control plants or lower after removal from the high temperature. Several authors have reported an increase in the activity of antioxidant enzymes during the initial stage of oxidative stress, which provides a certain degree of protection from the stress, followed by a decline in activity, owing to reduced synthesis or enhanced degradation or inactivation of the enzymes (Cakmak and Marschner 1988c, Zhang and Kirkham 1994, Yu *et al.* 1998, Yu and Rengel 1999). Huang *et al.* (2001) report a transient increase in SOD activity in creeping bentgrass in response to an increase in temperature from 20ºC to 35ºC, but this declined as the duration of temperature stress continued. Interestingly in the present experiments it was the Cu/Zn-SOD activity of the heat-stressed, low Zn plants (fertilised with 0 or 0.2 mg Zn kg^{-1} soil) that fell to below that of the control plants (an 80% and 35% decline, respectively), whereas the Cu/Zn-SOD activity of those plants that had received 2 or 20 mg Zn kg^{-1}

soil decreased to a similar level as that of the control plants. Huang *et al.* (2001) suggest that although the dismutation ability of SOD was able to increase transiently when the shoots of creeping bentgrass were exposed to high temperature, this increase in SOD activity was less than the increase in $O_2 \cdot^-$ production, since lipid peroxidation still occurred at this time. The subsequent decrease in the activity of SOD in the current experiments indicates that the scavenging ability in the cells of leaves was lowered to that of the control following removal from the high temperature conditions, however in the Zn deficient plants it declined further to much less than the control plants. It therefore appears that Zn fertilisation may improve a plant's ability to recover from an oxidative stress event.

Since Zn affects both the synthesis (Cakmak and Marschner 1993) and activity of antioxidative enzymes, Cakmak (2000) has suggested that Zn is an important factor in plant defence systems against destructive ROS. Plants with reduced Cu/Zn-SOD activity have been shown to be sensitive to a number of oxidative stress factors, including ozone toxicity (Pitcher and Zilinskas 1996), salinity (Van Camp *et al.* 1996), drought (Mittler and Zilinskas 1994, Yu and Rengel 1999) and low temperatures (Sen Gupta *et al.* 1993a). Bakardjieva *et al.* (2000) have shown that Zn ions added *in vitro* can increase the activity and thermostability of SOD isoforms from yew (*Taxus baccata* L.), Scots pine (*Pinus sylvestris* L.), lucerne and maize. The results of the present experiment indicate that elevated levels of Zn nutrition may be able to restrict the decline in Cu/Zn-SOD activity following a high temperature event, thus supporting Cakmak's (2000) speculation that the improvement of the Zn nutritional status of plants may be of great importance for their survival under oxidative stress conditions.

Results of chlorophyll fluorescence measurements in the present experiment are largely in support of those reported in previous chapters. The Fv/Fm ratio was significantly lowered by high temperature in all varieties during the heat stress period, but recovered rapidly upon return to control temperature conditions. A supplementary Zn fertilisation treatment of 2 mg Zn kg^{-1} soil improved the Fv/Fm of all varieties in comparison to the plants that had received no Zn supplementation during the first day of heat stress, after which there was no significant difference between these two Zn treatments. This

suggests that supplementary Zn can offer some protection to the photosynthetic apparatus against transient high temperature stress, as often occurs in the field, but not against long-term heat stress. The difference in Fv/Fm between Zn treatments under heat stress in this experiment was due to an increase in the Fo component of chlorophyll fluorescence, rather than any effect on Fm.

The increase in Fo by heat stress may be attributed to a physical separation of the chlorophyll *a/b* light harvesting complex of PSII (LHCII) from the PSII core complex and its subsequent migration from the appressed to the non-appressed thylakoid regions (Yamane *et al*. 1997), and this is associated with increased lipid fluidity of the thylakoid membranes at elevated temperatures (Armond *et al.* 1980, Berry and Björkman 1980, Gounaris *et al.* 1984, Sundby *et al.* 1986). It is therefore possible that adequate Zn fertilisation can limit the increase in Fo during heat stress through its role in the preservation of membrane integrity. Not only does the physical presence of Zn act as a stabilising influence on membrane components (Chvapil 1973, Welch *et al.* 1982, Cakmak and Marschner 1988a,b), but the function of Zn as a component of the Cu/Zn-SOD enzyme may also protect chloroplastic membranes under high temperature stress, since ROS have the potential to cause membrane damage through lipid peroxidation (Mead 1976, Fridovich 1986, Bowler *et al.* 1992, Scandalios 1993). However during the high temperature treatment in the present experiment it was found that a significant increase in Cu/Zn-SOD activity with Zn fertilisation occurred in one variety, Frame, only, whereas the Fo level was lower with Zn fertilisation in all varieties studied. This suggests that the improvement in Fo with Zn nutrition under heat stress is perhaps not entirely due to an increase in Cu/Zn-SOD activity, but instead may be the result of a more direct role of Zn in stabilising membrane components. By binding sulphydryl groups and phospholipids, Zn ions have been found to stabilise and protect cellular membranes against the oxidative attack of toxic O_2 species (Bray and Bettger 1990).

Damage to the chloroplast caused by high temperature mostly comes from detrimental effects on chloroplast envelope membranes (Bauer and Senser 1979, McCain *et al.* 1989) and thylakoid membranes (Santarius 1975, Armond *et al.* 1980). The physical dissociation of LHCII from the PSII core reaction centre has been attributed to an

increase in thylakoid membrane permeability, resulting in an alteration of the granum stacks and the replacement of the normal granal arrangement by modified thylakoid attachment sites (Gounaris *et al.* 1984, Sundby *et al.* 1986, Xu *et al.* 1995). The present results also showed a number of ultrastructural changes to the thylakoids and other cellular membranes under heat stress. When the high temperature stress was combined with Zn deficiency however, membrane damage was markedly increased. The tonoplast membrane was damaged, allowing the contents of the cytoplasm to enter the vacuole. Chloroplasts were swollen, and in some cases were ruptured completely, indicating an increase in permeability of the chloroplast envelope. This was also observed in the chloroplasts of maize (Ristic and Cass 1992) and bean (Stoyanova and Yordanov 1999) in response to a combination of heat stress and water deficit, and in wheat chloroplasts during plant maturation (Xu *et al.* 1995). The thylakoid membranes were also swollen, and the orientation of the granum stacks was altered. This has also been previously reported in the chloroplasts of maize (Ristic and Cass 1992) and wheat (Sharkova and Bubolo 1996) under high temperature stress. Under adequate Zn fertilisation however, these perturbations of the cellular and chloroplastic membranes under heat stress were considerably reduced, if not eliminated. The tonoplast and chloroplast envelope remained intact, and swollen chloroplasts were not observed. Some swelling of the thylakoids was noted, but the altered orientation of the granum stacks observed under Zn deficiency was not apparent.

Zinc deficiency alone in the present experiment was observed to cause much swelling of the chloroplasts and rupture of the chloroplast envelope, as well as some swelling of the thylakoid membranes in the Zn-inefficient variety, Goldmark. Wang *et al.* (1993) also observed chloroplast envelope rupture in Zn deficient wheat leaves, while Shrotri *et al.* (1978) noted some chloroplast swelling under Zn deficiency in maize. Both of these authors, together with Gui-chang and Zhao-ming (1984), who studied the chloroplast ultrastructure of tomato, reported some swelling of the thylakoid membranes under Zn deficiency, as well as a decreased number of grana per stack, which was also seen in the present experiment.

8.5 Conclusion

A number of authors have shown that a combination of stresses, such as water deficit and heat stress (Ristic and Cass 1992, Stoyanova and Yordanov 1999) or high light and Zn deficiency (Gui-chang and Zhao-ming 1984), can lead to a multiplication of the stress effect and thus cause significantly more damage to chloroplast ultrastructure than each stress applied independently. This was observed in the present experiment with heat stress and Zn deficiency, and it can be hypothesised that supplementary Zn fertilisation, added to a Zn deficient soil, will provide wheat plants with some ability to maintain photosynthetic activity during a short period of high temperature stress. The results demonstrated that adequate Zn nutrition can maintain the integrity of a number of cellular membranes during a high temperature event, including the tonoplast, chloroplast envelope and the thylakoid membranes. Maintenance of thylakoid membrane integrity resulted in the continuation of photosynthetic activity, seen in this experiment as a decrease in Fo fluorescence and a corresponding increase in Fv/Fm during heat stress. Some genotypic differences were observed, particularly with membrane structure, which was better in the Zn-efficient genotype, Frame, under Zn deficiency and heat stress, than in the Zn-inefficient genotype, Goldmark. Supplementary Zn fertilisation also improved the Cu/Zn-SOD activity of both genotypes following the heat stress treatment, which indicates that Zn nutrition may be of some importance in the recovery of cells following an oxidative stress event.

Overall the present results suggest that Zn can assist in the maintenance of photosynthetic activity during a short period of high temperature through its direct role in the stabilisation of membranes, rather than through its function as a component of the ROS scavenging Cu/Zn-SOD enzyme. This result is supported by the observation that Zn deficiency alone appeared to cause more damage to chloroplast ultrastructure than heat stress alone. Future work, which may involve a membrane-binding assay and/or an analysis of membrane binding sites using ^{65}Zn, should be undertaken to further elucidate the ways in which Zn ions bind to membrane components and thus stabilise and protect biomembranes under heat and other oxidative stress conditions.

CHAPTER 9
GENERAL DISCUSSION

Episodes of high temperature stress during the growing season are a common phenomenon throughout many of the world's cereal cropping areas, and often these regions also have soils that are low in plant available zinc (Zn). Individually both stresses are known to be responsible for limiting grain yield in cereals but the interaction between the two has been largely overlooked. A number of deleterious changes in plants are caused by high temperature stress, including damage to membrane integrity (Santarius 1973), denaturation of enzymes (Burke *et al.* 1988) and reduced photosynthesis (Berry and Björkman 1980, Al-Khatib and Paulsen 1984). Coincidently Zn deficiency affects similar processes in plants, resulting in leaky membranes and decreased photosynthetic activity (Welch *et al.* 1982, Marschner 1995).

The study presented in this thesis was designed to investigate the possible role of Zn in the provision of thermotolerance to wheat. A number of researchers in recent years have examined the involvement of Zn in plant stress tolerance, particularly with respect to the oxidative defence system (reviewed by Cakmak 2000), however this study is unique in that it has considered the overall plant response to supplementary Zn and high temperature stress, including both pre- and post-anthesis growth, as well as grain yield and grain quality. Investigations of photosynthetic activity, cell ultrastructure and enzyme activity were also undertaken to further elucidate the physiological mechanisms by which Zn may provide a plant with tolerance to heat stress.

The effect of micronutrient nutrition on wheat grain quality is also a topic that has received little attention in the literature to date. However fertiliser and temperature are two of the three major environmental factors, along with rainfall, known to alter the quality of grain (Wrigley 1996). There is much evidence from the field, supported by glasshouse experiments, to show that a few days of maximum temperatures above 32ºC will lead to the production of mature grain that has weaker-than-expected dough properties, and is consequently of a lower commercial standard (Randall and Moss

1990, Blumenthal *et al.* 1991a, 1993, Wrigley *et al.* 1994, Stone and Nicolas 1994, 1995a, Panozzo and Eagles 2000). This study presented the opportunity to examine the effect of supplementary Zn nutrition on some of the quality aspects of wheat grain, and in addition, since this grain was produced under different temperature regimes, also gave the opportunity to determine whether supplementary Zn nutrition can also provide wheat with some level of tolerance to the effects of growth environment on grain quality.

The purpose of this chapter is to present the main conclusions of this study and to interpret these in the context of the cropping environment of southern Australia. This study has also identified a number of gaps in our knowledge of plant nutrition and thermotolerance, and these will be addressed as a basis for future research.

9.1 The role of zinc in thermotolerance

Changes to the global climate from increased atmospheric concentrations of greenhouse gases are predicted to have important consequences for crop production (Parry and Carter 1988). Although yields of temperate crops increase with enhanced CO_2 concentrations, this may be offset by the negative effects of warmer temperatures in determinate crops (Mitchell *et al.* 1993, Conroy *et al*. 1994, Betts *et al*. 1997, 1998). In wheat, high maximum daily temperatures (>30ºC) imposed prior to anthesis can decrease grain yield (Wardlaw *et al*. 1989a, Tashiro and Wardlaw 1990a), while high temperatures after anthesis reduce both grain yield and grain quality (Randall and Moss 1990, Stone *et al*. 1995, Wardlaw and Moncur 1995). A reduction in grain yield of up to 23% has been reported from as little as 3 days exposure to very high temperatures (above 35ºC) (Hawker and Jenner 1993, Stone and Nicolas 1994). The greenhouse gas model predicts greater temperature fluctuations, and such periods of high temperature during the vegetative and grain filling periods of temperate cereals are likely to increase in both frequency and severity (Blumenthal *et al*. 1990b, 1996, Australian Greenhouse Office 2003).

Any mechanism by which some tolerance to these elevated temperatures may be imparted to crop plants would be welcomed in the ongoing efforts to meet the challenge of increasing global temperatures. The present study examined the responses of photosynthesis, membrane integrity, enzyme activity, vegetative growth, grain yield and grain quality to high temperatures when wheat plants were supplied with and without addition of the micronutrient Zn. The capability of Zn to provide each of these processes with thermotolerance is presented below.

9.1.1 Photosynthetic activity

It has been well established that both high temperature stress and Zn deficiency have a detrimental effect on the photosynthetic apparatus of plants. The results of this study have shown that supplementary Zn fertilisation can improve the photosynthetic activity of wheat, as measured by chlorophyll fluorescence, under high temperature conditions. Photosynthetic activity will be at its lowest when plants are subjected to both heat stress and low soil Zn availability concurrently. This finding was observed at the vegetative stage (Chapters 4 and 8) and during grain filling (Chapters 5 and 6), and in the field as well as under controlled environment conditions. Furthermore the results show that this effect of Zn will persist during up to three consecutive days of high temperatures (Chapters 6 and 8), indicating that supplementary Zn nutrition can offer some protection to the photosynthetic apparatus of wheat against transient high temperature stress, as occurs in the field, but not against a prolonged period of heat stress of greater than four days duration (Chapter 8).

Photosynthetic activity was measured in this study by chlorophyll fluorescence, a sensitive indicator of plant stresses that affect photosystem II (PSII) in the thylakoid membrane (Krause and Santarius 1975, Schreiber and Berry 1977, Schreiber and Bilger 1993). The improvement of the chlorophyll fluorescence ratio, Fv/Fm, with supplementary Zn under high temperature stress was primarily attributed to a stabilisation of chlorophyll initial fluorescence, Fo. This parameter is the dark level of fluorescence, the ground state value when Q_A is fully oxidised. The Fo level is known

to be affected by environmental stresses that cause structural alterations at the PSII pigment level (Krause and Weis 1984). Increases in Fo by heat stress are associated with changes in the lipid-protein interactions caused by increased lipid fluidity of the thylakoid membranes at high temperature (Armond *et al*. 1980, Berry and Björkman 1980, Gounaris *et al*. 1984, Sundby *et al*. 1986). This causes a separation of the chlorophyll *a/b* light-harvesting complexes of PSII (LHCII) from the PSII core complexes and their movement from the appressed to the non-appressed thylakoid regions (Yamane *et al*. 1997). Membrane damage associated with an increase in Fo has been shown to result in an impairment of photosynthetic activity and reduced growth in a number of crop species, including spinach (Ouzounidou *et al*. 1998), faba bean (Guidi *et al*. 1999) and sorghum (*Sorghum bicolour* L.) (Peixoto *et al*. 2002), while stability of Fo during anthesis has been shown to have a good correlation with grain yield in wheat grown under Mediterranean conditions (Araus *et al*. 1998). It is proposed that adequate Zn fertilisation limits the increase in Fo during heat stress through the role of Zn in the preservation of membrane integrity. Zinc has been found to interact with both membrane phospholipids (Von Glós and Boursnell 1981) and with the sulfhydryl groups of membrane proteins (Chvapil 1973), thus providing a stabilising and protective effect on membrane components.

An important outcome from the field experiments of the current study was the observation that supplementary Zn improved photosynthetic activity at elevated temperatures even though the plants with no supplementary Zn could not be considered as Zn deficient (Chapter 5). With a Zn concentration of 20 mg kg^{-1} in the flag leaves, these plants were well within the adequate Zn range for this stage of plant growth, although much lower than the Zn concentration of 103 mg kg^{-1} in the flag leaves of plants which had received a foliar spray of Zn. This phenomenon was observed in the Zn inefficient variety only and warrants further investigation. It implies that Zn fertilisation, even on a Zn sufficient soil, may be able to provide some wheat genotypes with a degree of heat tolerance. It is possible that the high levels of solar radiation and light intensity experienced by plants during grain filling in this late-sown field experiment exacerbated the effects of low Zn supply. Light intensity has been shown previously to affect the requirement for Zn in plants, with high light intensities

magnifying the symptoms of Zn deficiency (Zhang and Wu 1989) and limiting the release of phytosiderophores in Zn-inefficient wheat genotypes (Cakmak *et al.* 1998b).

Results from the controlled environment experiments differed slightly from those obtained in the field. The protective effect of Zn on the photosynthetic apparatus was observed both at 35ºC and at 40ºC in the growth room studies, whereas no detrimental effect on chlorophyll fluorescence was observed at 35ºC in the field, and only in the Zn inefficient variety at 37ºC in the field. Little difference was found between genotypes in the controlled environment experiments, with all varieties, with the exception of the most thermosensitive, showing an improvement in photosynthetic activity with supplementary Zn fertilisation under high temperature conditions.

The results of this study also showed that the chlorophyll content of the flag leaf was markedly reduced under heat stress and Zn deficiency in a Zn inefficient variety (Chapter 6). A loss of chlorophyll in plants subjected to heat stress is associated with irreversible damage to the photosynthetic apparatus (Al-Khatib and Paulsen 1984, Harding *et al.* 1990a, Havaux and Tardy 1999), which was exacerbated in the Zn inefficient variety by low Zn fertilisation. The results indicate that adequate Zn nutrition can limit the loss of chlorophyll in wheat leaves following a high temperature event, at least in a Zn inefficient variety, and add support to the hypothesis that adequate Zn fertilisation can reduce the damage to the photosynthetic apparatus under heat stress.

9.1.2 Membrane integrity

The physical dissociation of LHCII from the PSII core complex under elevated temperatures has been attributed to an increase in thylakoid membrane permeability, resulting in an alteration of the granum stacks and the replacement of the normal granal arrangement by modified thylakoid attachment sites (Gounaris *et al*. 1984, Sundby *et al*. 1986, Xu *et al.* 1995). The hypothesis that supplementary Zn fertilisation may be able to stabilise Fo during a period of heat stress by preserving thylakoid membrane integrity was supported in the present study by an examination of chloroplast ultrastructure

(Chapter 8). Damage to chloroplastic membranes was observed under both Zn deficiency and heat stress independently, but was greatest when the two stresses occurred in combination. In particular the thylakoid membranes were considerably more swollen and the grana were much more irregular in shape and orientation in the chloroplasts of plants grown under Zn deficiency and a period of high temperature stress. Some genotypic differences were observed with respect to thylakoid membrane structure; less damage to these membranes was apparent in a Zn efficient variety under Zn deficiency and heat stress than in a Zn inefficient variety. These differences were minor however, and were not great enough to be seen in the photosynthetic response to Zn deficiency and high temperature stress.

9.1.3 Enzyme activity

Damage to plant membranes by heat stress has often been attributed to increased generation of reactive oxygen species (ROS) (Kraus and Fletcher 1994, Liu and Huang 2000). It has been suggested that supplementary Zn nutrition may reduce a plant's sensitivity to heat stress by increasing the activity of the ROS-scavenging enzyme, copper/zinc-superoxide dismutase (Cu/Zn-SOD) (Cakmak *et al*. 1997, Obata *et al*. 1999, Cakmak 2000, Bakardjieva *et al*. 2000). However although the results from the current study showed an increase in Cu/Zn-SOD activity with an increase in Zn fertilisation, the increase in the activity of this enzyme in response to the period of heat stress was similar at all levels of Zn fertilisation (Chapter 8). Since there was greater membrane damage at low Zn supply, but a comparable increase in Cu/Zn-SOD activity there must be another mechanism by which Zn is involved in maintaining membrane stability and photosynthetic activity under elevated temperatures, rather than its function in Cu/Zn-SOD activity and the detoxification of ROS.

Supplementary Zn fertilisation did improve the Cu/Zn-SOD activity of wheat seedlings relative to those that had received little or no supplementary Zn *following* the high temperature treatment in the present study, but not *during* the heat stress episode. Following removal from the high temperature conditions, the Cu/Zn-SOD activity of

plants fertilised with 0 or 0.2 mg Zn kg^{-1} soil fell to below that of the control (unheated) plants, whereas the Cu/Zn-SOD activity of those plants that had received 2 or 20 mg Zn kg^{-1} soil fell back to a similar level as that of the control plants (Chapter 8). This suggests that Zn nutrition may be of some importance in the recovery of cells following a period of high temperature, through the maintenance of Cu/Zn-SOD activity. Such a result supports the speculation of Cakmak (2000), who suggested that the improvement of the Zn nutritional status of plants may be important for their survival under oxidative stress events. However the present results of chlorophyll fluorescence and SOD activity suggest that Zn is more likely to assist in the maintenance of photosynthetic activity *during* a short period of high temperature through its direct involvement in the stabilisation of membrane integrity, rather than through its function as a component of the Cu/Zn-SOD enzyme. It is also possible that the two functions of Zn are acting in unison; i.e. Zn may assist both in maintaining membrane integrity during heat stress and in improving the scavenging of ROS during recovery from a high temperature event.

9.1.4 Vegetative growth

The present study revealed little effect of supplementary Zn nutrition on the vegetative growth of wheat under high temperature stress. One experiment did demonstrate that supplementary Zn fertilisation could prevent the suppression of leaf elongation in Zn inefficient wheat genotypes under high temperature stress (Chapter 4). However in general there was little effect of high temperature on shoot dry matter production in the current study. It can be argued that the temperature treatments imposed (35/25ºC and 40/20ºC day/night for between two and six days) were not extreme enough to cause substantial reductions in growth, and further research using a more severe high temperature regime (hotter temperature/longer duration) could be undertaken. However the temperature treatments used in the current study were chosen to represent the high temperature conditions that plants are likely to encounter in the field, and a more severe heat stress regime may be less realistic in terms of mimicking field conditions.

9.1.5 Grain yield

Results from both the field and controlled environment experiments of this study found no relationship between the enhancement of photosynthetic activity observed under high temperature stress with supplementary Zn nutrition and the final kernel weight or grain yield of these wheat plants (Chapters 5 and 6). In the controlled environment experiment supplementary Zn nutrition did not prevent a decline in kernel weight or grain yield of a Zn inefficient or thermosensitive variety under heat stress during grain filling, despite an improvement in chlorophyll fluorescence. Some researchers have found that deprivation of assimilates in wheat does not account for the reduction in kernel weight under high temperature conditions (Wardlaw *et al*. 1980, Nicolas *et al*. 1984, Bhullar and Jenner 1986). Instead it is the reduction in the activity of the soluble starch synthase enzyme that is a major limitation to grain filling in wheat under high temperature, as the conversion of sucrose to starch is impaired (Caley *et al*. 1990, Jenner *et al*. 1991, Jenner 1994). This may explain the lack of correlation between photosynthetic activity and kernel weight under Zn deficiency and heat stress in the present study. Other researchers have demonstrated a strong correlation between photosynthetic activity and grain yield in plants exposed to heat stress during both the vegetative and grain filling stages (Al-Khatib and Paulsen 1990, Blum *et al*. 1994, Reynolds *et al*. 2000), but it may be that this is simply a correlated response, rather than cause and effect. It is possible that an enhanced photosynthetic source (such as that provided by supplementary Zn) may improve the supply of carbon assimilate, but that this carbon cannot be synthesised into starch at high temperatures unless another form of thermotolerance is involved, such as a heat tolerant form of soluble starch synthase (Jenner 1994, Fokar *et al.* 1998). Clearly further research is necessary to determine whether the improvement in photosynthetic activity with supplementary Zn nutrition under high temperature stress observed in the present study can also improve the grain yield of wheat under certain conditions.

The field experiments of the present study also revealed that certain wheat varieties will be less sensitive to the stresses of low yielding environments (such as high temperature or drought) when also grown with supplementary Zn nutrition. The thermosensitive variety, Meering, was more sensitive to changes in the environment when grown under

low available Zn. Meering produced lower yields in low yielding environments than the other, more thermotolerant, varieties when grown without adequate Zn fertilisation, but its yields were higher and comparable to the other varieties when grown without Zn under more favourable conditions. The addition of supplementary Zn fertilisation to varieties such as Meering will increase yield stability, thus again supporting the hypothesis that Zn has the ability to provide wheat plants with some tolerance to environmental stress. The exact mechanisms by which this occurs is not well understood, however the field experiments of the current study demonstrated that supplementary Zn fertilisation could increase the canopy temperature depression (CTD) of certain, mainly Zn inefficient, wheat varieties (Chapter 5). Since a high CTD is indicative of a cooler crop canopy and has been associated with improved yields and heat tolerance in wheat in warm environments, due to greater soil moisture extraction and/or higher stomatal conductance (Amani *et al*. 1997, Reynolds *et al*. 1998), these results add further support to the hypothesis that supplementary Zn nutrition can improve the heat tolerance of some wheat varieties.

9.1.6 Grain quality

Maximum temperatures above 32ºC during grain filling, even for just a few days, have been found to have a detrimental effect on grain quality (Finney and Fryer 1958, Blumenthal *et al*. 1991a, Wrigley *et al*. 1994, Borghi *et al*. 1995). A weakening of dough properties occurs as a result of changes in protein composition associated with high temperatures during grain filling (Blumenthal *et al*. 1991a, 1993, 1995a, Wrigley 1994, Stone *et al*. 1997 and many others). A hypothesis of the present study was that supplementary Zn nutrition could limit some of the deleterious alterations to grain protein composition that occur under high temperature during grain filling, possibly through its role in protein synthesis, or its role in the linking of protein sulfhydryl groups. Although the results showed no significant interaction between Zn nutrition and high temperature on protein composition, principal component analysis did show a negative association between grain Zn concentration and the number of days of maximum temperatures over 35ºC during grain filling. Furthermore, elevated levels of

Zn nutrition at Zn responsive sites were found to increase the ratio of glutenins to gliadins in the grain, a ratio that has been associated with an increase in baking quality (Wrigley 1994), and is known to decrease with high temperature stress (Blumenthal *et al*. 1991a, 1993, Graybosch *et al*. 1995, Stone *et al*. 1997). The possibility that the negative effects of high temperature stress on grain protein composition will be compounded when plants are grown on soils of low Zn availability cannot be ruled out, and further investigation is warranted.

9.2 The effect of zinc nutrition on grain protein composition

The results of this study have indicated that supplementary Zn nutrition may reduce the negative effects of high temperature stress on grain quality, however there were other results that showed a more general effect of Zn nutrition on grain quality. The storage protein composition of wheat grain is significantly affected by a number of environmental factors, including soil mineral composition (Huebner and Bietz 1988). Deficiencies of various macro- and micro-nutrients have been found to alter both the seed storage protein fingerprint and dough quality (Flynn *et al* 1987, Bonfil *et al*. 1997). Similarly the concentration of certain nutrients in the grain has been found to influence baking quality (Bequette *et al*. 1963, Douglas and Dyson 1985), although the relationship between Zn nutrition or grain Zn concentration and grain protein composition seems to have received little attention. The results of the present study revealed that the application of supplementary Zn fertilisation, at a Zn-responsive site, decreased the percentage of gliadins in the flour, and produced an increase in the glutenin:gliadin ratio (Chapter 7). This finding was supported by a principal component analysis of the field sites, which showed that the concentration of Zn in the grain was positively correlated with the percentage of glutenins in the grain and negatively correlated with gliadin percentage. Since an increase in the glutenin:gliadin ratio is associated with an increase in dough quality, it is possible that supplementary Zn nutrition may, under conditions of Zn deficiency, improve the baking quality of wheat. This result is encouraging, but clearly requires confirmation. The present study did not

investigate the response of dough quality from flours produced from grain with various concentrations of Zn. An analysis of these doughs is the next fundamental step required in order to clarify the effect of Zn on the storage protein composition of wheat grain and its corresponding baking quality.

The present study also found that the ratio of high molecular weight (HMW) to low molecular weight (LMW) glutenin subunits decreased with the application of Zn fertiliser at a Zn-responsive site (Chapter 7). A decrease in this ratio has been found to produce flours with weaker dough properties (Gupta *et al*. 1993, Wrigley 1994, Blumenthal *et al*. 1994, Stone and Nicolas 1996a, Corbellini *et al*. 1998). This would suggest a decrease in dough strength with supplementary Zn nutrition and may negate the positive effects of additional Zn on the total glutenin percentage in the flour. Clearly further analysis of dough quality from such wheats is necessary for a more complete understanding of the relationship between Zn nutrition and grain quality.

9.3 Recommendations for future research

This study has raised some important questions regarding the role of Zn in the provision of thermotolerance to wheat. A number of areas for future research can be identified.

(i) The present study focussed on the response to supplementary Zn nutrition under a single short period of high temperature stress only. However crop plants in the field are more likely to experience a series of intermittent episodes of high temperature during their life cycle. It is necessary to establish whether the protective effect of Zn on the photosynthetic apparatus under heat stress can be observed at each consecutive heat stress event, and further, whether any cumulative effects of this protection can be identified.

(ii) The results of the present study showed that Zn can maintain the photosynthetic activity of wheat during a short period of high temperature stress, and suggested that this is achieved through the direct involvement of Zn in the stabilisation of membrane integrity. Further research is required to elucidate the ways in which

Zn ions may bind to these membrane components, and thus stabilise and protect biomembranes under heat stress, and possibly other oxidative stress conditions.

(iii) There is some debate in the literature as to whether deprivation of assimilates can account for the reduction in kernel weight under high temperature conditions. This study has further confounded the issue by suggesting that the improvement in photosynthetic activity under supplementary Zn and heat stress does not necessarily bring about an increase in grain yield. However the study only examined the grain yield response after heat stress during grain filling. Al-Khatib and Paulsen (1990) assert that stability of photosynthesis during vegetative growth is essential for the productivity of wheat, while Nicolas and co-workers (1984) suggest that sink growth potential is particularly limited by heat injury when the stress is imposed during the early sink developmental stages. Therefore an investigation of the grain yield response to a period of heat stress at the vegetative stage with supplementary Zn should be undertaken. The improvement in photosynthesis with supplementary Zn under high temperature stress at the vegetative stage may result in a significant yield improvement.

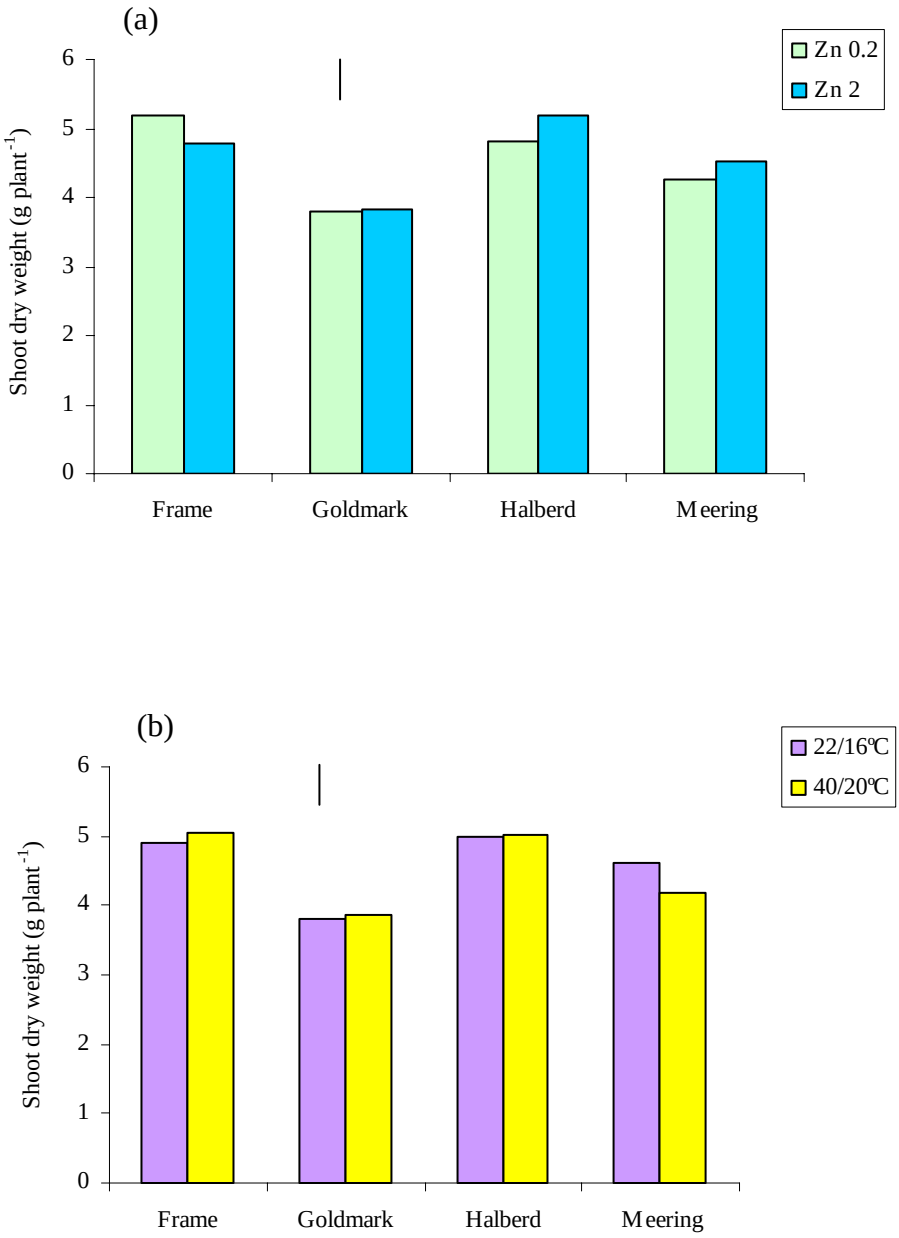
(iv) The mechanism by which supplementary Zn can alter the composition of wheat grain protein, and the glutenin:gliadin ratio in particular, needs to be determined. A number of areas for this research can be proposed, including investigations of the higher requirement of glutenin for Zn, the role of Zn in enzyme activity, and the function of Zn in DNA transcription and translation. This is an important area of future research, particularly if a link between a high grain Zn concentration and an improvement in dough quality can be established. Any incentive for plant breeders to pursue improving the micronutrient density of staple food crops, such as wheat, is important in the effort to find a sustainable food-based approach to the problem of micronutrient malnutrition in humans throughout the world (Welch and Graham 2004). The improvement in grain Zn concentration may therefore not only have important consequences for the baking industry, but also for the elimination of micronutrient malnutrition among the resource-poor people of the world.

APPENDICES

Appendix 5.1. Days on which the maximum temperature exceeded 30ºC after anthesis for each wheat variety grown at the Waite Institute in 1998.

Kronos		Excalibur		Goldmark		Frame		Meering		Halberd		Trident	
SD 1	SD 2	SD 1	SD 2	SD 1	SD 2	SD 1	SD 2	SD 1	SD 2	SD 1	SD 2	SD 1	SD 2
0	11	11	2	9	4	8	4	7	4	6	4	6	4
17	12	14	3	12	5	11	5	10	5	9	5	9	5
20	17	33	8	31	10	30	10	29	10	28	10	28	10
39	18	34	9	32	11	31	11	30	11	29	11	29	11
40	***25***	39	***16***	37	***18***	36	***18***	35	***18***	34	***18***	34	***18***
45	***26***	40	***17***	38	***19***	37	***19***	36	***19***	35	***19***	35	***19***
46	***27***	***47***	***18***	***45***	***20***	***44***	***20***	***43***	***20***	***42***	***20***	***42***	***20***
53	***28***	***48***	19	***46***	***21***	***45***	***21***	***44***	***21***	***43***	***21***	***43***	***21***
54	35	***49***	26	***47***	28	***46***	28	***45***	28	***44***	28	***44***	28
55	39	***50***	30	***48***	32	***47***	32	***46***	32	***45***	32	***45***	32
56	***40***	57	***31***	55	***33***	54	***33***	53	***33***	52	***33***	52	***33***
	41		32		34		34	57	34	56	34	56	34
	47		38		40		40		40	57	40	57	40
	48		***39***		***41***		***41***		***41***		***41***		***41***
	49		***40***		***42***		***42***		***42***		***42***		***42***
	50		41		43		43		43		43		43
	51		***42***		***44***		***44***		***44***		***44***		***44***
	52		***43***		***45***		***45***		***45***		***45***		***45***
			44										

NB. Days in bold and italics denote maximum temperatures above 35ºC.



Appendix 6.1. Effects of Zn fertilisation (mg kg^{-1} soil) (a) and temperature treatment (b) on the shoot dry matter production at maturity of wheat genotypes grown under controlled environment conditions.

The vertical bar represents the $LSD_{0.05}$ for the Genotype x Zinc interaction (a) or Genotype x Temperature interaction (b).

Appendix 7.1. Regression equations for the relationships between mean maximum temperature during grain filling (Figures 7.2 and 7.3), mean grain yield (Figures 7.4 and 7.5) and mean kernel weight (Figures 7.6 and 7.7) and flour protein concentration presented in Chapter 7. Also presented are the regression equations for the relationships between grain zinc concentration and some of the grain protein fractions (Figure 7.8).

Figure Number	Variety (if applicable)	Regression Equation
Figure 7.2		y = 0.779x – 4.26
Figure 7.3	Frame	y = 1.128x – 12.91
	Goldmark	y = 0.317x + 6.61
	Halberd	y = 0.806x – 4.57
	Meering	y = 0.869x – 6.22
Figure 7.4		y = -0.015x + 19.94
Figure 7.5	Frame	y = -0.017x + 21.44
	Goldmark	y = -0.008x + 16.90
	Halberd	y = -0.032x + 24.42
	Meering	y = -0.011x + 19.02
Figure 7.6		y = -0.606x + 35.46
Figure 7.7	Frame	y = -0.571x + 36.23
	Goldmark	y = -0.114x + 18.28
	Halberd	y = -0.324x + 27.19
	Meering	y = 0.095x + 13.06
Figure 7.8	(a)	y = 0.567x + 17.37
	(b)	y = -0.095x + 47.35
	(c)	y = 0.164x + 33.96
	(d)	y = 0.927x + 72.41
	(e)	y = -0.141x + 45.41
	(f)	y = 0.225x + 34.04

Appendix 7.2. Effects of site, genotype and zinc fertilisation (kg ha^{-1}) on the proportion of gliadin in flour protein of wheat grown at Lameroo, Tintinara and Minnipa in 1998.

Genotype	**Gliadin (% total protein)**											
	Lameroo				**Tintinara**				**Minnipa**			
	0	**7.5**	**22.5**	***Mean***	**0**	**7.5**	**22.5**	***Mean***	**0**	**7.5**	**22.5**	***Mean***
Frame	48.4	46.3	46.0	*46.9*	47.0	46.4	45.8	*46.4*	49.4	50.6	50.0	*50.0*
Goldmark	44.0	43.4	41.5	*43.0*	44.8	45.1	44.9	*44.9*	46.6	45.7	46.2	*46.2*
Mean	*46.2*	*44.9*	*43.8*	*44.9*	*45.9*	*45.8*	*45.4*	*45.7*	*48.0*	*45.8*	*45.4*	*48.1*
LSD$_{0.05}$												
Site	**n.s.**											
Genotype	**2.5**											
Zinc	**n.s.**											
Site x Genotype	**n.s.**											
Site x Zinc	**n.s.**											
Genotype x Zinc	**n.s.**											
Site x Genotype x Zinc	**n.s.**											
CV (%)	**2.7**											

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