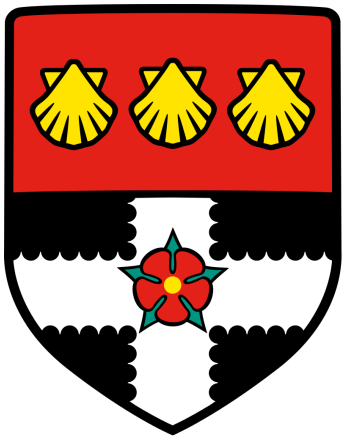

UNIVERSITY OF READING

SPIKE TEMPERATURE DEPRESSION OF WHEAT (*TRITICUM AESTIVUM* L.) AT ANTHESIS

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Thesis submitted for Degree of Doctor of Philosophy (PhD)

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Tempora mutantur, nos et mutamur in illis

DECLARATION OF ORIGINAL AUTHORSHIP

‘Declaration

I confirm that this is my own work and the use of all materials from other sources has been properly and fully acknowledged.’

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Frederick T. Steinmeyer

ABSTRACT

Global climate change has been forecast to result in significant alterations to current temperature and precipitation patterns in cereal growing regions worldwide. The increased occurrence of elevated temperature stress at anthesis is likely to result in significant yield losses in wheat (*Triticum aestivum* L.). The ability of plant tissue to depress its temperature has been reported in relation to the canopy but recent work has demonstrated that spike temperature depression (STD) can be assessed in controlled environments (CE). The findings from two consecutive years of pot-based CE experiments, a field-based experiment under polytunnel cover and a rhizobox-based experiment, in which the thermal dynamics of the spike and flag leaf under contrasting conditions of elevated temperature and water-deficit stress at anthesis, are reported. Flag leaf temperature depression (FLTD) was significantly greater than STD at anthesis. The data do not demonstrate an increased cooling capacity of the spike in the early stages of anthesis but rather in the latter stages, a phenomenon hypothesized to be primarily associated with the onset of senescence in the canopy. The inconsistent relationship observed between FLTD/STD at anthesis and grain yield (GY) does not currently elucidate whether a failure to depress tissue temperature at anthesis is associated with a yield penalty. The effect of experimental design on the physiological response to abiotic stress at anthesis was explored. The plant-wide distribution of photoassimilates at mid-anthesis was examined. Starch and water-soluble carbohydrate content in the flag leaf, peduncle and glumes was not found to correlate to GY. Further examination of the effects that abiotic stress at anthesis have on the photoassimilate distribution and GY need to take place in field-grown wheat.

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ABBREVIATIONS AND SYMBOLS

ANOVA	Analysis of Variance
AT	Ambient temperature
ATP	Adenosine triphosphate
^{12}C	Carbon-12
^{13}C	Carbon-13
$C_6H_{12}O_6$	Glucose
CBOT	Chicago Board of Trade
CE	Controlled environment
CIMMYT	Centro Internacional de Mejoramiento de Maíz y Trigo
cm	Centimetre
CO_2	Carbon dioxide
CT	Canopy temperature
CTD	Canopy temperature depression
DAS	Days after sowing
DW	Dry weight
DSI	Drought susceptibility index
e.g.	exempli gratia (for example)
EPT	External polytunnel temperature
et al.	et alia (and others)
etc.	et cetera (and so forth)
EU	European Union
FC	Field capacity
FDS	Floret development stage
FDS (F)	Female floret development stage
FDS (M)	Male floret development stage
Fig.	Figure
FIR	Far infrared
FLTD	Flag leaf temperature depression
FM	Fresh mass
g	Gram
G6PD	Glucose-6-phosphate dehydrogenase
GMO	Genetically modified organism

GMP	Gross mean productivity
GOPOD	Glucose oxidase/ peroxidase
GS	Growth stage
GY	Grain yield
GxE	Genotype x Environment interaction
h	Hour
H	Hydrogen
ha	Hectare
HSA	High sensitivity assay
HCl	Hydrochloric acid
HK	Hexokinase
HSI	Heat susceptibility index
HST	Heat stress tent
HT	Elevated temperature
i.e.	id est (that is)
Inc.	Incorporated
INV	Invertase
IPCC	Intergovernmental Panel on Climate Change
IPT	Internal polytunnel temperature
IR	Infrared
IWIS	International Wheat Information System
K_2O	Potassium oxide
kg	Kilogram
km	Kilometre
KOH	Potassium hydroxide
L	Litre
LWIR	Long-wave infrared
Ltd.	Limited
m	Meter
M	Mole
m.a.s.l.	Meters above sea level
$MgCl_2$	Magnesium chloride
MgO	Magnesium oxide
min	Minute

mg	Milligram
ml	Millilitre
mM	Millimole
mm	Millimetre
MP	Mean productivity
MWIR	Mid-wavelength infrared
N	Nitrogen
NAD^+	Nicotinamide adenine dinucleotide
NIR	Near-infrared
nm	Nanometre
nmol	Nanomole
O_2	Oxygen
P	Probability
P_20_5	Phosphorus pentoxide
PEL	Plant Environment Laboratory
PGI	Phosphoglucose isomerase
pH	Potential of hydrogen
REML	Restricted maximum likelihood
RH	Relative humidity
RLD	Root length density
RDW	Root dry weight
rpm	Revolutions per minute
RuBP	Ribulose-1,5-bisphosphate
Rubisco	Ribulose-1,5-bisphosphate carboxylase/oxygenase
RWC	Relative water content
s	Second
SB	Seri/Babax
SG	Stay green
SI	Stress indices
SSI	Stress susceptibility index
STD	Spike temperature depression
STI	Stress tolerance index
Subf.	Subfamily
t	Ton

T.	Triticum
T_{cr}	Critical threshold temperature
TD	Temperature depression
*T*Leth	Lethal temperature
T_{lim}	Temperature limit
*T*Max	Maximum temperature
*T*Min	Minimum temperature
TOL	Tolerance Index
*T*Opt	Optimal temperature
UK	United Kingdom
LU	Lancaster University
UoR	University of Reading
USA	United States of America
UV	Ultra violet
v/v	Volume/volume
VPD	Vapour pressure deficit
w/v	Weight/volume
WS	Water-stressed
WSC	Water soluble carbohydrate
WW	Well-watered
µg	Microgram
µl	Microlitre
µm	Micrometre
µmol	Micromole
T_a	Air temperature
T_c	Canopy temperature
T_f	Flag leaf temperature
T_s	Spike temperature
%	Percent
°	Degree
°C	Celsius (degree)
°K	Kelvin (degree)
<	less than
>	greater than

LIST OF EQUATIONS

[1] Stefan-Boltzmann Law: $E = \varepsilon\sigma T^4$

[2] Canopy temperature depression: $T_a - T_c$

[3] Spike temperature depression: $T_a - T_s$

[4] Flag leaf temperature depression: $T_a - T_f$

[5] Photosynthesis: $6CO_2 + 6H_2O \rightarrow C_6H_{12}O_6 + 6O_2$

[6] Relative water content: $\frac{(FM-DM)}{TM-DM} \times 100$

[7] Tolerance Index: $Y_s - Y_p$

[8] Stress/Heat/Drought Susceptibility Index: $\frac{1-(\frac{Y_S}{Y_p})}{1-(\frac{X_S}{YX_p})}$

[9] Stress Tolerance Index: $\frac{(Y_s \times Y_p)}{X_p^2}$

[10] Geometric Mean Productivity: $\sqrt{(Y_s} \times Y_p)$

[11] Mean Productivity: $\frac{(Y_s+Y_p)}{2}$

[12] Vapour pressure deficit: $VP_{sat} - VP_{air}$

[13] Root Length Density: $RL_s \times VSV$

[14] D-Glucose (nmol): $\frac{(\Delta - c)}{m}$

[15] Starch: $D-glucose\ (nmol)\times\frac{GOPOD\ V}{Sample\ V}\times FW\times TW\times MWG\times\frac{162}{180}$

[16] Water Soluble Carbohydrate: $GCont/\square Cont\ /SCont\times\frac{Eth\ V}{Sample\ V}\times TW\times MW\times\frac{1}{1000}$

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CHAPTER 1

GENERAL INTRODUCTION

1.1. THE WHEAT CROP

1.1.1. The Importance of Wheat in Global Trade

Wheat (*Triticum aestivum* L.) is ranked third amongst the 'big three' cereal crops, after rice and corn. In 2014, wheat was grown on 246 million hectares worldwide, which yielded 728 million tons of grain (FAO, 2016). In contrast, corn production in the same year stood at 1.02 billion tons, followed by rice with a yield of 740 million tons. However, when compared to rice and corn, wheat has a vast global geographical distribution, being cultivated at every latitude between 67°N in Northern Europe and 45°S in New Zealand and Argentina (Trethowan *et al.,* 2005). Therefore, there is a 12,400km wide belt spanning the Earth in which humans are able to cultivate wheat. Wheat grows at elevations of between 0 and 3,000 m.a.s.l., although Percival (1921) reported wheat cultivation in Tibet as high up as 4,570 m.a.s.l.. The average global wheat yield is 3.0t/ha^{-1}, although yields vary considerably between countries and regions, from 15.6 t/ha^{-1} in New Zealand to 0.5 t/ha^{-1} in some North African countries (FAO, 2016).

Since its domestication 8-10,000 years ago, wheat has become a staple food crop for approximately one-third of the world's population. Wheat is a particularly important crop in developing countries where there is a strong dependence on noodles, bread and related wheat products (e.g. bulgur wheat) to make up a large proportion of the daily calories consumed. The importance of wheat to humanity lies not only in its nutritional value to humans but also its ability to be used as a livestock feed. Wheat is commonly used in a range of other food and non-food products. These include thickeners for soups and sauces, adhesives, paper, livestock bedding and, if harvested green, as silage for livestock. Wheat, similar to rice, is not only a food and feed commodity to societies around the globe but has deep religious and cultural roots as well. From the bread used during the Christian Eucharist to the matzo shared at the Jewish Passover, wheat forms an integral part of religious

ceremonies the world over. Whilst wheat primarily consists of starch (60-70%), it also contains between 8-15% protein and consequently provides more protein in the form of food and feedstocks than the combined global soybean crop (Shewry, 2009).

Wheat is the most widely traded cereal grain in the world, with 156 million tons being moved around the world in 2015 (FAO, 2016). Total wheat utilization for 2016/17 is forecast to top 724 million tons globally, with a 1% increase in the proportion of wheat utilized for food rather than feed (FAO, 2016). Although exact figures vary year-on-year, the largest exporters of wheat in the world are the USA, Canada, Australia, the EU-27, the Former Soviet Union (consisting of Russia, the Ukraine and Kazakhstan) and Argentina. Combined, these top export countries account for approximately 90% of the wheat traded. As a consequence of the geographical distribution of exporting countries, wheat prices are able to remain relatively stable. Production shortages in the Southern Hemisphere, in Australia or Argentina for example, may be balanced by production from top exporters in the Northern Hemisphere, and *vice versa*. However, the domination of global trade by the top five producers does not act as a guarantee against volatility in the market. Uncertainty about harvest sizes, a spike in energy prices which led to increased production costs for cereal producers, export restrictions enforced by certain producers and a weak US dollar, all contributed to the food price spike of 2007/08. As a result of the price upheaval during 2007 and the first half of 2008, civil unrest was witnessed in several developing countries throughout Africa and Asia as uncertainty was cast over the availability and affordability of staple food stocks. Over the 14-month period from January 2007 until March 2008, the average price of wheat rose by 118%, with the largest increases in price witnessed in emerging economies (FAO, 2016).

The international wheat trade represents approximately 20% of annual production. The top importers of wheat on the global market are concentrated in developing countries, particularly in sub-Saharan Africa, North Africa, South-East Asia and Latin America. Growing populations, coupled with increased economic wealth, mean that demand for cereals has increased greatly in these regions since the 1960s. Increasing cereal yields are required in coming decades to meet the global population rise that is forecast to top 9.1 billion by 2050. If demand patterns remain

unchanged until then, annual demand for wheat will top 880 million tons (Weigand, 2011). The import demand from countries such as Brazil, the Philippines, Indonesia as well as a number of Middle Eastern and North African countries, will increase between now and 2050.

1.1.2. Origins and Distribution

Early domestication of wheat took place around the start of the Neolithic period, as human civilisations moved from their hunter-gather lifestyles to settled forms of early agriculture. This period, known as the Neolithic Revolution, resulted in the transition of human society from small, isolated groups of largely nomadic people, to the creation of larger, sedentary and complex societies. The domestication of animals and food crops provided the nutrition needed for the development of these societies which led to the creation of trade between people, the division of labour and the formation of the political structures we know today. Without the domestication of our food, the formation of complex human societies would not have been possible. Domestication of wheat took place in the areas surrounding the 'Fertile Crescent', today's Egypt, Israel, Syria and Iran (Feldman, 2001).

Wheat is a member of the grass family *Poaceae* (*subf. Pooideae*) and the genus *Triticum*. There are four cultivated species of wheat in the genus *Triticum*, along with numerous sub-species. Einkorn wheat (*T. monococcum*) is a diploid wheat containing two sets of chromosomes. As the translation from Germanic suggests, Einkorn ('one grain') wheat varieties typically only yield one grain per spikelet. It is the oldest of the wheat species, having been domesticated from natural grass populations approximately 10,000 years ago. Due to its obvious shortcomings in yield, Einkorn wheat was gradually replaced by Emmer wheat as of the start of the Bronze Age (approx. 3,000 BC). Today, Einkorn is considered a 'relic crop' and its cultivation is limited to the Balkans and isolated parts of Central Europe, such as Switzerland and Germany. Emmer wheat (*T. turgidum*) is a tetraploid species, containing four sets of chromosomes. Domestication was based on the selection of traits that provided farmers with a clear yield benefit over Einkorn. These traits included multiple grains per spikelet, a larger grain size, a non-shattering spike at maturity and increased ease of threshing (Jantasuriyarat *et al.,* 2004; Nalam *et al.,*

2006; Simons *et al.,* 2006). The subspecies of *T. turgidum*, durum, is the only remaining tetraploid wheat species of commercial significance remaining today. Bread wheat (*T. aestivum* L.) is a hexaploid wheat, containing six sets of chromosomes and it is the most economically important wheat grown today. This species was produced by means of hybridisation of Emmer wheat and a wild grass species, *A. tauschii*, about 8,000 years ago. Bread wheat is classified as either a spring wheat or a winter wheat. A winter wheat is sown in autumn so that it can develop into its vegetative phase during the winter months before resuming growth in spring. This gives a winter crop the advantage of exploiting the moisture associated with the autumn growing period and gives the plant a head start in light interception in spring. Spring wheat, as the name suggests, is sown in the spring and winter and spring wheat are harvested in late summer. In the UK, winter wheats are more popular for producers than spring wheats although the importance of spring-sown crops is recognized (FWI, 2016). Grain yields are usually higher in a winter wheat crop than in a spring crop. Today, 95% of all wheat grown is hexaploid bread wheat. The remaining 5% is largely accounted for by tetraploid durum wheat, grown primarily for pasta production.

1.1.3. Plant morphology

Cereal breeding has resulted in the creation of 'modern' wheat varieties which differ from their ancestors in numerous aspects. The grain yield of modern wheat cultivars is greater than that of older varieties (Morgunov *et al.,* 2013), in part due to breeding efforts but also due to changes in agronomic practices. Historically, breeders have made improvements to virtually every quality aspect of modern wheat, including grain protein content, micronutrient composition and bread-making traits. Wheat breeding targets have been adapted to meet the local environmental conditions and therefore differ depending on the region in question e.g. Hard Red Wheat in Canada and Soft Wheat in the UK. In the UK, the Agriculture and Horticulture Development Board issues an annual Recommended List that provides yield, quality and market data for a range of UK grown wheat varieties in each end-use category. The classification of recommended varieties in this way enables farmers and agronomists to select the most suitable variety to meet their specific needs.

Zadocks *et al.* (1974) created a standardised measurement scale of cereal development. Based on ten principle growth stages in cereals, each stage is sub-divided into ten further stages so that the Zadocks scale ranges from 00 (dry seed) to 99 (loss of secondary dormancy). Since its inception, the Zadocks growth scale is used by researchers, agronomists and farmers to assess the growth and development of wheat. In a commercial setting, the scale is used primarily as a standardised method of sample collection and for the correct timing of agro-chemical applications.

Both above and below-ground parts of wheat plants are subject to substantial variation between varieties grown today. The shoot system of a mature wheat plant consists of a central stem, from which leaves branch off on either side. The stem is built from repeating phytomers, each of which contain a node, a leaf, a hollow internode and a tiller bud (Kirby, 2002). Wheat has two distinctive types of roots, namely seminal (roots which originate from the root primordial of the grain) and nodal roots (roots which develop at the same time that tiller development is initiated). Every sown wheat seed is capable of producing a number of tillers with each shoot terminating in the spike at the top of the stem. The mature wheat spike is formed from two parallel rows of spikelets, branching off from a centrally located rachis. Each spikelet contains between two and four fertile florets (Kirby, 2002). The floret contains the floral organs, namely two lodicules, the carpel (comprising the ovary and stigma) and three stamens. The anthers of wheat are approximately 3mm long and consist of four loculi that hold the pollen grains (Kirby, 2002). The floral organs within the floret are encapsulated by an inner (palea) and outer (lemma) sheathing structure. The outer spikelet is protected by a lemma-like structure called the glume.

At the start of anthesis, the lodicules at the base of the floret swell, which forces the palea and lemma to part. The extent to which the palea and lemma are forced apart varies with genotypes, producing both open and closed pollinated varieties. The filaments, on which the stamens are located, elongate thus forcing them upwards. During this elongation process, the stamen will dehisce, causing a split to form along the length of the anther. The lobes of the stigma swell and are forced apart, exposing the white-hairy receptive structures, which are used for pollen capture.

Percival (1921) found that this process takes approximately five minutes. Turgor pressure from the lodicules may keep the floret open for up to an hour in order for pollination to occur. The stigma may remain receptive to pollen for up to five days after the end of anthesis if successful pollination does not occur (Kirkby, 2002). The viability of pollen is dependent on a range of environmental stress factors, both during development and after release. These stress factors include fluctuations in temperature (Rao *et al.,* 1992), relative humidity (Hong *et al.,* 1999; Nepi *et al.,* 2001), water stress during development (Lalonde *et al.,* 1997; Saini, 1997), UV concentration (Murphy, 1983; Torabinejad *et al.,* 1998) and the composition of atmospheric gasses (Aloni *et al.,* 2001). Upon successful germination of the stigma by pollen, a pollen tube is formed. This tube will develop downwards towards the embryonic sac. Two sperm cells from the pollen then migrate down the length of the pollen tube to the embryotic sac, where the nuclei then fuse with the egg nucleus and the polar nuclei. The egg nucleus develops into the embryo and the polar nucleus develops into the endosperm.

The pattern of floral development in wheat is asynchronous (Lukac *et al.,* 2012). Centrally located florets on the spike of the main tiller are generally the first to flower, with anthesis progressing synchronously towards the apex and the base of the spike (Pask *et al.,* 2012). Anthesis within an ear lasts between four and seven days, although genotypic variation of this trait exists. As wheat is largely cleistogamous (Frankel and Galun, 1977), it is a primarily self-pollinating plant. Florets have however been reported to be chasmogamous (Lukac *et al.,* 2012). Due to the absence of nectaries in a wheat flower, cross-pollination by insects has been found to be of minimal importance (Glover, 2002).

1.2. PROBLEM STATEMENT AND JUSTIFICATION OF STUDY

1.2.1. The Green Revolution

The Green Revolution refers to a twenty-year period of technology transfer, market development, crop research and infrastructure improvements that took place in developing countries between 1966-1985. During the 1960s, the demand for staple crops far outstretched the production capability of the time. In 1965, India and Pakistan imported a combined 450 tons of new wheat seed developed by the *Centro Internacional de Mejoramiento de Maíz y Trigo Centro* (CIMMYT) in Mexico. The following year, significant drought events in northern India led to the importation of an additional 15 million tons of food grain to meet its population's demands. Between 1966 and 1970, interventionist strategies spearheaded by CIMMYT and the Indian government distributed the new seed, novel irrigation techniques and agronomic practices, as well as market development projects throughout the country. In 1970, India harvested 20.1 million tons of wheat and Pakistan harvested 7.3 million tons of wheat. This represented a 61% and a 63% increase on 1965 wheat yields respectively. Two years later in 1972, India harvested 27 million tons of wheat. Coupled with the success of increasing wheat yields during the period of 1966-1970, it was the arrival of IR8 from the Philippines, a semi-dwarf rice variety which responded well to irrigation and fertilizer application, that it became evident that the agricultural revolution taking place had been a success. By the 1980s, CIMMYT had distributed seed to 113 countries and CIMMYT-derived wheat covered 45 million hectares of land in the developing world. Africa largely missed out on the first wave of the Green Revolution that happened in India and Pakistan during the 1960s.

1.2.2. High Temperature Stress

1.2.2.1. Climate Change

Since field crops were first domesticated, the global environment has undergone gradual changes. From the start of the Industrial Revolution, the period from approximately 1760-1840, human consumption of fossil fuels has increased

dramatically (Kostic, 2007). The global climate is in a constant state of flux and changes in it before the Industrial Revolution were due to changes in the Earth's orbit, increased volcanic activity or changes in solar patterns. However, it is the historic consumption of fossil fuels, and its continued use at the current rate, which has resulted in the global climate experiencing a rapid increase in the severity and frequency of these changes (IPCC, 2014). There is strong evidence to support the hypothesis that the global climate has warmed significantly since the mid-1800s as a result of increased use of fossil fuels (IPCC, 2014). This change in the global climate coincides with an unprecedented rise in atmospheric carbon dioxide, methane and nitrous oxide levels. As a result of the rise in mean global temperature, a changing climate will also result in alterations to current precipitation patterns and the increased occurrence of extreme weather events. Mean global temperature is predicted to rise by between 1-6°C by the end of the century (IPCC, 2014). Under the 'business as usual' scenario developed by the IPCC, mean global temperatures will increase by 0.3°C per decade until 2100 (Houghton *et al.,* 1990). Coupled with an increase in daytime temperatures, elevated night-time temperatures will occur over a sizable portion of cereal producing regions due to increased cloudiness trapping radiant heat (Alward *et al.,* 1999; IPCC, 2014; Vose *et al.,* 2005).

1.2.2.2. Impact of Climate Change on Global Wheat Yields

An increased understanding of the potential impacts that climate change is likely to have has meant that research has progressively focused on estimating the effects of increased temperature stress on crops (Amir and Sinclair, 1991; Rosenzweig and Tubiello, 1996). However, the changes predicted by climate scenarios do not necessarily mean that yield losses are inevitable (Ortiz *et al.,* 2008). Some wheat growing regions in the Northern Hemisphere, such as the United Kingdom, the American Northern Plains or the North China Plain, are likely to benefit from a changing climate (Nakicenovic and Swart, 2000; Izaurralde *et al.,* 2003; Richter and Semenov, 2005). In Europe, a portion of the yield increases can be attributed to higher seasonal temperatures extending the growing period and making more land suitable for cultivation (Olesen and Bindi, 2002). The occurrence of extreme weather events associated with climate change is, however, likely to result in yield losses in the long term. Despite potential yield gains of between 7-36% in these

regions, there are large wheat producing regions in the world that will experience yield losses as a result of an altered climate. Wheat yields in Southern Australia for example, are set to decrease by between 13.5-32%, depending on the climate scenario under consideration (Lu *et al.,* 2005). The negative effects of climate change are likely to be more pronounced in tropical regions where the adaptation capacity is far lower than in temperate growing regions (IPCC, 2014).

In recent years, numerous crop models have demonstrated the vulnerability of the food production system through changes in our current climate (Lobell and Field, 2007; Challinor *et al.,* 2010). Despite the inherent uncertainty contained within these models, the general consensus arising from their findings show that increases in high temperatures, water-deficit stress and extreme weather events in the main cereal growing regions of the world are a threat to food stability between now and the end of the century (Kang *et al.,* 2009; Jaggard *et al.,* 2010).

Whilst in the UK, temperatures in early June may not be consistently high, the maximum daily temperatures in wheat producing areas during the 2010 growing season reached up to 30°C during the period immediately before, and during, anthesis (Lukac *et al.* 2012). In June 2015, the highest daily maximum temperature recorded was 32.5°C (June 30th). The extreme temperatures recorded during May/June 2010 indicate that high temperature stress has the impact of potentially reduce wheat yields in the UK. Recent years have seen above average temperatures being reported in June/July for many regions in the UK (Met Office, 2016b).

1.2.2.3. Long-term Trends in Wheat Yields

The gains in wheat production experienced during the Green Revolution have recently plateaued and, in some cases, started to decline (Grassini *et al.,* 2013). A number of key wheat producing regions are currently experiencing stagnating yields despite earlier periods showing linear increases in growth. These 'Upper Yield Plateaus' have been identified in a number of globally important, high-yielding wheat systems. Up to 27% of global wheat production may have plateaued (Grassini *et al.,* 2013). Reasons to explain this plateau include: (i) intensive wheat producing regions may be reaching a biophysical yield ceiling, (ii) land degradation, (iii)

changes in the use of agrochemicals, (iv) poorly suited/inappropriate varietal selection, as well as (v) poorly targeted research and development initiatives.

These upper yield plateaus, coupled with the overwhelming evidence supporting anthropogenically driven climate change and the threat posed from a growing global population, should serve as an important early warning sign that despite the gains in food production made during the first Green Revolution, the world is in need of a Green Revolution 2.0. The fundamental challenge facing food production today is much the same as it was during the 1960s: how to produce sufficient food to meet the growing demands of the global population? However, today there are strong calls to meet these demands in a sustainable way that minimises agro-ecological damage. The impending threat posed by high temperature stresses and other forms of abiotic stress brought about as a result of climate change must be considered as well when developing a new approach to feeding the world. Cereal production has to be improved greatly in order to ensure global food security into the future. In order to effectively meet the growing demand for food from future generations it is essential to develop new, heat tolerant varieties of wheat. For this to be successful, it is vital to have a complete understanding of the effects of high temperature stress on wheat.

1.2.2.4. Vulnerability Hotspots

By 2080, Battisti and Naylor (2009) reported that most cropped areas of the world are likely to be exposed to record temperatures during the growing season. Many authors have reported a trend of increasingly high growing season temperatures in a number of key wheat growing regions around the world (Alexander *et al.,* 2006; Hennesey *et al.,* 2008). A *vulnerability hotspot* is a cereal-growing region likely to be negatively impacted by climate change during the 21st century. It has been defined by Fraser *et al.* (2012) as: *"a region that models project as likely to experience both a decline in adaptive capacity and in available soil moisture".* The two key criteria used to identify vulnerability hotspots are susceptibility to climatic stress and a limited capacity to adapt to this change in abiotic stress. Fraser *et al.* (2012) concluded that the key *vulnerability hotspots* for wheat during the 21st

century are located in *"The south-eastern USA, south-eastern South America, north-eastern Mediterranean and parts of central Asia"* (Fraser *et al.,* 2012).

A central issue associated with climate change in cereal growing areas in temperate regions, is the occurrence of short and extreme periods of high temperature, known as 'heat waves', which are likely to increase in frequency (Barrow and Hulme, 1996). These short, non-seasonal heat stresses can have a negative impact on the yields of a number of key crops (Wheeler *et al.,* 1996a; Porter and Semenov, 2005) and pose a threat to global food security. Teixeira *et al.* (2013) found an increase in the occurrence of heat stresses during the "thermal sensitive period" in wheat, mid-anthesis, when modelling future climate scenarios.

With unprecedentedly high temperatures affecting twenty percent of Russia's agricultural area during the 2010 growing season, price volatility and speculative pricing practices resulted in wheat prices rising by as much as 50% on the international market during that year (see Fig. 1.1 in Appendix). Many of the issues surrounding food pricing are highly dependent on the socio-political and financial stability of a region to respond to global market fluctuations (Kharas, 2011). However, if farmers are insufficiently adapted to the threats posed by a changing climate in the future, serious yield reductions will further worsen the problem of food supply and availability in already volatile regions around the world. Providing farmers with the adaptive tools and capacities will address these issues of food production volatility. A key part of this is providing farmers with suitable seed to ensure crops are able to withstand increasingly severe stresses throughout the growing season.

1.2.2.5. Effect of Heat Stress on Plants

In order to survive, plants must adapt to the prevailing biotic and abiotic constraints they face. All stages of plant growth are sensitive to temperature and the physiological effects of heat stress on plants are consistent between crop species. For example, during the reproductive period, heat stress has been correlated to reduced flowers per plant, impaired anther dehiscence (Saini and Aspinall, 1982), pollen sterility (Saini and Aspinall, 1982; Sakata *et al.,* 2000), reduced pollen

production (Prasad *et al.,* 2006a) and reduced flower fertility (Young *et al.,* 2004). Heat stress during grain filling has been found to reduce both grain number and weight (Prasad *et al.,* 2006a; Prasad *et al.,* 2006b; Farooq *et al.,* 2011). Abiotic stress during the period between spikelet initiation and anthesis in wheat, has been identified as having the largest negative impact on yield amongst a number of species (Saini and Aspinall, 1982; Ferris *et al.,* 1998; Porter and Gawith, 1999). The sensitivity of plants to temperature events that may occur outside of the optimal range varies greatly between cultivars. Barnabas *et al.* (2008) concluded that the timing of the onset, duration and severity of a heat stress event determines which combination of response mechanisms a plant utilizes.

1.2.2.6. Tolerance, Avoidance and Escape

A great variation in tolerance to heat stress exists in plant species. Larcher (1995) classified plant species into three possible categories based on thermotolerance: heat-sensitive species, relatively heat-resistance species and heat-tolerant species. The range of adaptations are present in plants that allow for survival in hot and arid environments. These adaptation mechanisms can be classified into three groups: *avoidance, tolerance* and *escape*.

Avoidance adaptations are those that can prevent or delay the negative effects of an environmental stress on a plant. Examples of heat/drought avoidance mechanisms include increased stomatal resistance (Oosterhuis and Walker, 1987), the deposition of waxes on leaf surfaces (Clark and Richards, 1988), a reduction in the overall canopy size and a change in the leaf angle and/or orientation (Morgan, 1984; Araus *et al.,* 1992). Differences in the root/shoot ratio exist between varieties (Williams *et al.,* 2013). Transpirational cooling is a further example of possible avoidance mechanisms (Reynolds *et al.,* 1997; Pinto *et al.,* 2010). Another key stress avoidance mechanism in wheat is plasticity in the rate of growth, duration of anthesis and the duration of grain filling.

Tolerance relates to the ability of a crop to acclimate to and withstand stressful environmental conditions. Drought tolerance is expressed primarily through changes in osmotic adjustment to prevent disruption to the intercellular metabolic

pathways (Munns, 1988) and through cellular elasticity (Steudle *et al.,* 1977; Joly and Zaerr, 1987). Thermotolerance can be divided into four main sub-categories: (i) the enzymatic tolerance to high temperatures in order to prevent denaturation of key enzymes (Senioniti *et al.,* 1986; Mahan *et al.,* 1987; Burke, 1990), (ii) thermostability of cell membranes (Ahrens and Ingram, 1988; Chaisompongopan *et al.,* 1990), (iii) thermotolerance as a result of the syntheses of heat shock proteins (Burke *et al.,* 1985; Pelham, 1986), and (iv) thermotolerance through maintained photosynthetic productivity at supra-optimal temperatures (Al-Khatib and Paulsen, 1990).

Escape through phenophase modification has been a valuable tool for some wheat varieties to address the negative effects of abiotic stress on development and yield (Richards, 1991; Loss and Siddique, 1994). The selection of appropriate phenology - such as timing the sensitive flowering period with the coolest part of the wheat growth cycle - has shown some success. Extending the period of anthesis over a long timescale and changing the diurnal pattern of flowering has been hypothesized to reduce yield losses (Lukac *et al.,* 2012). However, with the increased threat of high temperature stresses occurring with greater frequency and out of season, merely relying on early flowering as an adaptation mechanism cannot be considered sufficient.

1.2.2.7. Effect of Heat Stress on Wheat

The extent of heat stress damage is highly dependent on the physiological developmental stage a stressed plant is in. Wollenwebber *et al.* (2003) concluded that heat stress during the reproductive phase of development is more damaging to the plant than during the vegetative phase of growth. Several other authors have also reported the sensitivity of sexual reproduction in plants to thermal extremes (Hedhly, 2011; Thakur *et al.,* 2010). The sensitive stage of microsporogenesis is negatively affected by heat and/or drought stress and disruption of this process is associated with yield losses (Lalonde *et al.,* 1997; Sakata *et al.,* 2000; Sakata and Higashitani, 2008). Heat stress at anthesis has been found to cause tissue dehydration, reduce pollen viability (Saini and Aspinall, 1982), increase rates of floret abortion (Wardlaw and Wrigley, 1994) and reduce fertilization (Ferris *et al.,* 1998). The

negative effect of a heat stress event, even only for a few hours, is particularly pronounced during the sensitive developmental stage of mid-reproduction (Porter and Semenov, 2005). Any heat stress event is further exacerbated by the effect of any potential water stress that may occur simultaneously.

Heat stress has also been found to speed up the rate of development of the spike, leading to fewer spikelets being formed which results in fewer grains per spike (Saini and Aspinall, 1982; Porter and Gawith, 1999). In addition to heat stress during the reproductive phase, heat stress during grain filling (terminal heat stress) has also been identified as a vulnerable stage of plant development (Wardlaw *et al.,* 1980; Viswanathan and Khanna-Chopra, 2001; Streck, 2005; Dias and Lidon, 2009).

The critical threshold temperature (T_{cr}), defined as the temperature above which a stress response is initiated in wheat, varies between genotypes. For wheat, the T_{cr} has been identified as ranging from 22°C (Modhej *et al.,* 2008), to 24°C (Porter and Gawith, 1999), 25°C (Ferris *et al.,* 1998; Spiertz *et al.,* 2006) and 27°C (Semenov and Shewry, 2011; Teixeira *et al.,* 2011). This variability in T_{cr} would suggest two likely explanations: (1) there is an adaptive capacity within wheat to heat stress, or (2) there is a large amount of variation in the methodology used to establish T_{cr} values. This variation in experimentation is due to a number of differing variables existing between experiments including differences in the genotypes used for analysis, the duration, timing and severity of heat stress, differences in the relative humidity and vapour pressure deficit, differences in light intensity and whether the experiment is conducted in the field or in a controlled environment. The limiting temperature (T_{lim}), the temperature above which leaf development ceases, has been identified by Porter and Gawith (1999) as being 31°C, by Ferris *et al.* (1998) as being 35°C and by Teixeira *et al.* (2013) as being 40°C. This variation in T_{lim} suggests that similarly to T_{cr}, a significant adaptive capacity to high temperatures exists in wheat or that experimental variation in the establishment of T_{lim} is great.

1.2.3. Photosynthesis

Photosynthesis is the process by which water and carbon dioxide are assimilated and converted using harvested solar energy and a series of enzymic reactions, into synthesized carbohydrates (Tanaka and Makino, 2009). Photosynthesis consists of a series of light dependent reactions, the products of which drive the light independent reactions.

The light dependent reactions take place in the thylakoids. Here, photolysis splits water into the components required for non-cyclic photophosphorylation, i.e. hydrogen ions, electrons and oxygen. The hydrogen ions pass through adenosine triphosphate (ATP) synthase from which ATP is produced.

The light independent reactions, also called the Calvin Cycle, take place in the chloroplast and are comprised of three stages. The first is carbon dioxide fixation in which ribulose bisphosphate (RuBP) is carboxylated under the influence of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco). This conversion results in the synthesis of glycerate 3-phosphate (GP). The next step of the light independent reactions is carbon dioxide reduction, which utilizes the GP from the previous stage to synthesize triose phosphate (TP) using reduced nicotinamide adenine dinucleotide phosphate (NADP) and ATP. Some of this TP is converted into the carbohydrates, lipids or proteins that are then made available to the plant. The final stage of the light independent reactions is ribulose bisphosphate regeneration. In this stage, the remaining TP is converted into RuBP with the help of ATP, which is used to facilitate the continued carboxylation taking place in the first stage of the light independent reactions, this keeps the cycle operating.

Photosynthesis is limited primarily by three main factors: carbon dioxide concentration, light intensity and temperature. If any of these factors become limiting, the rate of photosynthesis will plateau. The concept of the rate of photosynthesis being limited by the pace of the ‘slowest factor’ dates back to the beginning of the 20th Century (Blackman, 1905).

The light dependent reactions are, as suggested by the name, dependent on the light intensity. As the intensity moves from low to high levels, the rate of reaction increases. The greater the light intensity, the greater the chlorophyll ionization and consequent ATP/NADP synthesis takes place. At very high light intensity levels however, chlorophyll may be damaged which leads to a steep decline in photosynthetic rate. However, the photosynthetic response to light is not unlimited, as a limiting factor is likely to be encountered before these high light levels are reached. An increase in the carbon dioxide concentration is dependent on the rate of the light dependent reactions and therefore will increase the rate of photosynthesis until a limiting factor results in a plateau.

As the light dependent reactions are not enzyme driven, they are not affected by changes in temperature. The light independent reactions however are enzyme driven and therefore changes in temperature will affect the function of the stage. As the temperature approaches the optimum, the rate of activity increases. If the temperature increases above this optimal threshold, denaturation of the enzyme takes place and the activity declines sharply until it ceases entirely. The negative effects that high temperature stress has on the rate of photosynthesis have been well documented (Berry and Björkman, 1980).

Over 25 years ago, Weis (1981) demonstrated that elevated temperatures inhibited Rubsico activity in spinach. Kobza and Edwards (1987) subsequently reported that high temperature stress in wheat resulted in damage to Rubisco, confirming the sensitivity of the photosynthetic apparatus of wheat to heat stress. It was Feller *et al.* (1998) who demonstrated that under light conditions, temperatures in excess of 30°C resulted in Rubisco activation inhibition in wheat. High temperature stress disrupts enzymic activity and consequently, the CO_2 fixation associated with photosynthesis (Crafts-Brandner *et al.,* 1997). Genetic variation of Rubsico sensitivity to high temperature stress exists and the exploitation of this trait has the potential to improve crop photosynthesis and consequently crop yields (Prins *et al.,* 2016).

Many current cereal-breeding programs recognize the historic lack of focus on increasing photosynthetic capacity in grain crops (Long *et al.,* 2006; Parry *et al.,* 2011). As an enzyme driven process, photosynthesis is limited by heat stress. Rubisco is an important enzyme associated with carbon dioxide assimilation and is a key determinant of the photosynthetic efficiency of a plant. In wheat plants, Rubisco constitutes between 60-80% of the total soluble proteins of the flag leaf. The photosynthetic assimilation rate of a leaf increases with temperature until it reaches a maximum level at an optimal temperature. The rate then decreases, as temperatures become supraoptimal. Several authors have demonstrated that photorespiration in leaf tissue has a significant effect on the net photosynthetic assimilation rate when tissue temperatures exceed optimal levels (Ku and Edwards, 1977; Monson *et al*, 1982). A complete discussion of the processes involved in assimilate production and storage, remobilization of carbohydrates and the role of photoassimilates in yield production is available in Chapter 4.

Water soluble carbohydrates (WSC) in the vegetative tissue consist primarily of fructan, a macromolecule of the simple sugar fructose, but with a smaller quantity of sucrose. Wheat cultivars have demonstrated a differing ability to store WSC, which can be used as a reserve source of assimilates for grain filling in case of late season water stress (Reynolds *et al.,* 2007). The accumulation of WSC in a plant occurs when the source production of assimilates is greater than the sink consumption. Schnyder (1993) concluded that WSC are primarily stored within the stem internodes of the plant. Further literature relating to the distribution of WSC in other wheat organs, such as the spike, is limited. Under conditions of terminal drought, WSC have been found to contribute significant amounts of carbohydrates used for grain filling (Bidinger *et al.,* 1977; Blum, 1998).

1.2.4. Breeding for Extreme Environments

By 2050, global agricultural production is required to increase by approximately 70% in order to meet the demands placed by a growing population (Tilman *et al.,* 2011; FAO, 2016). The demand for crops is forecast to increase by up to 110% in the same period (Tilman *et al.,* 2011). This additional output must be achieved through crop yield increases rather than expansion of cultivated land (Godfray *et al.,*

2010; Foley *et al.,* 2011). Ray *et al.* (2013) reported that wheat yield is currently increasing at an average rate of 0.9% per year. This increase is insufficient to meet future demand pressures.

Although efficient management of cropped land is important in maintaining yields, agronomic practices alone will not continue to raise crop yields indefinitely. With an understanding of the negative impacts that agricultural inputs, such as fertilizers, herbicides, insecticides and fungicides, have on the environment, genetic yield gains are required to take place with increasingly fewer inputs. With the knowledge that essential resources such as fossil fuels and potash are becoming increasingly scarce, along with ecological issues associated with runoff from fields, it is imperative that food production considers its global impact. Furthermore, these yields gains are required to take place despite the increase in the severity of extreme weather events as well as a changing climate.

Breeding successes since the 1970s have largely been due to an increase in the Harvest Index (HI) as a result of the introduction of dwarfing genes (Richards, 1996; Richards, 2000). The Japanese variety Akakomugi provides the *Rht8* gene to many of the dwarf and semi-dwarf wheat varieties grown in Europe today. The introduction of dwarfing genes into modern wheat varieties resulted in improved lodging resistance which was required as a result of the increased grain mass in the spike. In terms of empirical breeding successes however, the largest gains have been observed under optimal growing conditions, with yield gains in the UK for example being ten times greater than in Australia (Richards, 1996). The increases in wheat yield seen since the 1960s have become increasingly difficult to replicate (Reynolds *et al.,* 1996; Mann, 1999). In order to achieve the necessary yield gains, an increase in the development of analytical breeding programs is required. The targeting of key physiological traits, as well as an integration of these traits into empirical breeding programs, has been predicted to increase the rate at which yield improvements are realized (Araus, 1996; Slafer and Araus, 1998).

Crop biomass production is determined primarily by the photosynthetic rate of the crop throughout its life cycle. Consequently, increases in photosynthetic rate will result in increases in crop yields (Ainsworth and Long, 2005). When considering

opportunities to increase the photosynthetic rates of our food crops, a number of opportunities exist. These include the optimization of light capture by the canopy (Horton, 2000) and extending the possible duration of light capture with the help of 'stag green' phenotypes (Dohleman *et al.,* 2009). Once interception optimization has been achieved, increasing photosynthesis is limited to increasing the photosynthetic rate per unit of leaf area (Long *et al.,* 2006; Parry *et al.,* 2007). A significant increase in the photosynthetic efficiency of crops is possible (Reynolds *et al.,* 2000). In order to create wheat varieties adapted to stress prone environments, it is vital the development of novel screening tools, which are capable of detecting advantageous secondary screening traits, takes place.

1.2.5. Canopy Temperature and Thermography

The motivation behind the development of thermography (the study of heat distribution on a surface with the use of a thermogram) was the wish to thermographically inspect the radiative heat transfer from an object, such as a plant. Sullivan and Edmondson (2008) defined temperature as: *"the property of a system, which determines whether or not heat is transferred to or from an object."* The Stefan-Boltzmann Law is a key concept when examining the basic physics surrounding thermography. The law states that *"the total energy radiated per unit surface area of a black body per unit time is directly proportional to the fourth power of the black body's thermodynamic temperature"*. The Stefan-Boltzmann law is expressed as:

$$E= \varepsilon\sigma T^4$$

where ε is the emissivity dependent on the material properties (plant tissue typically ranges from 0.92-0.99), σ is the Boltzmann constant (5.67 x 10^{-8}W/m^2 · K) and *T* is the temperature in Kelvin.

Thermal radiation, the rate at which thermal energy is emitted from an object, is found in all matter above 0°K (-273.15°C). Thermal radiation is considered to be a surface phenomenon, i.e. it is localised to the surface from which it originates. Thermal radiation relating to heat transfer occurs in the section of the

electromagnetic radiation spectrum between 0.1µm to 100µm. As illustrated in Fig.1.2, thermal radiation on the electromagnetic spectrum includes the top range of ultraviolet, all visible light and all infrared radiation.

As the human eye is incapable of detecting electromagnetic radiation outside of the visible spectrum (400-700nm), IR cameras allow for measurements of radiant energy of a body emitted in infrared wavelengths. This information is then converted to electrical signals corresponding to the amount of energy radiating from a surface. These signals are further processed to produce thermograms, a false colour picture in which each pixel corresponds to a specific temperature value. Therefore, thermography is used to precisely pinpoint where energy losses on an object are occurring and which component organs are operating at differing temperatures.

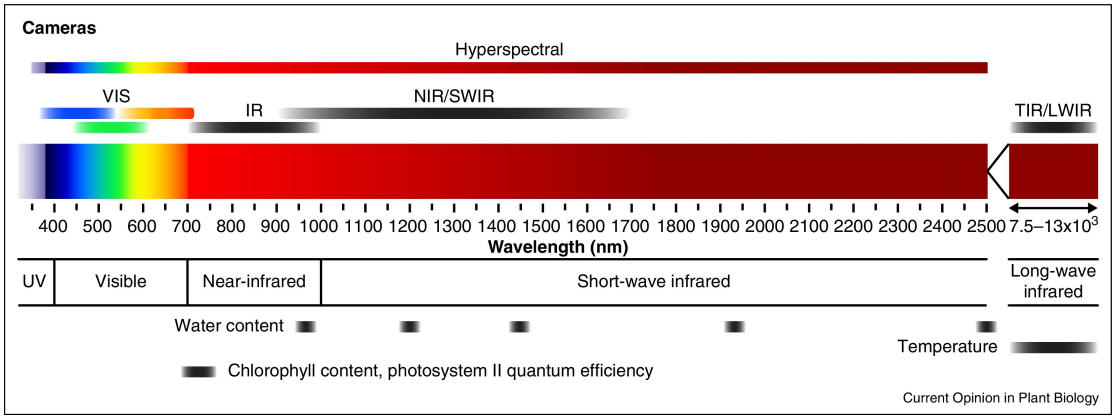

Fig. 1.2 – Wavelengths of the visible spectrum (VIS), the infrared spectrum (IR), the near-infrared (NIR) and the long-wave infrared (LWIR). Source: Fahlgren *et al. (*2015).

Recently, there has been an increased interest in using plant tissue temperature as an indicator of plant water relations. Leaf temperature is determined by both external environmental factors (relative humidity, tissue water status, solar radiation and air temperature) as well as physiological factors specific to plant tissue in question (Oerke *et al.,* 2006). Raschke (1960) was among the first to outline the principles of leaf energy exchanges. Following from this work, Fuchs and Tanner (1966) were the first to study plant water relations remotely by use of canopy temperature measurements in the field. It was only in the 1970s, that thermograhic equipment

became readily available for crop physiologists to use. Early IR cameras were used primarily for monitoring plant water stress from high altitudes. However, Bartholic *et al.* (1972) concluded that the use of IR remote sensing from high altitudes may reduce the accuracy of readings as a result of factors such as planting geometry, leaf shading and soil surface imagery. Ferguson *et al.* (1973) found that IR thermography readings taken at ground level (i.e. where the IR camera is directly aimed at the plant organ being investigated) reduced some of the problems outlined by Bartholic *et al.* (1972).

In the field of plant physiology, the 1980s saw the introduction of IR technology to study temperature distribution within plant leaves (Omasa *et al.,* 1981; Hashimoto *et al.,* 1984). The equipment used in these early days of experimentation was heavy and bulky. With the continued development of new IR technology, many of these problems have been addressed. It was the introduction of the handheld IR cameras, where the sensor did not require cooling with liquid nitrogen, that expanded the potential uses of IR and made it a viable option for researchers to use. IR cameras today keep falling in price and size, whilst continuously increasing their strength, accuracy and functionality. It is now possible to purchase high-quality IR cameras for under $10,000.

Numerous studies have identified leaf-canopy temperature as being a reliable indicator of plant water stress (Aston and Van Bavel, 1972; Bartholic *et al.,* 1972; Nixon *et al.,* 1973; Jackson *et al.,* 1977; Hatfield *et al.,* 1979; Jackson 1981). Canopy temperature depression (CTD) denotes the difference in air temperature and canopy temperature (CT) and is expressed by the following formula:

$$CTD = T_a - T_c$$

where T_a is the air temperature and T_c is the canopy temperature.

CTD and thermography are now being widely used as breeding tools to select for yield potential in key cereal crops grown in heat stressed conditions (Reynolds *et al.,* 1997; Araus *et al.,* 2001; Romano *et al.,* 2011; Zia *et al.,* 2013). Pinto *et al.* (2010), showed that heat-tolerant wheat genotypes display great CTD than susceptible

genotypes as a result of transpirational cooling from the canopy. Recently, thermography has been utilized in novel areas of crop physiology, highlighting the versatility of the technology as a tool for research and exploration (Steinmeyer *et al.,* 2013).

1.3. – AIMS AND HYPOTHESES

In order to provide the reader with a clear overview of the experiments conducted in this thesis, the individual research objectives and hypotheses to be tested for Chapters 2-4 are summarised on a chapter-by-chapter basis below.

Chapter 2 (pp. 27 - 108)

This chapter details two years of experiments that took place at the University of Reading Plant Environment Laboratory (PEL). The experiments took place in controlled environment growth cabinets and utilized pot-grown wheat plants to examine the effect of heat and drought stress during anthesis on tissue temperature depression, grain yield and a range of other physiological traits.

The objectives of this chapter were to: (i) assess the relationship between spike/flag leaf temperature depression at individual floret development stages during anthesis, and to (ii) determine the relationship between spike/flag leaf temperature depression at anthesis and grain yield. The following hypotheses were therefore constructed for testing:

Objective (i)

H_1: Increased spike/flag leaf temperature depression will be observed in the early stages of anthesis and decline during the latter stages.

H_0: Increased spike/flag leaf temperature depression will not significantly differ at any stage of anthesis.

Objective (ii)

H_1: Increased spike/flag leaf temperature depression during anthesis will result in increased grain yield under conditions of abiotic stress.

H_0: Increased spike/flag leaf temperature depression during anthesis will not affect grain yield under any combination of abiotic stress.

Chapter 3 (pp. 109-163)

This chapter discusses the findings made from a field-scale and a rhizobox experiment, which took place at the University of Reading Sonning Farm and PEL, respectively. The experiments took place in order to examine the relationship between rooting depth, temperature depression and grain yield at anthesis.

The objectives of this chapter were to: (i) measure spike/canopy temperature depression at anthesis of field-grown wheat in the UK, (ii) assess the relationship between spike/canopy temperature depression at anthesis and deep rooting in field grown wheat in the UK, (iii) assess the relationship between spike/flag leaf temperature depression at anthesis and rooting depth in rhizobox-grown wheat, and (iv) examine the relationship between spike/flag leaf/canopy temperature depression at anthesis and the grain yield of field- and rhizobox-grown wheat. The following hypotheses were therefore constructed for testing:

Objective (i)

H_1: Spike/canopy temperature depression can be accurately measured in the field-grown wheat in the UK.

H_0: It is not possible to accurately measure spike/canopy temperature depression in field-grown wheat in the UK.

Objective (ii)

H_1: Deep rooting in field-grown wheat will result in greater spike/canopy temperature depression at anthesis.

H_0: There is no association between deep rooting in field-grown wheat and increased spike/canopy temperature depression at anthesis.

Objective (iii)

H_1: Deep rooting in rhizobox-grown wheat will result in increased spike/canopy temperature depression at anthesis.

H_0: There is no association between deep rooting of rhizobox-grown wheat and increased spike/canopy temperature depression at anthesis.

Objective (iv)

H_1: In both field- and rhizobox-grown wheat, greater spike/flag leaf/canopy temperature depression at anthesis results in increased grain yields under conditions of abiotic stress.

H_0: In both field- and rhizobox-grown wheat, greater spike/flag leaf/canopy temperature depression at anthesis is not associated with increased grain yield under conditions of abiotic stress.

Chapter 4 (pp. 164-202)

This chapter describes the research findings that arose from a collaboration between the University of Reading and Lancaster University, looking at how the starch and WSC content in the flag leaf, peduncle and glumes of the genotypes of wheat used during Experiment 2 and 4 differs and how these relate to yield tolerance under abiotic stress.

The objectives of this chapter were to: (i) examine how the composition, at mid-anthesis, of starch and WSC in the flag leaf, peduncle and glumes is affected by abiotic stress, and to (ii) identify the relationship between organ starch/WSC content and grain yield, under contrasting conditions of abiotic stress at anthesis. The following hypotheses were therefore constructed for testing:

Objective (i)

H_1: The peduncle is the primary site of starch/WSC storage, with the flag leaf and glumes playing a smaller role in carbohydrate storage. Abiotic stress at anthesis results in significant reductions in starch/WSC content in individual organs.

H_0: Starch and WSC content do not vary between organs and abiotic stress at anthesis has no effect on the carbohydrate content of the flag leaf, peduncle or glumes.

Objective (ii)

H_1: Greater starch/WSC content in the flag leaf, peduncle and glumes is associated with increases grain yields under conditions of abiotic stress.

H_0: Greater starch/WSC content in the flag leaf, peduncle and glumes is not associated with increased grain yields under conditions of abiotic stress.

Chapter 5 (pp. 203-214)

This chapter serves as a general conclusion to the thesis. It details how the empirical findings discussed in Chapter 2, 3 and 4, relate to the research objectives set and discusses the theoretical implications of the findings made. It highlights the limitations of the thesis and outlines recommendations for future research that have arisen as a result.

CHAPTER 2

TEMPERATURE DEPRESSION IN CONTROLLED ENVIRONMENTS

2.1. CHAPTER SUMMARY

The relationship between spike temperature depression (STD), flag leaf temperature depression (FLTD), floret development stage (FDS) and grain yield (GY) was investigated over the course of two controlled environment experiments that took place at the Plant Environment Laboratory at Reading University. Additional data relating to chlorophyll content of the flag leaf and glumes as well as the relative water content (RWC) of the flag leaf was also collected. Five genotypes of the elite recombinant inbred line Seri-Babax were used in Experiment 1, which was reduced to three in Experiment 2 to enable higher replication. Tissue samples of the flag leaf, peduncle and glumes from Experiment 2 were frozen for the determination of starch and water soluble carbohydrates detailed in Chapter 4. Plants were grown in pots and exposed to a combination of two contrasting temperature and irrigation treatments at anthesis. FDS and organ temperature depression (TD) were recorded daily in a three-hour window around solar noon. Organ TD was measured using a hand-held infrared camera. Chlorophyll content was significantly higher in the flag leaf compared to the glume. RWC was a reliable indicator of plant water status but failed to correlate to higher yields. Variation in STD was consistently lower than FLTD and negative average STD as low as -0.53°C indicated that the spike was not preferentially cooled over the flag leaf at anthesis. Organ TD was found to be greater in the latter stages of anthesis compared to the early stages. It was not confirmed that increased STD at anthesis is associated with increased GY. Without this crucial link it is not possible to develop STD further into a viable, high-throughput screening tool for wheat breeders to use in a commercial setting. Further examination on a field-grown crop is required to establish the relationship between STD and GY without the root zone restrictions and thermal loads placed on the plants by the growth chambers used for experimentation in this chapter.

2.2. CHAPTER INTRODUCTION

2.2.1. Justification of Study

Agriculture is highly dependent on both climatic conditions and prevailing weather patterns of a region. Farmers have historically been able to adjust management practices to take into account year-to-year changes in weather events so, not only have agricultural systems in the past been highly adapted to their given environments but also have some flexibility depending upon the given variability within a growing season.

Climate change is likely to have unprecedented impacts on crop production largely due to the predicted increased variability of weather patterns. The effects of anthropogenic climate change are likely to be regional, although Battisti and Naylor (2009) found that by the 2080 most cropped regions globally will experience record temperatures during the growing season. Of particular importance to cereal producing areas in temperate regions, such as the UK, is the increased frequency of short and extreme periods of high temperature, known as 'heat stresses' (IPCC, 2014). Furthermore, yields in arable agriculture are underpinned by the consistent availability of fresh water. As a consequence of a changing climate, shifts in previously stable precipitation patterns are predicted to take place (Trenberth, 2011). The future productivity of agriculture is threatened by climate change. Faced with these threats, along with increased societal pressure on resources as a result of a growing global population, adaptation strategies to climate change that are not dependent on increasing inputs but are rather focused on harnessing the genetic potential contained within crops must become a priority.

In order to be adequately prepared for the challenges that climate change is likely to impose on plant breeding efforts in the future, an understanding of the genotypic responses of key biochemical processes is necessary. Furthermore, the interplay between these processes and novel selection tools will be required to further develop our ability to screen genotypes for their unique adaptive capabilities.

2.2.2. Abiotic Stress in Wheat

2.2.2.1. Heat Stress During Development

Temperature and water are the two major abiotic factors that effect the growth, development and yield potential of cereal crops. Porter and Gawith (1999) extensively reviewed literature to determine the critical temperature ranges of key physiological processes in wheat (*Triticum aestivum* L.). Table 2.1 (adapted from Porter and Gawith, 1999) summarizes the minimum (*T*Min), the optimal (*T*Opt) and the maximum temperature (*T*Max) for key phenological phases in wheat. Collectively, *T*Min, *T*Opt and *T*Max are referred to as the cardinal temperatures. As well as cardinal temperatures, lethal temperatures (*T*Leth) have been established for wheat. The difference between cardinal and lethal temperatures is that recovery of function is possible if the temperature remains within the cardinal range, but recovery is not possible when temperature exceeds lethal limits.

Although a useful summary of temperature thresholds for a range of phenological phases in wheat, Table 2.1 is not a definitive guide to these thresholds. Differences in experimental objectives and designs, differences in cultivar selection and differences in temperature regimes complicate the establishment of thermal limits for wheat. The differing tolerance cultivars exhibit to extreme temperature stress has been highlighted by the findings of several authors (Pomeroy and Fowler, 1973; Blum and Sinmena, 1994; Asseng *et al*., 2011). In general terms, based on Porter and Gawith (1999), and over the course of the crop's life cycle, the *T*Opt for wheat is between 17-23°C, with a *T*Min of 0°C and a *T*Max of 37°C. From Table 2.1 it is evident that the period of anthesis, compared to the period of sowing to emergence for example, has historically received little attention with regards to temperature threshold studies.

Although modern wheat varieties exhibit a range of tolerance, avoidance and escape mechanisms (see Chapter 1.2.2.6) to respond to abiotic stress and to heat and drought stress in particular, Bita and Gerats (2013) concluded that stress events can negatively affect all vegetative and reproductive stages in crops. However, there are stages in the life cycle of wheat that appear to be more sensitive to high temperature

stress than others, namely reproductive development (Saini and Aspinall, 1982; Wollenweber *et al.,* 2003). In many cereal-producing regions, such as Southern Australia, high temperature stress is commonly observed in combination with other abiotic stresses, such as drought or salinity. The compounding negative effects of multiple stresses on a plant are greater than the effect of a single, isolated stress (Gregorio *et al.,* 2002; Barnabás *et al*., 2008).

Table 2.1 – Summary of the lethal (*T*Leth), minimum (*T*Min), optimum (*T*Opt) and maximum (*T*Max) temperature thresholds for key phenological phases in wheat. Values in brackets represent standard error. *n* is the number of literature sources used to calculate means and standard error. Adapted from Porter and Gawith (1999).

Processes	Temperature Thresholds	Mean Temperature (°C) (±se)	*n*
Lethal Limits	*T*Leth (Min)	-17.2 (1.2)	21
	TLeth (Max)	47.5 (0.5)	2
Phenological Phases	-	-	-
Sowing - Emergence	*T*Min	3.5 (1.1)	8
	*T*Opt	22.0 (1.6)	11
	*T*Max	32.7 (0.9)	10
Vernalization	*T*Min	-1.3 (1.5)	6
	*T*Opt	4.9 (1.1)	11
	*T*Max	15.7 (2.6)	7
Terminal Spikelet	*T*Min	1.5 (1.5)	2
	*T*Opt	10.6 (1.3)	5
	*T*Max	>20.0	1
Anthesis	*T*Min	9.5 (0.1)	3
	*T*Opt	21.0 (1.7)	2
	*T*Max	31.0	1
Grain Filling	*T*Min	9.2 (1.5)	6
	*T*Opt	20.7 (1.4)	7
	*T*Max	35.4 (2.0)	5

2.2.2.2 Water Stress during Development

Along with high temperature, drought (a prolonged period of reduced rainfall resulting in low water availability) is a major constraining factor of cereal production in rain-fed systems. Without supplementary irrigation to replace soil moisture, drought will lead to water-deficit stress in a crop. Water stress occurs when a plant is unable to extract sufficient moisture from the soil to meet its evaporative demands. Depending on the timing, duration and severity, this water-deficit stress will negatively affect plant growth, physiology, morphology and ultimately yield (Saini and Westgate, 2000; Boyer and Westgate, 2004). Water deficit stress imposed on a crop early in its life cycle will result in poor germination and crop establishment (Bouaziz and Hicks, 1990) whereas water-deficit stress experienced during vegetative growth can severely reduce leaf expansion and growth (Acevedo *et al*., 1971; Eastham *et al*., 1984). Water deficit stress experienced immediately prior to floral initiation will negatively impact the number of spikelet primordial in wheat (Oosterhuis and Cartwright, 1983). Anthesis is accelerated by water-deficit stress (Simane *et al*., 1993) and the plants ability to synthesize and accumulate water soluble carbohydrates in the stem tissue is negatively impacted by stress occurring before and during this period (Nicholas and Turner, 1993). As a highly sensitive stage of development, water-deficit stress at anthesis will reduce grain number as well as the number of fertile spikelets per spike (Hochman, 1982). If a crop encounters water-deficit stress during grain filling, a reduction in grain weight is observed as a result of early senescence shortening the duration of grain filling (Hochman, 1982).

Unlike thermal stress that can be easily quantified using degrees Celsius, water-deficit stress is more complicated to apply, monitor and quantify precisely. It is possible to apply drought by monitoring pot weights on a daily basis and supplying sufficient water accordingly so that a consistent level of water is present in each pot used. This method is accurate when applying drought stress conditions to plants at the seedling stage. However, once the size of the plant increases significantly, this methodology becomes cumbersome for larger trials in which plants that are grown to seed. Many authors therefore report drought conditions in terms of overall water provision to the crop or plant in the form of frequency of irrigation events.

Commonly used terms to quantify these treatments are “well-watered” to denote sufficient water provision to the crop in order to prevent drought-like conditions or “water-stressed”, which denotes the restriction of irrigation to simulate drought-like conditions. Consequently, the extent of drought stress is likely to vary between individual experimenters and the designs they utilize.

2.2.2.3. Interaction Between Heat and Water Stress

In controlled environment (CE) and glasshouse experiments in particular, the interactive effects of air temperature and relative humidity on plants are important to consider. Vapour pressure deficit (VPD) is a measure of the capacity of air to absorb moisture. It is calculated using air temperature and relative humidity and is expressed in the following equation:

$$\text{VPD} = VP_{sat} - VP_{air}$$

where VP_{sat} is the saturated vapour pressure (maximum water holding capacity of the air at a given temperature) and VP_{air} is the actual vapour pressure, which is a function of relative humidity and VP_{sat}. Transpiration rate from the leaf, as well as stomatal conductance, are intrinsically linked to the VPD of the air surrounding the leaf (Monteith, 1995). Consequently, VPD is one of the most important environmental variables capable of affecting stomatal conductance in CE experiments.

Bhargava and Sawant (2013) noted that that although a plant may be able to survive a stress event, it is the ability to sustain yields as a result of the stress event that is key in determining stress tolerance. Although abiotic stress tolerance shows a high level of genotypic adaptation (Ramirez-Villegas *et al.,* 2015), breeding genotypes for this tolerance is a much more complex task than breeding for yield under favourable conditions.

2.2.2.4. Stress During Reproductive Development

Wheat is sensitive to heat stress (Slafer and Satorre, 1999) but the extent of heat stress damage which occurs is highly dependent on the phenological stage a plant is in when the stress occurs. Wollenwebber *et al.* (2003) concluded that heat stress during the reproductive phase of development is more damaging to the plant than during the vegetative phase of growth. Within the reproductive phase, heat stress during anthesis has been found to cause tissue dehydration, reduce pollen viability (Saini and Aspinall, 1982), increase rates of floret abortion (Wardlaw and Wrigley, 1994) and result in poor fertilization (Ferris *et al.,* 1998). Heat stress also accelerates the rate of development of the spike leading to fewer spikelets being formed which results in fewer grains per spike (Saini and Aspinall, 1982; Porter and Gawith, 1999). In addition to heat stress during the reproductive phase, terminal heat stress (heat stress during grain filling) has also been identified as a vulnerable stage of plant development (Wardlaw *et al.,* 1980; Viswanathan and Khanna-Chopra, 2001; Streck, 2005; Dias and Lidon, 2009). The negative effect of a heat stress event, even if it is sporadic and lasts for only a few hours, is particularly evident during the most sensitive developmental stage of mid-reproduction (Porter and Semenov, 2005).

2.2.2.5. Abiotic Stress and Photosynthesis

A complete discussion of the process of photosynthesis, as well as the effects of high temperature stress on its rate, is available in Chapter 1. Briefly, photosynthesis is the process by which plants capture and convert solar energy into chemical energy. Carbon dioxide and water are catalysed into carbohydrates and oxygen during this process. Photosynthesis consists of both light dependent and light independent reaction stages. Due to the involvement of enzymes, it is the light independent reactions involved in photosynthesis that are affected by supra-optimal temperature. The enzyme driven reactions are significantly inhibited when plant tissue temperature exceeds the optimal threshold. Enzyme activity initially increases up to the optimal temperature, after which further increases in temperature reduce the enzymic rate of activity until temperatures become limiting and enzyme activity

stops due to damage to the pathways involved. Consequently, the rate of photosynthesis is limited by high temperature stress.

Significant adaptations to high temperature stress exist between wheat cultivars (Al-Khatib and Paulsen, 1984; Wardlaw *et al.,* 1989a; Wardlaw *et al.,* 1989b; Viswanathan and Khanna-Chopra, 2001). A complete discussion of the topic of stress adaptation mechanisms in wheat is available in Chapter 1.2.2.6. A reduced grain filling capacity in wheat has been found as a result of thermal damage to the photosynthetic structures in the flag leaf and spike (Blum *et al.,* 1994). 'Stay Green' (SG), the prolonged maintenance of chlorophyll content and high photosynthetic rate through the growing season, is considered a key indicator of heat tolerance in crops (Fokar *et al.,* 1998). Consequently, cultivars with the ability to maintain SG are able to sustain rates of carbon assimilation under conditions of elevated temperature stress. Breeding efforts for the incorporation of the SG trait into wheat have accelerated in recent years (Christopher *et al.,* 2008). The extent of tolerance or susceptibility in wheat to heat stress has been found to be determined by the plants physiological response to the stress (Almeselmani *et al.*, 2012).

The positive correlation between photosynthetic rate and chlorophyll content has long been established (Emerson, 1929; Fleischer, 1935). Significant genotypic variation of both chlorophyll content and photosynthetic rate have been reported (Parry *et al*., 2011; Hamblin *et al*., 2014). Consequently, chlorophyll content determination has been identified as a suitable proxy measure of photosynthetic rate in crops (Buttery and Buzzell, 1977). Chlorophyll content measurement is a far simpler method to examine proxy photosynthetic rate than alternative methodologies that exist, for example the use of an infrared gas analyser (IRGA).

2.2.3. Controlled Environment Experiments

The applicability of a secondary screening trait may be assessed in a controlled environment (CE) but the trait must associate with improved yield under field-scale conditions for it to be considered viable for inclusion into a breeding program. Field-scale experimentation plays a central role in breeding efforts aimed at developing commercially viable cultivars. However, the limitations of field-scale

experiments have been raised in the past. Firstly, there is an inherent difficulty in examining the effect of a single stress on a crop, as the effect of compounding biotic and abiotic stresses encountered in the field can be complex. This is particularly problematic when attempting to impose heat and/or drought stresses on a field-scale. The naturally occurring heterogeneity of the soil profile and microclimates within the field can further confound the ability to effectively apply these stresses at key growth stages. Secondly, the variation in developmental stages between cultivars makes timing of stress application in field-scale experiments complex. A stress event on one day may affect two genotypes in marginally different growth stages in different ways. Thirdly, the stress response between a secondary screening trait and other physiological factors in the field (such as rooting depth) is complex and without having a complete understanding of all the physiological interactions occurring, the applicability of results may be limited. In order to study a single trait in isolation, the screening process must move from the field to a CE facility. Although it addresses a large proportion of the issues facing field-scale experiments, this move also creates new challenges. The limitations of CE experiments are discussed in detail in Chapter 2.5 and must be considered when findings are interpreted.

A CE experiment is one that is conducted under tightly monitored and regulated conditions, which in turn allows for accurate measurements to be taken. The factors that can be controlled range from temperature and humidity, to carbon dioxide/ozone concentration, light intensity and photoperiod, nutrient availability and soil moisture. The replicability, uniformity and control over the intensity of these abiotic factors makes CE experiments essential to many research efforts. In the context of plant physiology, CE experiments can take place under a range of growing systems, depending on the crop species in question. Common CE systems used to examine the effects of elevated temperature on wheat include greenhouses (Gibson and Paulsen, 1999; Shah and Paulsen, 2003), glasshouses (Saini and Aspinall, 1982; Wardlaw *et al*., 1989a; Wardlaw *et al*., 1989b; Brien *et al.,* 2013), polytunnels (Wheeler *et al.,* 1996a; Ferris *et al*., 1998), growth chambers (Prasad *et al*., 2008; Steinmeyer *et al.,* 2013), polybags in a polyhouse (Kaur and Behl, 2010), mobile heat chambers in the field (Ugarte *et al*., 2007; Talukder *et al*., 2013) and walk-in chambers (Evers *et al.,* 2010).

There are several key benefits that CE experiments have over field-scale experiments, which include: (1) the ability to examine key plant processes without the inherent abiotic variability found in field-scale studies disrupting measurements; (2) the capacity to simulate likely future environments; (3) the capability of conducting experiments with potentially harmful and biohazardous materials (e.g. GMO's); (4) the opportunity to easily observe below-ground plant processes; and (5) the capability of conducting experiments out of season. Additionally, accessing a CE site is generally faster, safer and more convenient than conducting an experiment in the field.

2.2.4. Thermography

Significant advances in the development of high-throughput phenotyping platforms (HTPP) have taken place in the past decade. The progress has resulted in significant advances in our understanding of model crops. In order to translate these advances to food crops, the development of novel HTPP is necessary. Whilst field-scale platforms are able to use plants grown under natural conditions, they often lack any control over the environment and are affected by seasonal weather trends. CE platforms are able to modify the plants environment at the cost of having to grow the plants in restrictive pots and under conditions not encountered in the plants natural environment. CE phenotypng platforms are however making significant progress in the examination of below-ground phenotyping techniques (Topp *et al.,* 2013; Bucksch *et al.,* 2014). Hyperspectral imaging is a central component in most HTPP today. This specialist imaging technology is capable of non-destructively assessing individual plants for phenotypic traits such as stomatal conductance, chlorophyll content, photosynthetic efficiency, morphology and geometry of the leaves, as well as leaf thickness, leaf water content and plant tissue temperature (Fahlgren *et al.,* 2015).

2.2.5. Tissue Temperature Depression

There has been an interest in using plant tissue temperature as an indicator of plant water relations. Leaf temperature is determined by both external, or environmental, factors (relative humidity, tissue water status, solar radiation and air temperature) as

well as internal factors related to plant physiology (Oerke *et al.,* 2006). Raschke (1960) was among the first to outline the principles of leaf energy exchanges. Following from this work, Fuchs and Tanner (1966) were the first to study plant water relations remotely by the use of canopy temperature measurements in the field. This work paved the way for the development of the Stress Degree Day (SDD) calculation used for predictions relating to the timing of irrigation to a crop (Idso *et al.,* 1977; Jackson *et al.,* 1977). SDD is calculated by establishing the difference between the daily maximum temperature and the peak optimal temperature of a crop. In a commercial setting, SDD is commonly calculated autonomously using field-based sensing stations (e.g. Smartfield, Lubbock, Texas, USA). There is a negative correlation between the water status of a plant and its leaf temperature (Kümmerlen *et al.,* 1999).

2.2.6. Spike Temperature Depression

Similarly to CTD discussed in Chapter 1, Spike Temperature Depression (STD) denotes the difference between the air temperature and the tissue temperature of the spike and it is expressed as:

$$\text{STD} = T_a - T_s$$

where T_a is the air temperature and T_s is the spike temperature.

Ayeneh *et al*. (2002) examined tissue temperature of the spike during grain filling and found that although hotter than the canopy, the spike temperature was cooler than the air temperature. The significance of the work presented by these authors related primarily to grain quality. However, the finding that the spike is capable of depressing its temperature under supra-optimal thermal conditions provided an opportunity to examine STD further. The regulation of spike temperature has been hypothesised to be associated with rooting depth and habit (Reynolds *et al.,* 2007), stomatal conductance (Reynolds *et al.,* 2007) and the remobilisation of sugars within the plant (Wardlaw and Willenbrink, 1994).

Earlier work by Steinmeyer *et al*. (2013) attempted to address several key questions relating to flowering phenology and stress adaptation in wheat. Their findings illustrated that under certain environmental conditions, wheat spikes display a capacity to down regulate their tissue temperature, with the potential to significantly cool the spike at anthesis. It is hypothesised that the underlying cooling mechanism responsible for temperature depression of both the spike and the flag leaf is transpirational cooling. Differing rates of the transpirational cooling capacity may be associated with differences in plant water status, differences in photosynthetic/respiratory rates or differences in stomatal density or conductance. In contrast to their hypothesis, that STD would be greatest at mid-anthesis, their work found that it was the early stages of anthesis that correlated most strongly with increased TD of the spike. Steinmeyer *et al*. (2013) did not explore the effect that abiotic stress had on grain yield or how alternative adaptation mechanisms may be utilized in thermoregulation. The findings of the authors were informative as they demonstrated that TD of wheat spikes can be manipulated and measured relatively easily in a CE.

2.2.7. Thermoregulation in Plants

Thermogenesis in plants is the ability to modify thermogenic properties to maintain an optimal tissue temperature under fluctuating environmental conditions. It is an interesting phenomenon in the plant kingdom and not one commonly encountered when discussing food crops. Several plant species have been shown to exhibit extraordinary examples of thermogenesis such as *Philodendron selloum*, capable of maintaining the tissue temperature of between 38°C and 46°C despite air temperatures falling to as low as 4°C (Nagy *et al.,* 1972). Seymour and Schulze-Motel (1996) reported thermoregulation in *Nelumbo nucifera* whilst Knutson (1974) reported thermoregulation in *Symplocarpus foetidus*.

Thermogenesis may be of significant benefit to plants that grow in cold conditions as it acts in a protective capacity towards the floral structures during cold periods (Knutson, 1974). As many of these plants are found in tropical areas in which frost rarely occurs, an alternative explanation for thermogenesis in these plants may be the volatilization of chemical compounds into the air in order to attract pollinators

(Skubatz *et al.,* 1996; Lamprecht *et al.,* 2002). However, thermoregulation has also been suggested as a possible mechanism utilized by plants in order to create optimal conditions under which floral development and pollen tube growth may occur (Ervik and Barfod, 1999). The mechanisms underpinning the process of heat production in thermogenesis relate to increased oxidase activity (Gonzalez-Meier *et al.,* 1999; Ito *et al.,* 1997).

Many of the plants in which thermogenesis has been described are in the Aracae family. The extent to which thermogenesis features in food crops remains unclear. Whether thermoregulation in wheat exists is unknown although Nevo *et al*. (1992) reported that when leaf tissue of *T. dicoccoides* and *Hordeum spontaneum* were exposed to low air temperatures, significant metabolic heat generation was observed. It is possible that wild progenitors of *T. aestivum* may contain thermogenic properties. The underlying physiological basis for thermoregulation in wheat is unknown.

With several cases of thermogenesis present in the plant kingdom, as well as within the *Triticum* genus, it is likely that thermoregulation takes place in some form in modern bread wheat. The thermoregulation likely to be of benefit to future breeding efforts will not relate to heat generation but rather the effective dissipation of heat from the plant.

2.2.8. Stress Indices

Stress indices (SI) are a measure of stress tolerance that provide breeders with information relating to yield stability under contrasting growing conditions. In SI, yields recorded under conditions of abiotic stress are compared to yields recorded under favourable growing conditions. Below, seven commonly used SI to rank yield stability are described.

Tolerance Index (TOL) is defined as the difference in yield between the stressed (Y_s) and non-stressed environments (Y_p), and is expressed as:

$$\text{TOL} = Y_s - Y_p$$

(Rosielle and Hamblin, 1981)

Stress Susceptibility Index (SSI) is the reduction in yield as a result of the combined effect of heat and drought stress in a given genotype compared to the mean reduction over all genotypes examined. It is expressed as:

$$\text{SSI} = \frac{1-(\frac{Y_S}{Y_p})}{1-(\frac{X_S}{X_p})}$$

(Fischer and Maurer, 1978)

where X_s and X_p are the mean yields of all genotypes under combined heat and drought stress and non-stress conditions, respectively.

Heat Susceptibility Index (HSI) is the reduction in yield as a result of heat stress in a given genotype, compared to the mean reduction over all genotypes examined. It is expressed as:

$$\text{HSI} = \frac{1-(\frac{Y_S}{Y_p})}{1-(\frac{X_S}{X_p})}$$

where X_s and X_p are the mean yields of all genotypes under heat stress and non-stress conditions, respectively.

The **Drought Susceptibility Index (DSI)** is the is the reduction in yield as a result of drought stress in a given genotype, compared to the mean reduction over all genotypes examined. It is expressed as:

$$\text{DSI} = \frac{1-(\frac{Y_S}{Y_p})}{1-(\frac{X_S}{X_p})}$$

where X_s and X_p are the mean yields of all genotypes under drought stress and non-stress conditions, respectively.

Stress Tolerance Index (STI) is used to identify genotypes that produce high yields under both stressed and non-stressed conditions, and is defined as:

$$\text{STI} = \frac{(Y_s \times Y_p)}{X_p^2}$$

(Fernandez, 1992)

Geometric Mean Productivity (GMP) is widely used by breeders wanting to assess relative performance between years, since drought severity may vary year-on-year, and is defined as:

$$\text{GMP} = \sqrt{(Y_s \times Y_p)}$$

(Fernandez, 1992)

Mean Productivity (MP) is the average of Y_s and Y_p, and is defined as:

$$\text{MP} = \frac{(Y_s + Y_p)}{2}$$

(Rosielle and Hamblin, 1981)

2.2.9. Chapter Objectives

The motivation behind this work was the desire to further develop the understanding of tissue temperature depression as a potential secondary screening trait in wheat. Therefore, the objectives of this chapter were to:

a. Assess the relationship between spike/flag leaf temperature depression at individual floret development stages during anthesis in a CE, and to;
b. Determine the relationship between spike/flag leaf temperature depression and grain yield at anthesis.

2.3. MATERIALS AND METHODOLOGY

2.3.1. Plant Material and Controlled Environment Cabinets

Two consecutive years of CE experiments were conducted at the Plant Environment Laboratory (UoR, UK) during December 2013 (Experiment 1) and July 2014 (Experiment 2). The plant material used in this study originated from a reciprocal crossing of two related parent lines, namely 'Seri M82' (IWIS CODE (Fox *et al.,* 1996), selection history: M31 IBWSN S-1 MXI96-97) and 'Babax' (IWIS CODE (Fox *et al.,* 1996)), selection history: CM92066-J-0Y-0M-0Y-4M-0Y-0MEX-48BBB-0Y). Both are considered to be highly adapted semi-dwarf lines (CIMMYT, 1986), with Babax being highly tolerant to severe drought, whereas Seri M82 is moderately susceptible to severe drought (Pfeiffer, 1988). Known as Seri-Babax (SB), this cross is widely used for phenotyping studies in heat and drought stress environments. SB has a relatively short period of flowering between 10 and 15 days, making it ideal for this type of work (Olivares-Villegas *et al.,* 2007). In Experiment 1, six lines of the SB cross were used, namely SB019, SB020, SB055, SB155, SB156 and SB165. The selection of these lines, as well as the experimental methodology used for this experiment, was as a result of previous work by Steinmeyer *et al*. (2013). Thermal and floral data was unavailable for SB055 in Experiment 1 due to replication affecting time constraints which resulted in SB055 being excluded from these analyses. Yield and chlorophyll data relating to SB055 was however collected and is therefore presented. Five genotypes of SB were therefore used during Experiment 1. In Experiment 2, the total number of genotypes used was reduced from six to three, as a result of the exclusion of SB019 and SB156, in addition to SB055, due to their performance determined by stress index ranking. The three genotypes of SB used in Experiment 2 were therefore SB020, SB155 and SB165.

In Experiment 1, five replicate pots containing three plants in each were sown for each of the six genotypes used in the four treatment combinations imposed, resulting in a total of 120 pots. Of these five replicates, four were selected on the basis of optimal plant growth and used for organ TD and floret development stage determination. In Experiment 2, eight replicate pots, each containing three plants,

were sown for each of the three genotypes used in the four treatment combinations imposed, resulting in a total of 96 pots. Consequently, the pot density during Experiment 1 was 59.7/m^2 and during Experiment 2 it was reduced to 47.7/m^2. As destructive measurements were taken at mid-anthesis in Experiment 2, three pots were used for destructive sampling and the remaining five were used for organ temperature depression and floret development stage determination. In each of these pots, a single plant that was not being used for IR imaging, was selected for determination of flag leaf relative water content (see Chapter 2.3.4 for details).

The pots used were 180mm plastic pots containing a substrate comprised of a sterilised mixture of vermiculate, sand, gravel and organic compost (in a ratio of 2 : 1 : 2: 0.5). Additionally, 2kg m^{-3} of Osmocote (LBS Horticulture Ltd., Colne, UK) slow release fertiliser containing N : P_2O_5 : K_2O : MgO (in a ratio of 15 : 11 : 13 : 2) was added. Prior to three seeds of each line being sown into the pots, the substrate was irrigated to field capacity (FC). Pots were randomly allocated across four 1.37 x 1.47 m^2 Saxcil growth cabinets. The growth cabinets were equipped with cool white fluorescent lamps contained within a clear glass barrier at the top of the growth cabinet. The plants were grown in these growth cabinets with day/night cycle temperatures being maintained at 25°C/16°C (±0.5°C) until Zadoks GS39 (Zadoks *et al.,* 1974), when the flag leaf ligule was just visible. The number of plants in each pot was then restricted to three plants per pot and three tillers per plant. Once 50% of tillers had reached GS59 (inflorescence fully emerged), the temperature in two cabinets was increased to 35°C/26°C (day/night cycle). These two cabinets were the 'elevated temperature' treatment (HT). The other two cabinets were maintained at 25°C/16°C (day/night cycle). These two cabinets were the 'ambient temperature' treatment (AT).

The plants were drip irrigated using an acidified complete nutrient solution which contained 100 mg L^{-1} of inorganic nitrogen. Half of the pots were irrigated to field capacity (FC) three times daily by an automated drip system. These were the pots in the 'well-watered' treatment (WW). The other half received minimal water to simulate drought conditions, which was defined as 'infrequent irrigation such that the water applied to the pot resulted in the potting mix reaching no more than 25% of the FC at any given time'. These were the pots in the "water-stressed" treatment

(WS) and they received 75ml of irrigation to each pot every two days. The 25% of FC value, was used because several authors have reported significant plant responses to drought at this level whereas plant responses to drought at higher FC values were less severe (Emam *et al.* 2010; Medeiros *et al*. 2012). Steinmeyer *et al.* (2013) had previously applied drought stress to wheat using the potting mix and irrigation regime used during Experiment 1 and 2. They concluded that the soil was sufficiently dry when voltmeter readings were between 100 mV and 120 mV. Expressed in FC terms, sufficiently dry soil in the ranged from 18.7-22.4% FC. In contrast, soil in the WW treatment was considered sufficiently irrigated when the voltmeter readings ranged from 275 mV to 500 mV (51.4-93.5% of FC). The water retention capacity of the potting mix declined by approximately 25% of FC within 24 hours and 32% of FC after 48 hours. Over a 24-hour period, electrical conductivity values did not fall to levels low enough at which water stress might be observed within plants (Fig. 2.1).

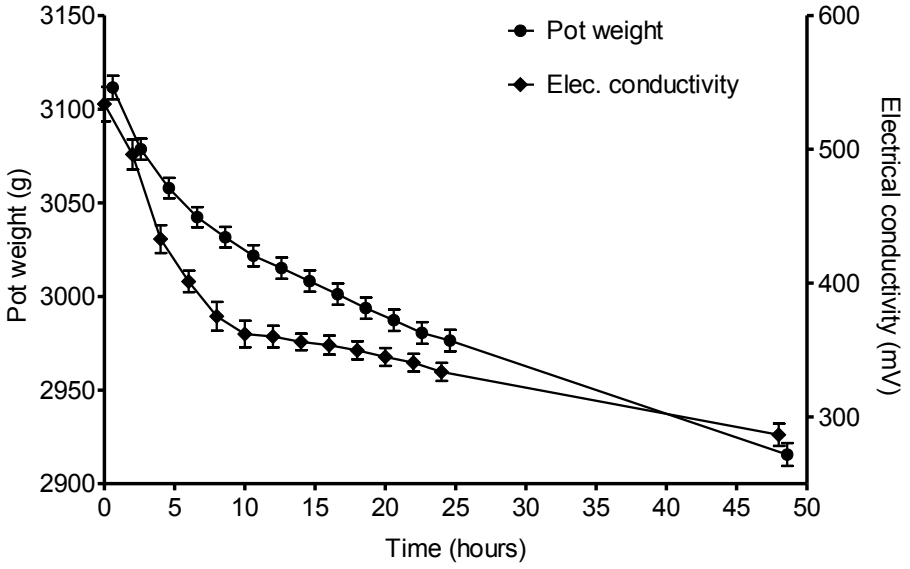

Fig. 2.1 – Pot weight and EC drying out curve from the potting substrate used for Experiment 1 and 2. The curve illustrates that sufficient water remains available in the pots in-between irrigation events such that differences in tissue temperature observations made are not due to significant changes in water availability. Water loss was assessed using a gravimetric approach (left Y axis) as well as by monitoring the change in electrical conductivity of the substrate (right Y axis). Results are based on 20 replicate pots and bars represent standard error. Reproduced from Steinmeyer *et al.* (2013).

A timeline illustrating when, in Experiment 1 and 2, the elevated temperature and water-deficit stress treatments around the period of anthesis were applied, can be found in the Appendix (see Fig. 2.37).

The photosynthetically active radiation (PAR) at canopy level was 650µmol $m^{-2}s^{-1}$ (±20 µmol $m^{-2}s^{-1}$) and the photoperiod lasted for 16h. Irrigation was turned off to pots in the WS treatment three days before the anticipated onset of anthesis in order for the soil moisture in the pots to decline to sufficiently low levels. The plants were kept in the growth cabinets until flowering was complete (GS69) and senescence had begun (GS70). Upon completion of flowering, all cabinets were maintained at a 25°C/20°C cycle until harvest. The night-time temperature was increased in order to simulate the higher temperatures experienced during grain filling by field-grown crops in arid growing regions.

2.3.2. Infrared Imaging Assessment Protocol

The methodology used was developed and first detailed in Steinmeyer *et al.* (2013). Infrared images were taken using a recently calibrated, hand-held thermal imaging camera (FLIR Systems, Wilsonville, OR, USA). The infrared (IR) camera used (FLIR T335) operated in the spectral range of 7.5-13µm, with a reported accuracy of ±2% (FLIR, 2013). IR image analysis took place daily between 11.00h and 14.00h for the duration of anthesis. The odd side of the spike was exclusively used for IR image analysis as the even side was used for floret development stage (FDS) determination (see Chapter 2.3.3. for details). This was done to avoid imaging glumes that may have been damaged as a result of FDS determination. IR images were captured prior to FDS assessment in order to avoid the plant tissue temperature being altered by handling. The IR image was captured at a distance of between 30-35cm from the plant. The background of the IR image did not interfere with the assessment of organ temperature.

2.3.3. Floret Development Stage Assessment Protocol

The protocol surrounding FDS determination was developed by Lukac *et al.* (2012). The scale is based on distinct physiological stages of the male (anther) and female

(stigma) floral organs. The scale detailing the development of the anthers is a numerical system that uses the numbers '1', '2', '3' and '4' to define distinct stages in anther development. The stages of stigma development were recorded using a similar, however non-numerical, scale, namely: 'Half-Fluffy' (HF), 'Fluffy' (F) and 'Grain' (G). Images illustrating these stages can be found the in the Appendix (Fig. 2.38A-D and Fig. 2.39A-C). This method of assessing the floral organs is rapid and relatively non-intrusive.

2.3.4. Relative Water Content

In Experiment 2 the relative water content (RWC) of the three SB lines used were examined in order to determine whether genotypic differences in water uptake and maintenance under stress conditions existed. The method used for RWC analysis was based on previous experimental protocols outlined by Turner (1981) and is calculated using the following equation:

$$\text{RWC } (\%) = \frac{(FM-DM)}{TM-DM} \times 100$$

where FW is the fresh mass (g), TW is the turgid mass (g) and DW is the dry mass (g).

RWC was measured at mid-anthesis when florets at FDS (F3) were most prevalent. Only healthy, fully expanded flag leaves of the primary tiller were used for analysis. Measurements were taken between 11.00h and 13.00h, a period during which leaf water potential has been reported to be stable (Fischer and Sanchez, 1979). Five pots were randomly assigned to each genotype x environment interaction (GxE), each serving as a replicate. A razor blade was used to excise a 2cm long, central section of the flag leaf before sealing it in a plastic bag and storing it in a chilled container. Each leaf cutting was gently blotted with filter paper to remove excess water from the leaf surface before it was placed in a pre-weighed and labelled, sterile plastic culture tube. The fresh weight of all samples was determined within 1 hour of harvest. A minimum of 0.5g of fresh weight plant tissue was required for analysis (Clausen and Kozlowski, 1965). 10ml of distilled water was pipetted into

the base of the centrifuge tube, with care being taken not to wet the sides of the leaf tissue. The tubes were resealed and placed in a dark room at a constant temperature for 24h, to allow for rehydration of the tissue samples. The leaf cuttings were gently blotted again with filter paper before being reweighed to determine turgid weight. The plant tissue was then dried in an oven at 80˚C for 24h until the tissue weight remained constant following two consecutive readings. The dried leaf tissue was then reweighed to determine the dry weight.

2.3.5. Chlorophyll Content Determination

Chlorophyll content was recorded at anthesis in both Experiment 1 and 2. A recently calibrated chlorophyll content meter (CCM-300, Optisciences Inc. Hudson, NH) was used to measure the chlorophyll content of the flag leaves and glumes in all GxE interactions. The output generated by the chlorophyll meter was as chlorophyll content per unit area and not per unit weight. The assessment took place at mid-anthesis between 11.00h and 14.00h. For flag leaf assessment, the central section for the leaf was utilized and for the glume assessment the centrally located glumes were utilized, with the three basal and three apical spikelets excluded from measurements.

2.3.6. Tissue Sample Collection Protocol

In Experiment 2, three replicate tissue samples were collected for the flag leaf, peduncle and glumes from each GxE interaction. Collection of the samples took place on a single day within three hours of the onset of the photoperiod. The samples were flash frozen in 15ml Falcon tubes before being stored at -80˚C in a freezer until analysis. In order to standardize sample collection, the following guidelines were used when determining how a single replicate of each plant organ was collected:

Glumes – all the glumes of an entire spike, with the awns removed
Flag leaf – an approximate 5cm long, central section of the flag leaf
Peduncle – an approximate 5cm long section of the peduncle starting immediately under the base of the spike

The samples were collected in order to determine the starch and water soluble carbohydrate content in the plant tissue. Analysis of the samples took place at the Lancaster Environment Centre (LU, UK). Details relating to the determination of starch and water soluble carbohydrates from these samples can be found in Chapter 4.3.

2.3.7. Yield Assessment

Grain harvest took place after grain filling was completed and the kernel was sufficiently hard. All samples were oven dried at 80°C for 72h prior to assessments being made. The variables recorded included spike weight, grain weight and chaff weight.

2.3.8. Statistical Data Analysis

Tissue temperature analysis of the IR images was carried out using FLIR Quick Report 1.2 SP1 (FLIR Systems). Restriction maximum likelihood analysis (REML), ANOVA, regression analysis and correlation analysis were performed using GenStat Version 16 (VSN International, Hemel Hempstead, UK). GraphPad Prism (GraphPad Software, San Diego, CA, USA) was used as a supplementary graphing tool. Separate pots within growth cabinets were considered as independent replicates. Effects were considered significant at $P<0.05$.

2.4. RESULTS

2.4.1. Growth Cabinet Environmental Conditions

2.4.1.1. Temperature and Relative Humidity

The day and night temperatures achieved in the growth cabinets during anthesis for both Experiment 1 and 2 were within ±1°C of the target temperature. The variation between the treatment cabinets was below ±0.5°C and therefore considered negligible. A significant difference in treatment temperature was created between the ambient temperature (AT) and the elevated temperature (HT) treatment. Table 2.2 summarizes the day and night temperatures recorded for each growth cabinet during vegetative growth, anthesis and grain filling.

Table 2.2 – Growth cabinet temperatures during vegetative growth, anthesis and grain filling phases during Experiment 1 and 2. Values in brackets represent standard deviation.

Year	Growth Cabinet Temperature (°C)						
	Growth Phase	Vegetative Growth		**Anthesis**		Grain Set	
	Cab	Day	Night	**Day**	**Night**	Day	Night
2013	Cab 1 (Cool)	25.0 (±0.2)	16.4 (±0.8)	**25.0 (±0.4)**	**16.1 (±0.9)**	25.0 (±0.5)	20.1 (±0.0)
	Cab 2 (Cool)	24.9 (±0.2)	16.1 (±0.5)	**25.1 (±0.4)**	**16.3 (±0.6)**	25.1 (±0.5)	20.2 (±0.0)
	Cab 3 (Hot)	25.0 (±0.4)	16.2 (±0.5)	**35.0 (±0.6)**	**26.3 (±0.7)**	25.0 (±0.6)	20.1 (±0.0)
	Cab 4 (Hot)	25.0 (±0.3)	16.1 (±0.6)	**35.0 (±0.6)**	**26.2 (±0.7)**	24.9 (±0.5)	20.4 (±0.0)
2014	Cab 1 (Cool)	24.9 (±0.3)	16.3 (±0.7)	**25.0 (±0.4)**	**16.8 (±0.9)**	25.1 (±0.5)	20.0 (±0.1)
	Cab 2 (Cool)	24.8 (±0.3)	16.1 (±0.6)	**25.9 (±0.4)**	**17.0 (±0.7)**	25.0 (±0.5)	20.2 (±0.0)
	Cab 3 (Hot)	25.1 (±0.3)	16.1 (±0.5)	**35.0 (±0.6)**	**26.3 (±0.7)**	25.1 (±0.6)	20.2 (±0.0)
	Cab 4 (Hot)	25.0 (±0.5)	16.2 (±0.8)	**34.9 (±0.6)**	**26.3 (±0.7)**	24.9 (±0.5)	20.5 (±0.0)

Relative humidity (RH) differed significantly between the AT and HT treatment cabinets, with a lower RH being recorded in the HT treatment compared to the AT treatment during anthesis. The average RH of the two AT treatment cabinets in Experiment 1 was 93.0% compared to 78.6% in the HT treatment cabinets (a 14.4% difference). A similar pattern was observed during Experiment 2 in which the average RH of the AT treatment was 13.6% lower than the HT treatment. The RH of cabinet 3 was consistently lower than the RH recorded in the other growth cabinets. RH was found to be higher at night than during the day in both Experiments 1 and 2. Table 2.3 summarizes the day and night RH recorded for each growth cabinet during vegetative growth, anthesis and grain filling.

Table 2.3 – Growth cabinet relative humidity during vegetative growth, anthesis and grain filling phases during Experiment 1 and 2. Values in brackets represent standard deviation.

Year	Growth Cabinet Relative Humidity (%)						
	Growth Phase	Vegetative Growth		**Anthesis**		Grain Set	
	Cab	Day	Night	**Day**	**Night**	Day	Night
2013	Cab 1 (Cool)	89.1 (±1.5)	96.4 (±1.6)	**93.7 (±1.0)**	**96.7 (±1.3)**	90.9 (±1.2)	94.7 (±0.7)
	Cab 2 (Cool)	90.1 (±1.0)	96.3 (±0.8)	**92.3 (±1.4)**	**97.0 (±1.1)**	90.1 (±0.9)	90.1 (±0.4)
	Cab 3 (Hot)	81.0 (±1.3)	87.2 (±0.8)	**74.5 (±1.5)**	**81.1 (±1.6)**	75.3 (±0.8)	80.4 (±0.1)
	Cab 4 (Hot)	81.2 (±0.5)	85.6 (±1.1)	**82.6 (±0.9)**	**90.2 (±1.0)**	82.3 (±1.1)	89.9 (±0.3)
2014	Cab 1 (Cool)	91.2 (±1.6)	96.1 (±1.4)	**92.9 (±1.0)**	**96.8 (±1.1)**	91.5 (±1.1)	93.5 (±0.7)
	Cab 2 (Cool)	89.8 (±1.0)	94.1 (±1.0)	**91.1 (±1.0)**	**96.3 (±1.0)**	90.0 (±0.6)	89.0 (±0.3)
	Cab 3 (Hot)	80.8 (±1.1)	86.5 (±1.0)	**74.5 (±2.2)**	**80.9 (±1.5)**	76.4 (±0.8)	79.6 (±0.4)
	Cab 4 (Hot)	80.7 (±0.6)	84.7 (±1.1)	**82.3 (±1.0)**	**90.0 (±1.0)**	83.3 (±1.0)	89.3 (±0.3)

2.4.1.2. Vapour Pressure Deficit

As a consequence of differences in growth cabinet temperature and RH, differences in vapour pressure deficit (VPD) were identified. In both Experiments 1 and 2, the VPD was lower in the AT treatment cabinets than in the HT treatment cabinets. VPD was also lower at night than during the day. The VPD was consistently higher for cabinet 3 during the day and night in both experiments. Table 2.4 summarizes the day and night VPD of each growth cabinet during vegetative growth, anthesis and grain filling.

Table 2.4 – Growth cabinet vapor pressure deficit during vegetative growth, anthesis and grain filling phases during Experiment 1 and 2.

Cab Vapour Pressure Deficit (VPD kPa)							
Year	Growth Phase	Vegetative Growth		**Anthesis**		Grain Set	
	Cab	Day	Night	**Day**	**Night**	Day	Night
2013	Cab 1 (Cool)	0.34	0.07	**0.20**	**0.06**	0.29	0.13
	Cab 2 (Cool)	0.31	0.07	**0.24**	**0.06**	0.31	0.23
	Cab 3 (Hot)	0.60	0.23	**1.43**	**0.65**	0.78	0.46
	Cab 4 (Hot)	0.60	0.26	**0.98**	**0.33**	0.56	0.24
2014	Cab 1 (Cool)	0.27	0.07	**0.23**	**0.06**	0.27	0.15
	Cab 2 (Cool)	0.32	0.11	**0.30**	**0.07**	0.32	0.26
	Cab 3 (Hot)	0.61	0.25	**1.44**	**0.65**	0.75	0.48
	Cab 4 (Hot)	0.61	0.28	**0.99**	**0.34**	0.53	0.26

2.4.2. Yield

2.4.2.1. Grain Weight

2.4.2.1.1. Experiment 1

Significant differences in the average grain weight/spike (GW) were identified between the six genotypes examined (P<0.001). SB156 displayed the highest GW (2.36g) followed by SB020 (1.93g) and SB155 (1.70g). SB165 and SB019 had comparable GW values (1.55 and 1.54g, respectively) and SB055 was the genotype with the lowest GW (1.26g). No significant differences in GW were found between the AT and HT treatments applied (P=0.180). Significant differences were however found between the WW (2.02g) treatments and the WS (1.26g) treatments (P<0.001). Fig. 2.3 illustrates the average genotypic grain weight for each of the four treatment combinations applied during Experiment 1.

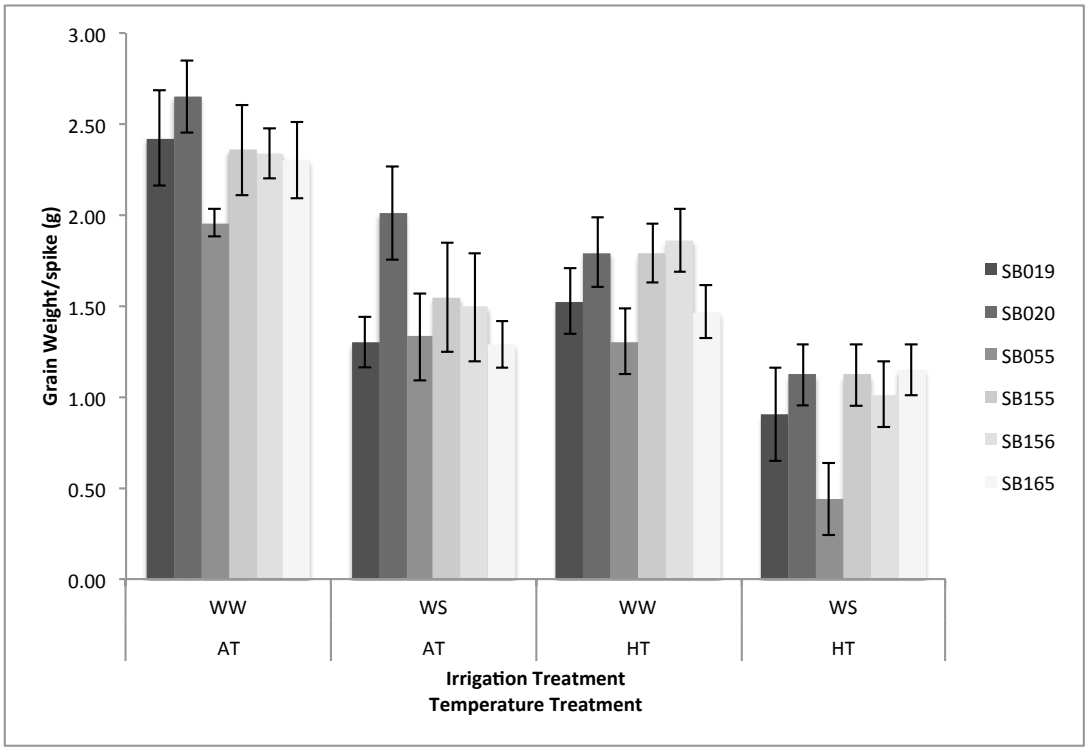

Fig. 2.3 – Average genotypic grain weight/spike of each of the four treatment combinations applied at anthesis during Experiment 1. Error bars represent standard error.

2.4.2.1.2. Experiment 2

No significant differences in grain weight/spike (GW) were identified between the three genotypes used (P=0.946). Significant differences were however uncovered between the two temperature treatments applied (P<0.001), where the GW in the AT treatment (1.60g) was significantly higher than the GW in the HT treatment (0.35g). Similarly for the two irrigation treatments applied, significant differences were uncovered (P<0.001). The GW in the WS treatment (0.75g) was found to be lower than the GW in the WW treatment (1.20g). When GW was examined under the combined temperature and irrigation treatments, significant differences were found (P<0.001). There were no difference in the HT treatment between the GW of the two irrigation treatments applied, but in the AT treatment, the addition of WS resulted in a significantly lower GW (1.2g) compared to the WW treatment (1.9g). Fig. 2.4 illustrates the average genotypic grain weight/spike for each of the four treatment combinations applied during Experiment 2.

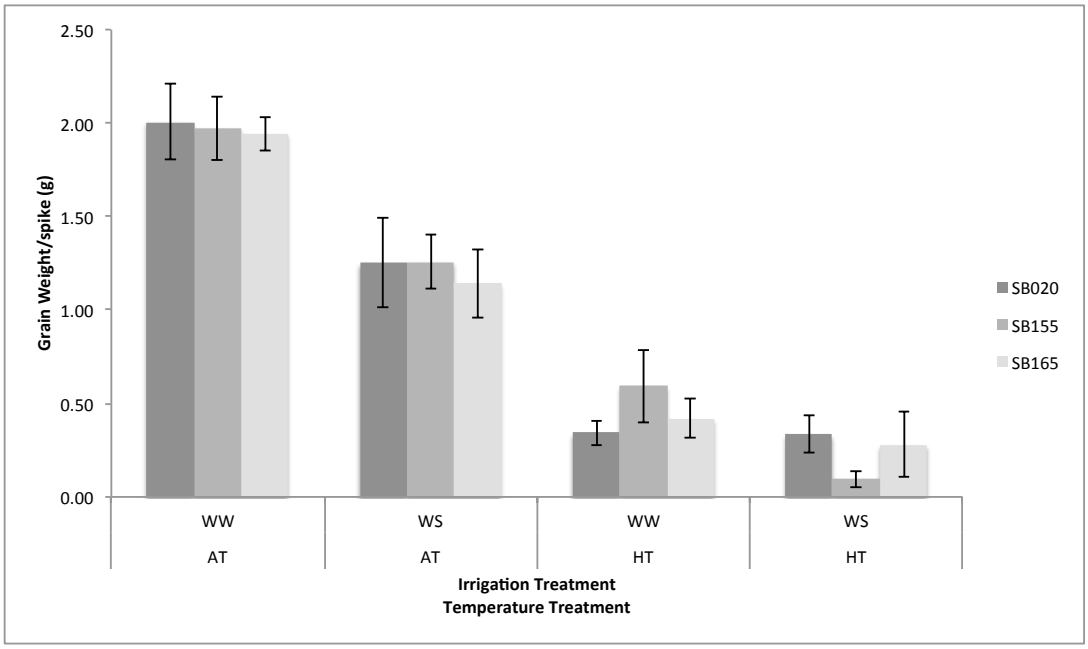

Fig. 2.4 – Average genotypic grain weight/spike of each of the four treatment combinations applied at anthesis during Experiment 2. Error bars represent standard error.

2.4.2.2. Spike Weight

Significant differences in the average spike weight (SW) were identified between the six genotypes examined in Experiment 1 ($P<0.001$). However, no such differences were found in the three genotypes examined in Experiment 2 ($P=0.545$). In Experiment 1, no significant difference in SW was identified between the two temperature treatments applied ($P=0.166$). However, differences in SW were identified between the two irrigation treatments ($P<0.001$), with the SW being significantly lower in the WS treatment (1.84g) compared to the WW treatment (2.75g). In Experiment 2, the two temperature and irrigation treatments applied resulted in significant differences in SW ($P<0.001$). Under HT conditions, SW was significantly reduced (0.86g) compared to the AT treatment (2.15g). The restriction of water at anthesis in the WS treatment resulted in a significantly lower SW (1.23g) than was found in the WW treatment (1.78g).

2.4.2.3. Chaff Weight

Similarly to the SW detailed above, significant differences in chaff weight (CW) were identified between the six genotypes examined in Experiment 1 ($P<0.001$) but not in Experiment 2 ($P=0.112$). In Experiment 1, no significant differences in CW were recorded as a result of the temperature treatments imposed ($P=0.504$). The two irrigation treatments applied significantly affected CW ($P<0.001$), with WS resulting in a lower CW (0.58g) than the WW treatment (0.73g). Significant differences in the CW of the WW (0.67g) and WS treatments (0.57g) in the AT treatment were also identified ($P=0.016$), as well as differences in the WW (0.59g) and WS treatments (0.79g) of the HT environment ($P=0.034$). In Experiment 2, no significant differences were uncovered between the two temperature treatments applied ($P=0.663$). Significant differences in CW were identified between the two irrigation treatments ($P=0.003$), with the CW in the WS treatment (0.49g) being significantly lower than that in the WW treatment (0.59g). In Experiment 2, no significant difference in CW of the WW or the WS irrigation treatments in the AT temperature treatment were identified but in the HT treatment, the addition of WS at anthesis (0.45g) significantly reduced the CW compared to in the WW treatment (0.62g).

2.4.2.4. Chaff Weight/Grain Weight Ratio

The chaff weight/grain weight (CW/GW) ratio was calculated for both experiments. A high ratio indicates greater grain number loss than a low ratio. No significant differences in CW/GW ratio were found between the six genotypes used in either Experiment 1 or 2 (P=0.326 and P=0.253, respectively). In Experiment 1, both the temperature treatments (P<0.001) and irrigation treatments (P=0.029) resulted in significantly different CW/GW weight ratios. The HT treatment increased the ratio significantly (0.63) compared to the AT treatment (0.37). WS produced a higher CW/GW ratio (0.56) than the WW treatment (0.45). No combined treatment (0.72) or genotypic interaction with the treatments was found (P=0.155). In Experiment 2, the two temperature treatments applied, significantly affected the ratio (P<0.001). The HT treatment (1.50) resulted in a significantly higher ratio than the AT treatment (0.43). No significant differences between the WW (0.96) and the WS treatments (0.96) were found (P=0.997). The CW/GW ratio for the two irrigation treatments was greater in Experiment 2 than in Experiment 1.

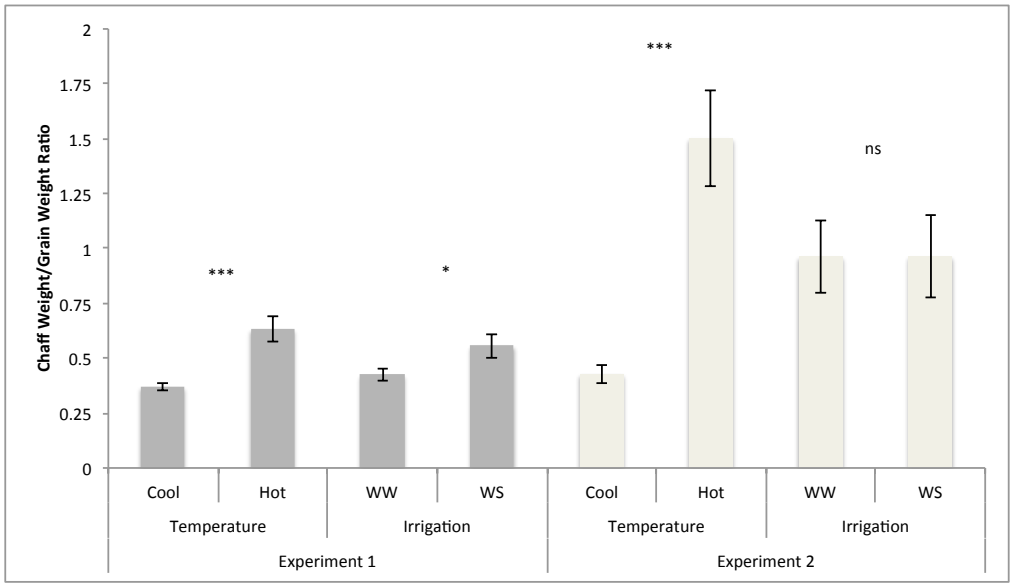

Fig. 2.5 – Chaff/grain weight ratio for each of the combinations of temperature and irrigation treatment applied during Experiment 1 and 2. Error bars represent standard error. ns *P>0.05*; * *P<0.05*; ** *P<0.01*; * *P=<0.001*.**

2.4.2.5 Stress Indices

Details relating to how these SI were calculated can be found in Chapter 2.2.7.

2.4.2.5.1. Experiment 1

Tables 2.5A-C summarize the SI calculated for each of the six genotypes used in Experiment 1: TOL (Tolerance Index), HSI (Heat Susceptibility Index), DSI (Drought Susceptibility Index), SSI (Stress Susceptibility Index), GMP (Geometric Mean Productivity), MP (Mean Productivity) and Y_S and Y_P (yield under stressed and non-stressed conditions, respectively). Table 2.5A shows SI categorized by temperature treatment, Table 2.5B shows the SI categorized by irrigation treatment and Table 2.5C shows the SI by the combined treatment effect calculated during Experiment 1. The genotypes were ranked in the following order of decreasing combined stress tolerance (most stress tolerant first) using the SI calculated in Table 2.5C: SB020, SB156, SB165, SB019, SB155, SB055 (see below).

Table 2.5A, 2.5B, 2.5C – A summary of the SI calculated for the six genotypes used in Experiment 1. Fig. 2.5A shows SI categorized by temperature treatment, Fig. 2.5B shows the SI categorized by irrigation treatment and Fig. 2.5C shows the SI by the combined treatment effect calculated during Experiment 1.

(A)

Genotype	TOL	HSI	STI	GMP	MP	Ys (g)	Yp (g)	Rank (STI)
SBS019	7.19	0.37	0.73	15.37	15.78	12.19	19.38	3
SBS020	8.66	0.41	0.82	16.33	16.90	12.57	21.22	1
SBS055	3.94	0.29	0.41	11.57	11.74	9.77	13.71	6
SBS155	3.33	0.20	0.67	14.75	14.84	13.18	16.51	4
SBS156	4.92	0.26	0.80	16.09	16.27	13.82	18.73	2
SBS165	7.39	0.40	0.63	14.23	14.70	11.01	18.40	5

(B)

Genotype	TOL	DSI	STI	GMP	MP	Ys (g)	Yp (g)	Rank (STI)
SBS019	7.49	0.30	0.70	20.90	21.23	17.48	24.98	4
SBS020	5.00	0.16	1.28	28.19	28.30	25.81	30.80	1
SBS055	2.11	0.11	0.58	18.94	18.97	17.92	20.03	6
SBS155	6.75	0.28	0.69	20.67	20.94	17.57	24.32	5
SBS156	7.00	0.27	0.79	22.16	22.44	18.93	25.94	2
SBS165	4.55	0.19	0.72	21.07	21.20	18.92	23.47	3

(C)

Genotype	TOL	SSI	STI	GMP	MP	Ys	Yp	Rank (STI)
SBS019	11.94	0.48	0.52	18.05	19.01	13.04	24.98	4
SBS020	17.30	0.56	0.67	20.39	22.15	13.50	30.80	1
SBS055	13.51	0.67	0.21	11.43	13.28	6.52	20.03	6
SBS155	11.14	0.46	0.52	17.90	18.75	13.18	24.32	5
SBS156	12.08	0.47	0.58	18.96	19.90	13.86	25.94	2
SBS165	9.33	0.40	0.53	18.22	18.81	14.14	23.47	3

2.4.2.5.2. Experiment 2

Table 2.5D-F summarize the SI calculated for each of the three genotypes used in Experiment 2. Table 2.5D shows SI categorized by temperature treatment, Table 2.5E shows the SI categorized by irrigation treatment and Table 2.5F shows the SI by the combined treatment effect calculated during Experiment 2. The genotypes were ranked in the following order of decreasing combined stress tolerance (most stress tolerant first) using the SI calculated in Table 2.5F: SB165, SB020 and SB155.

Table 2.5D, 2.5E, 2.5F – A summary of the SIs calculated for the three genotypes examined in Experiment 2. Fig. 2.5D shows SI categorized by temperature treatment, Fig. 2.5E shows the SI categorized by irrigation treatment and Fig. 2.5F shows the SI by the combined treatment effect calculated during Experiment 2.

(D)

Genotype	TOL	HSI	STI	GMP	MP	Ys (g)	Yp (g)	Rank (STI)
SBS020	16.41	0.80	0.21	9.28	12.39	4.18	20.59	2
SBS155	15.03	0.74	0.26	10.36	12.80	5.28	20.31	3
SBS165	14.84	0.74	0.25	10.25	12.65	5.23	20.07	1

(E)

Genotype	TOL	HSI	STI	GMP	MP	Ys (g)	Yp (g)	Rank (STI)
SBS020	3.58	0.17	0.85	18.71	18.80	17.01	20.59	2
SBS155	2.77	0.14	0.86	18.87	18.93	17.54	20.31	1
SBS165	3.14	0.16	0.82	18.43	18.50	16.93	20.07	3

(F)

Genotype	TOL	HSI	STI	GMP	MP	Ys (g)	Yp (g)	Rank (STI)
SBS020	16.39	0.80	0.14	9.30	12.40	4.20	20.59	2
SBS155	18.72	0.92	0.05	5.68	10.95	1.59	20.31	3
SBS165	15.42	0.77	0.15	9.66	12.36	4.65	20.07	1

2.4.3. Chlorophyll Content

2.4.3.1. Experiment 1

Significant differences in the flag leaf chlorophyll concentration were found between the six genotypes examined ($P<0.001$). The genotypes could be divided into two distinct categories: low chlorophyll content and high chlorophyll content. SB019, SB155 and SB156 had comparably low chlorophyll contents (570.1, 576.5 and 586.3mg/m^2, respectively) whereas SB020, SB055 and SB165 had comparably high chlorophyll contents (647.0, 642.4 and 634.83mg/m^2, respectively). Although no significant differences in chlorophyll content were identified as a result of the temperature treatments imposed ($P=0.087$) the difference in chlorophyll content of the flag leaf at mid-anthesis between the two irrigation treatments was found to be significant ($P<0.001$). The chlorophyll content in the WS treatment (579.1mg/m^2) was significantly lower than in the WW treatment (639.9mg/m^2). When chlorophyll content of individual genotypes in the two temperature treatments was examined, SB019, SB155 and SB156 had significantly less chlorophyll than the other GxE combinations ($P<0.001$). Similarly, significant differences were also identified between genotypes when categorized by irrigation treatment, with SB019, SB155 and SB156 containing significantly less chlorophyll than the other genotypes ($P<0.001$). The flag leaf chlorophyll content was significantly lower in the HT+WS treatment than for the other three treatment combinations ($P<0.001$).

Similarly to the flag leaf chlorophyll content, no significant differences in glume chlorophyll concentration were found as a result of the two temperature treatments ($P=0.372$) but the two irrigation treatments affected the glume chlorophyll concentration significantly ($P=0.001$) with plants receiving the WS treatment (300.9mg/m^2) containing more chlorophyll than the than plants receiving the WW treatment (281.5mg/m^2). At mid-anthesis, significant differences in the chlorophyll content of the glumes were found between the genotypes examined ($P<0.001$), with SB155 and SB165 having lower chlorophyll concentrations than the other three lines. Compared to the chlorophyll content in the flag leaves, the glumes contained significantly less chlorophyll in each GxE interaction.

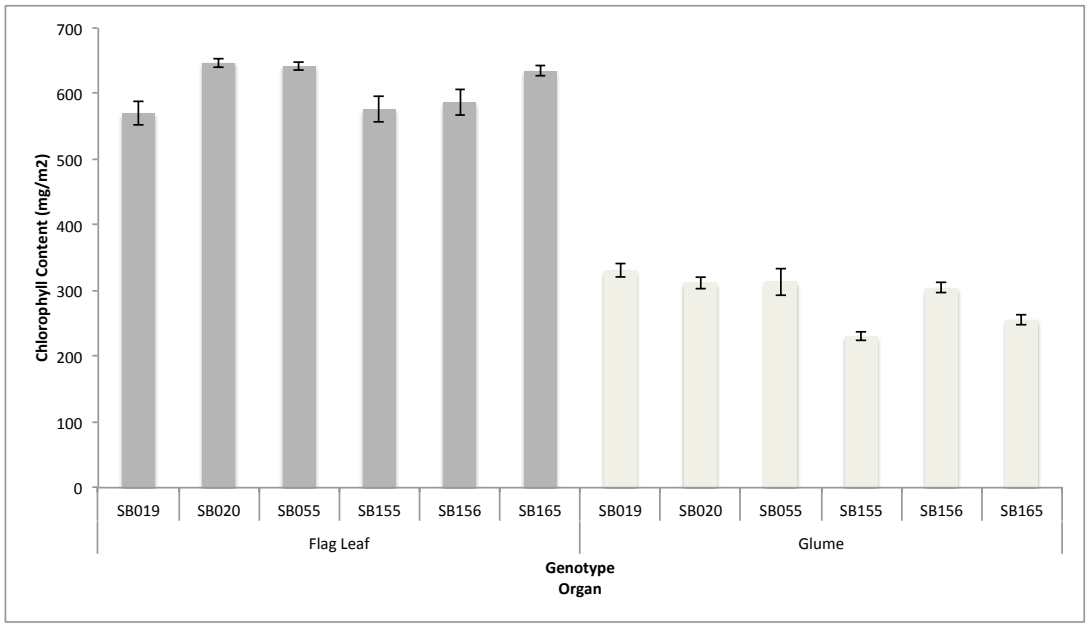

Fig. 2.6 – The chlorophyll content, recorded at mid-anthesis, of the flag leaves and glumes for all genotypes examined in Experiment 1. Error bars represent standard error.

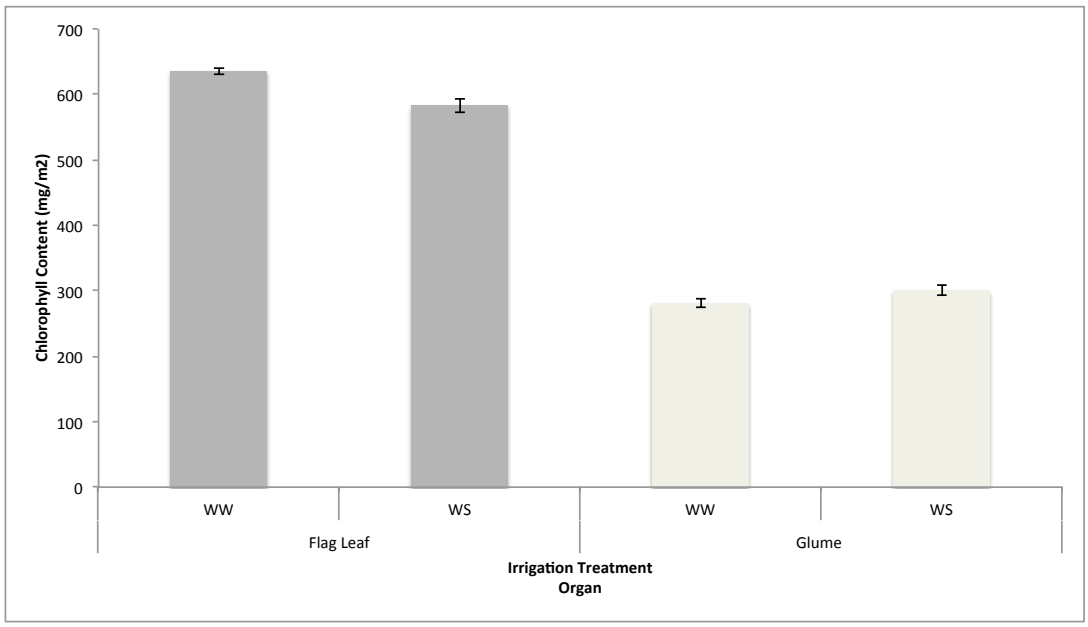

Fig. 2.7 – The chlorophyll content, recorded at mid-anthesis, of the flag leaves and glumes for the two irrigation treatments applied in Experiment 1. Error bars represent standard error.

2.4.3.2. Experiment 2

No significant differences in flag leaf chlorophyll content were identified between the three genotypes examined in Experiment 2 (P=0.100). Significant differences were however identified as a result of the two temperature treatments imposed (P=0.010), with the flag leaf chlorophyll concentration in the HT treatment being significantly lower than in the AT treatment (378.6 compared with 595.7 mg/m^2, respectively). Similarly, significant differences were identified as a result of the two irrigation treatments imposed (P<0.001) with the WS treatment resulting in a significantly lower flag leaf chlorophyll content than the WW treatment (399.4 compared with 574.8mg/m^2, respectively). When the combined effect of the temperature and irrigation treatments on flag leaf chlorophyll content was examined, the HT+WS treatment (183.6mg/m^2) resulted in significantly lower concentrations than the other treatment combinations (P<0.001), with flag leaf chlorophyll content being the highest in the AT+WS treatment (615.2mg/m^2).

Significant differences in the glume chlorophyll content were identified between the three genotypes examined (P<0.001). SB055 contained the lowest glume chlorophyll content (294.3mg/m^2). SB165 contained significantly more chlorophyll in the glumes than SB055 (350.7mg/m^2) but SB020 was identified as the line with the highest glume chlorophyll concentration (387.4mg/m^2). The HT treatment was found to significantly reduce glume chlorophyll concentration (P=0.009). No significant differences in glume chlorophyll content were observed between the irrigation treatments (P=0.508). Significant genotypic differences as a result of the two temperature treatments imposed were uncovered (P=0.037), with the lowest glume chlorophyll content being recorded in the HT treatment for SB055 (236.1mg/m^2). SB020 and SB165 displayed the highest glume chlorophyll contents in the AT treatment (421.8 and 410.3mg/m^2, respectively). Similarly, significant genotypic variation in glume chlorophyll content was identified as a result of the two irrigation treatments imposed (P<0.001). Unlike the flag leaf chlorophyll content, there was no effect of the combined temperature and irrigation treatments on glume chlorophyll content (P=0.177).

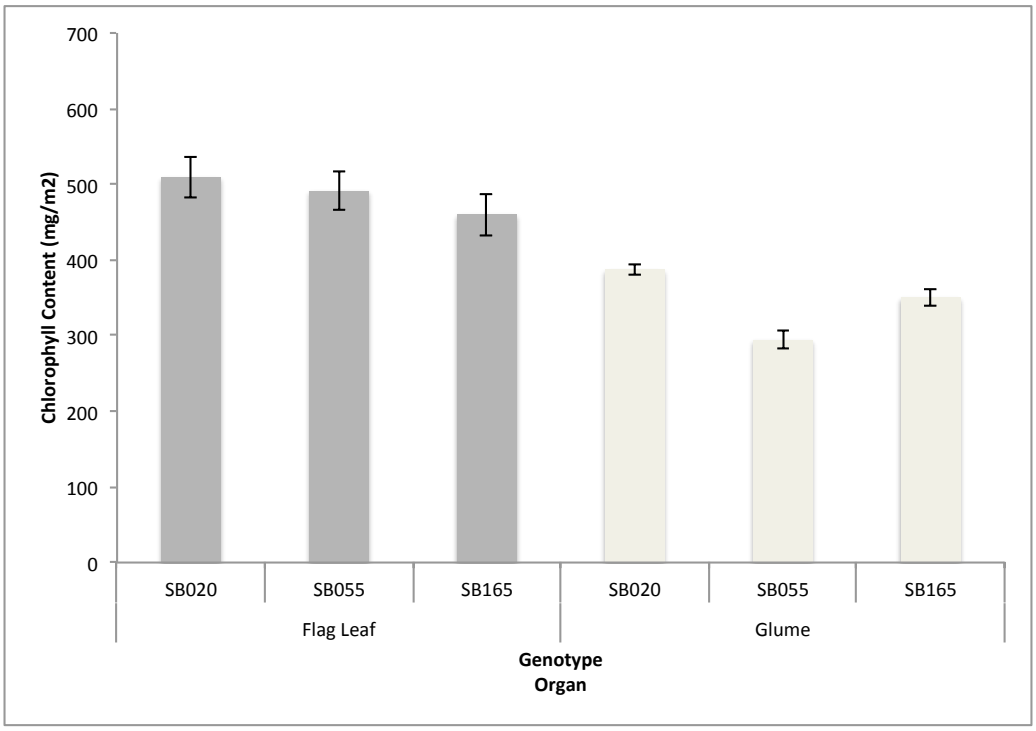

Fig. 2.8 – The chlorophyll content, recorded at mid-anthesis, of the flag leaves and glumes for all genotypes examined in Experiment 2. Error bars represent standard error.

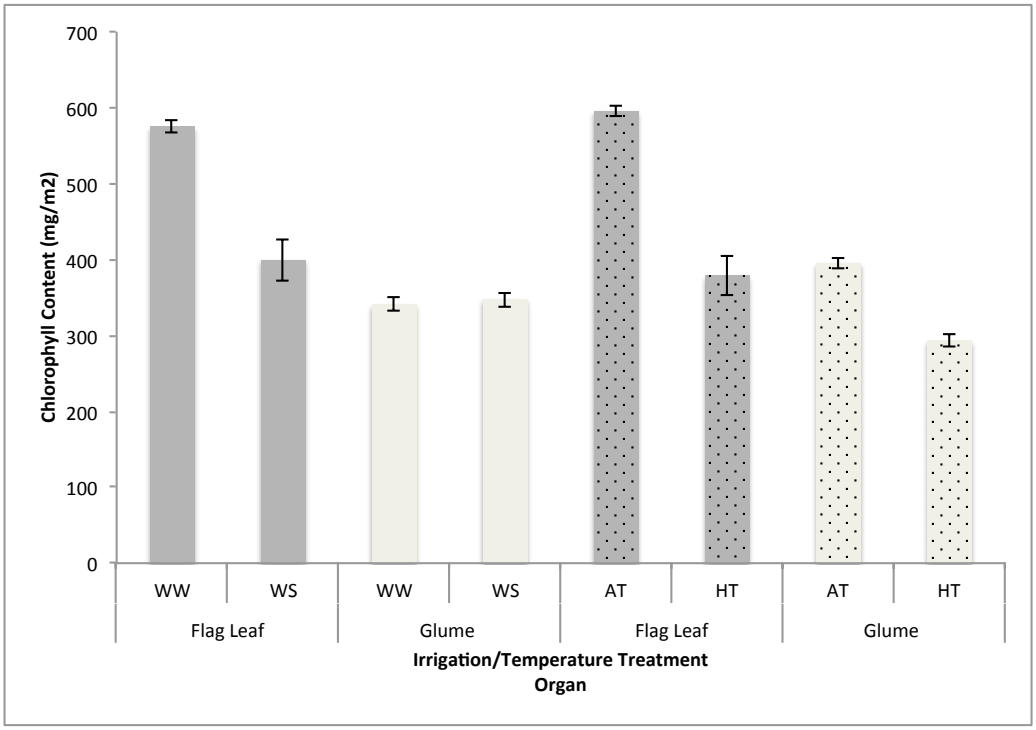

Fig. 2.9 – The chlorophyll content, recorded at mid-anthesis, of the flag leaves and glumes for both the irrigation and temperature treatments applied in Experiment 2. Error bars represent standard error.

2.4.4. Relative Water Content

Flag leaf relative water content (RWC) was assessed only in Experiment 2. Significant differences in RWC were identified between the three genotypes examined (P=0.006). SB155 was found to have a significantly lower RWC (66.8%) than SB020 and SB165 (79.6 and 75.1%, respectively). There was no significant effect of the two temperature treatments on the RWC (P=0.080) but irrigation was found to significantly affect RWC (P<0.001). The RWC in the WW treatment (91.5%) was significantly greater than the RWC in the WS treatment (56.1%). There was no significant interaction between the genotypes examined and the temperature treatments imposed (P=0.340), but irrigation was found to significantly affect RWC on the genotypic level (P=0.027). The RWC was found to be greater in the WW compared to the WS treatments for all three genotypes, although these did not differ significantly from one another. In the WS treatment, SB155 had the lowest RWC (44.4%), followed by SB165 (56.4%) and SB020 had the highest RWC (67.2%). A significant interaction between the temperature treatments and the irrigation applied was uncovered (P=0.023). Under conditions of WS, plants in the AT treatment had a higher RWC (62.2%) compared to those in the HT treatment (49.8%). However, no significant differences were found between the RWC of plants grown in the AT+WW (91.1%) or the HT+WW treatments (92.2%).

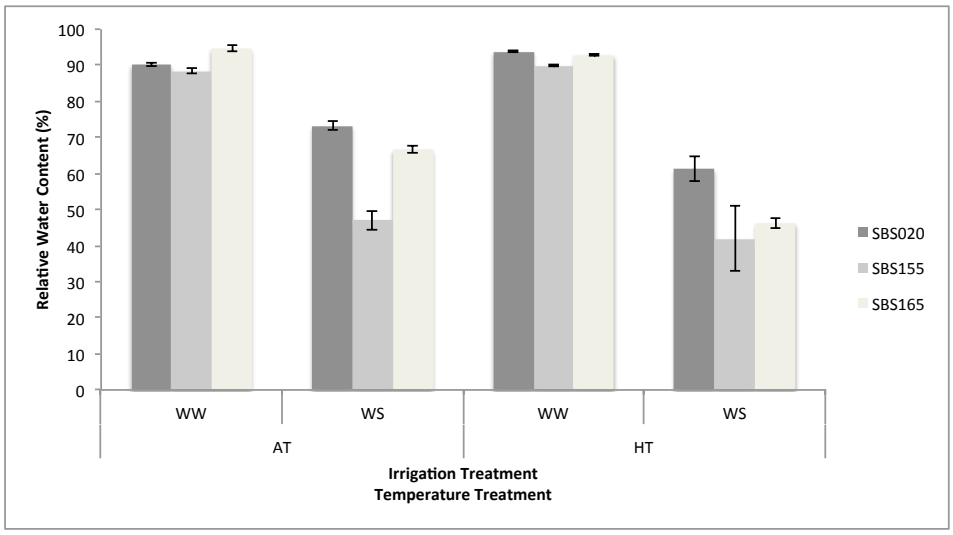

Fig. 2.10 – Flag leaf relative water content of each of the genotypes used, categorized by the two irrigation and temperature treatments applied at anthesis in Experiment 2. Bars represent standard error.

2.4.5. Temperature Depression

2.4.5.1 Experiment 1

2.4.5.1.1. Flag Leaf Temperature Depression

Table 2.6 – A three-way ANOVA analysis table for FLTD on Day 1 during Experiment 1.

Source of variation	d.f.	*P*-value
Genotype	4	0.061
Heat	1	<.001*
Irrigation	1	0.005*
Genotype x Heat	4	0.112
Genotype x Irrigation	4	0.141
Heat x Irrigation	1	0.425
Genotype x Heat x Irrigation	4	0.349
Residual	60	
Total	79	

Table 2.7 – A three-way ANOVA analysis table for FLTD on Day 2 during Experiment 1.

Source of variation	d.f.	*P*-value
Genotype	4	0.217
Heat	1	<.001*
Irrigation	1	<.001*
Genotype x Heat	4	0.003*
Genotype x Irrigation	4	0.833
Heat x Irrigation	1	0.767
Genotype x Heat x Irrigation	4	0.104
Residual	60	
Total	79	

Table 2.8 – A three-way ANOVA analysis table for FLTD on Day 3 during Experiment 1.

Source of variation	d.f.	*P*-value
Genotype	4	0.005*
Heat	1	0.003*
Irrigation	1	0.011*
Genotype x Heat	4	0.047*
Genotype x Irrigation	4	0.048*
Heat x Irrigation	1	0.255
Genotype x Heat x Irrigation	4	0.209
Residual	60	
Total	79	

Tables 2.6, 2.7 and 2.8 summarize the results of a three-way ANOVA analysis looking at the interactions affecting FLTD for each day of observation during Experiment 1. Significant genotypic differences in flag leaf temperature depression (FLTD) were identified ($P<0.001$). SB020 had the highest average FLTD (1.51°C) followed by SB156 (1.2°C). SB155, SB165 and SB019 all had comparably low FLTD values (0.3, 0.1 and 0.1°C, respectively). Significant differences in FLTD were identified as a result of the two temperature treatments applied ($P<0.001$), with average FLTD being significantly lower in the AT treatment (0.2°C) compared to the HT treatment (1.1°C). The FLTD was also significantly affected by the irrigation treatments ($P<0.001$), with the WW treatment resulting in an average FLTD 1.1°C greater than the WS treatment. No significant differences in FLTD were uncovered as a result of the genotypic interaction between the two irrigation treatments applied ($P=0.276$) or as a result of the interaction between the temperature and irrigation treatments ($P=0.981$). Average FLTD differed significantly between the three days of observations ($P<0.001$). FLTD was the greatest on Day 1 (2.0°C) and declined significantly on Day 2 (0.8°C), with the lowest FLTD being recorded on Day 3 (-0.8°C). A reduction in the average cooling capacity of the flag leaf in the range of 2.8°C from the start to the end of the observation period was recorded.

A significant interaction was found in the FLTD of the genotypes used in the two contrasting temperature treatments ($P<0.001$), with SB020 exhibiting the greatest difference in FLTD between the HT and AT treatments (3.1°C). SB156 also

exhibited a FLTD difference of above 1.0°C between the HT and the AT treatments (1.6°C). SB019, SB155 and SB165 all exhibited a significant FLTD difference between the two temperature treatments, although this difference was far smaller than in the case of SB020 and SB156 (0.5, 0.6 and 0.4°C, respectively). FLTD also differed significantly when the five genotypes were categorized by each day of observation (P=0.034). On Day 1, SB156 and SB020 displayed the greatest FLTD (3.1 and 2.6°C, respectively) and SB165 and SB019 had the lowest FLTD (1.3 and 1.1°C, respectively). On Day 2, SB165 and SB020 had the highest FLTD (1.3 and 1.1°C, respectively) and SB155 and SB019 had the lowest (0.5 and 0.1°C, respectively). On Day 3, SB020 was the only genotype examined with a positive FLTD value (0.9°C), whereas FLTD varied from -0.3°C (SB156) to -2.2°C (SB165) for the other genotypes. The effect of the two irrigation treatments on FLTD, when categorized by day, was found to be non-significant (P=0.769) but significant for the two temperature treatments (P<0.001). FLTD on Day 1 and 2 was greater in the HT treatment compared to the AT treatment (2.8 and 1.8°C, respectively). On Day 3, FLTD for the AT treatments was significantly greater than in the HT treatment, although not significantly different from the FLTD in the AT treatment on the previous day (Day 2). A 4.8°C reduction in the average flag leaf cooling capacity was observed from Day 1 to Day 3. These results are illustrated by Fig. 2.11.

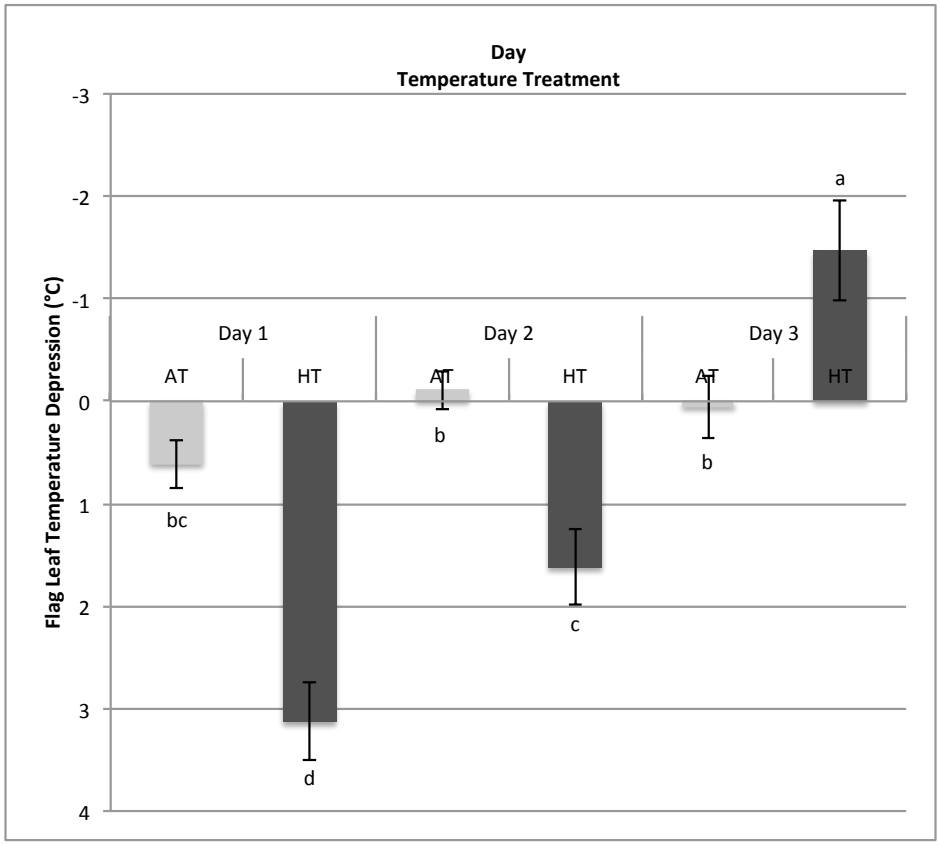

Fig. 2.11 – The average FLTD recorded in each of the two temperature treatments, categorized by observation day in Experiment 1. Error bars represent standard error. Different letters above bars indicate significant differences (*P*<0.05).

2.4.5.1.2 Spike Temperature Depression

Table 2.9 – A three-way ANOVA analysis table for STD on Day 1 during Experiment 1.

Source of variation	d.f.	*P*-value
Genotype	4	0.518
Heat	1	<.001*
Irrigation	1	0.162
Genotype x Heat	4	0.615
Genotype x Irrigation	4	0.013*
Heat x Irrigation	1	0.628
Genotype x Heat x Irrigation	4	0.046*
Residual	60	
Total	79	

Table 2.10 – A three-way ANOVA analysis table for STD on Day 2 during Experiment 1.

Source of variation	d.f.	*P*-value
Genotype	4	0.352
Heat	1	<.001*
Irrigation	1	0.123
Genotype x Heat	4	0.060
Genotype x Irrigation	4	0.638
Heat x Irrigation	1	0.451
Genotype x Heat x Irrigation	4	0.812
Residual	60	
Total	79	

Table 2.11 – A three-way ANOVA analysis table for STD on Day 3 during Experiment 1.

Source of variation	d.f.	*P*-value
Genotype	4	0.099
Heat	1	0.027*
Irrigation	1	0.189
Genotype x Heat	4	0.077
Genotype x Irrigation	4	0.142
Heat x Irrigation	1	0.601
Genotype x Heat x Irrigation	4	0.405
Residual	60	
Total	79	

Tables 2.9, 2.10 and 2.11 summarize the results of a three-way ANOVA analysis looking at the interactions affecting STD for each day of observation during Experiment 1. Significant differences in STD were identified between the five genotypes examined (P=0.028). SB020 displayed the greatest STD (0.4°C), followed by SB156 (0.2°C), SB165 (-0.2°C) and finally SB019 and SB155, both of which had comparable STD values (-0.5°C). However, STD differed significantly between the WW and the WS treatments applied (P=0.017). STD was significantly lower in the WS treatment (-0.4°C) than the WW treatment (0.1°C). Significant differences in the STD were identified between the genotypes in the two temperature treatments (P=0.003). The difference in STD between the AT and HT treatment was greatest in SB020 (2.2°C) followed by the difference in SB156 (1.0°C) and finally the difference in SB165 (0.5°C). No significant differences were

identified between the two temperature treatments of SB019 or SB155. The effect of the two irrigation treatments on STD, when categorized by day, was found to be non-significant (P=0.931) but significant for the two temperature treatments (P<0.001). STD on Day 1 and 2 was greater in the HT treatment compared to the AT treatment (1.4 and 1.6°C, respectively). On Day 3, STD in the AT treatment was significantly greater than in the HT treatment (1.1°C). STD decreased significantly in both the HT and AT treatments from Day 1 to 3 (3.4 and 0.9°C, respectively). These results are illustrated by Fig. 2.12.

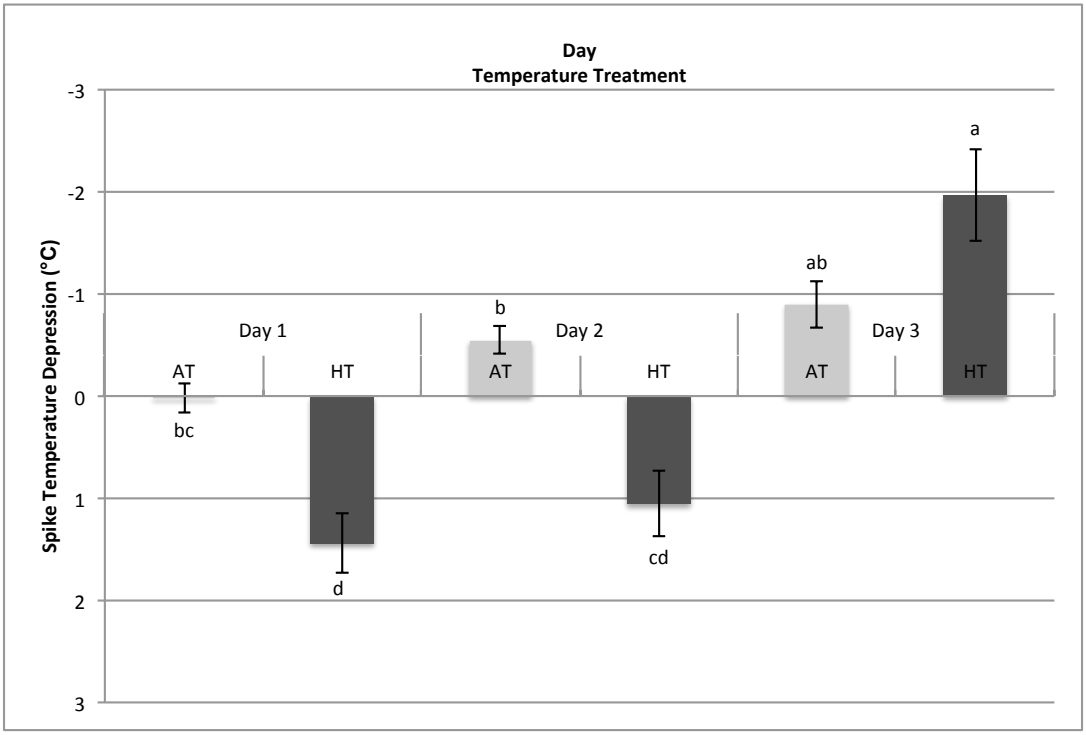

Fig. 2.12 – The average STD recorded in each of the two temperature treatments, categorized by observation day in Experiment 1. Error bars represent standard error. Different letters above bars indicate significant differences (P<0.05).

2.4.5.1.3. Floral Development Observations

2.4.5.1.3.1. Anther

Between Days 1 and 3, a distinct change in average FDS (3) was observed for the anthers in Experiment 1. On Day 1, only 1% of all florets were at FDS (3) with the proportion rising to 10% on Day 2. On Day 3, the proportion of florets at FDS (3) remained low at 4%. Fig. 2.13 illustrates the changes observed in the male floral structures on all three days of observation, pooled for all genotypes and treatments. Fig. 2.14 and 2.15 illustrate the pooled genotypic change in male FDS, on all three days of observation in Experiment 1, in the AT and HT temperature treatments, respectively.

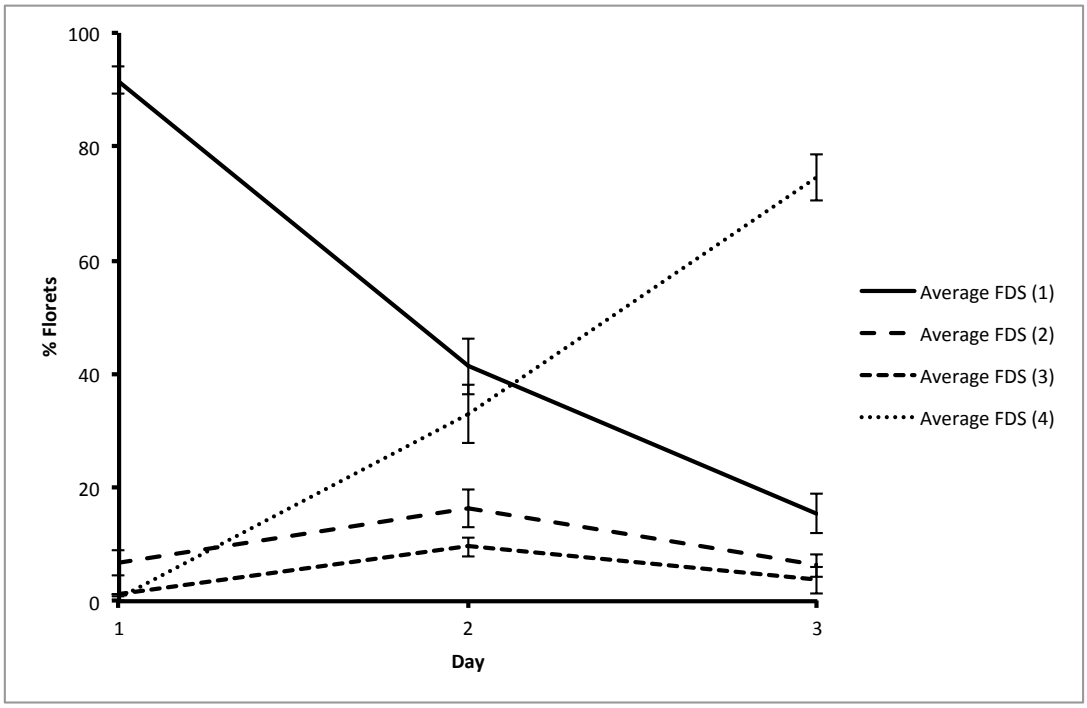

Fig. 2.13 – Daily change in the average percentage of anthers at each male FDS, pooled for all genotypes and treatments during Experiment 1. Error bars represent standard error.

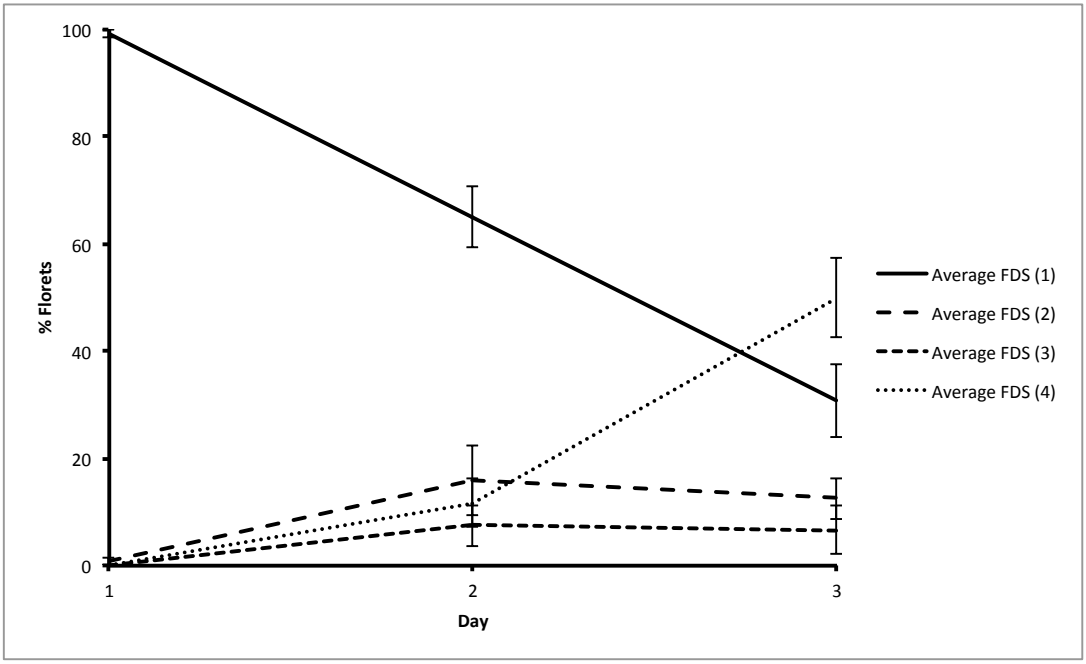

Fig. 2.14 – Daily change in the average percentage of anthers at each male FDS, pooled for all genotypes in the AT temperature treatment during Experiment 1. Error bars represent standard error.

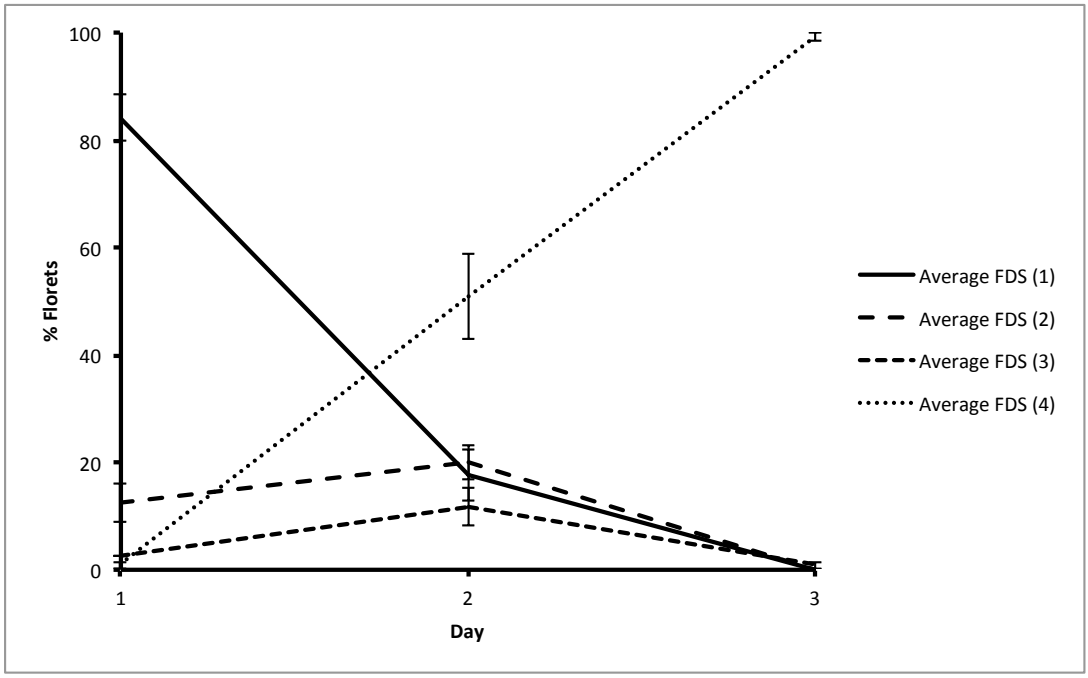

Fig. 2.15 – Daily change in the average percentage of anthers at each male FDS, pooled for all genotypes in the HT temperature treatment during Experiment 1. Error bars represent standard error.

Fig. 2.14 and 2.15 illustrate that in the HT treatment, a significantly faster decline in the percentage of florets at FDS (1) was observed with a concurrent and rapid rise in the occurrence of florets at FDS (4). In the AT treatment, a greater proportion of florets remained at FDS (1), (2) and (3) on Day 3 than under conditions of elevated temperature (HT). Consequently, elevated temperature stress was found to accelerate the progression of anthesis. Table 2.6 and 2.7 (see Appendix) summarize the daily change in the average percentage of male florets recorded at each development stage for each of the five genotypes examined during Experiment 1, in the AT and HT temperature treatments, respectively. On each day of observation, in both the AT and HT temperature treatments, the proportion of florets at FDS (3) did not significantly differ between the five genotypes examined ($p=0.059$ and $P=0.673$, respectively).

2.4.5.1.3.2. Stigma

Similarly for the stigma, a distinct change in the proportion of florets at FDS (F) was observed between Day 1 and 3. On Day 1, florets at FDS (F) constituted 33% of total florets. On Day 2, this proportion rose to 56% with the percentage of florets at FDS (3) falling to 23% on the final day of anthesis, Day 3. Fig. 2.16 illustrates the changes observed in the female floral structures on all three days of observation, pooled for all genotypes and treatments. Fig. 2.17 and 2.18 illustrate the pooled genotypic change in female FDS, on all three days of observation in Experiment 1, in the AT and HT temperature treatments, respectively.

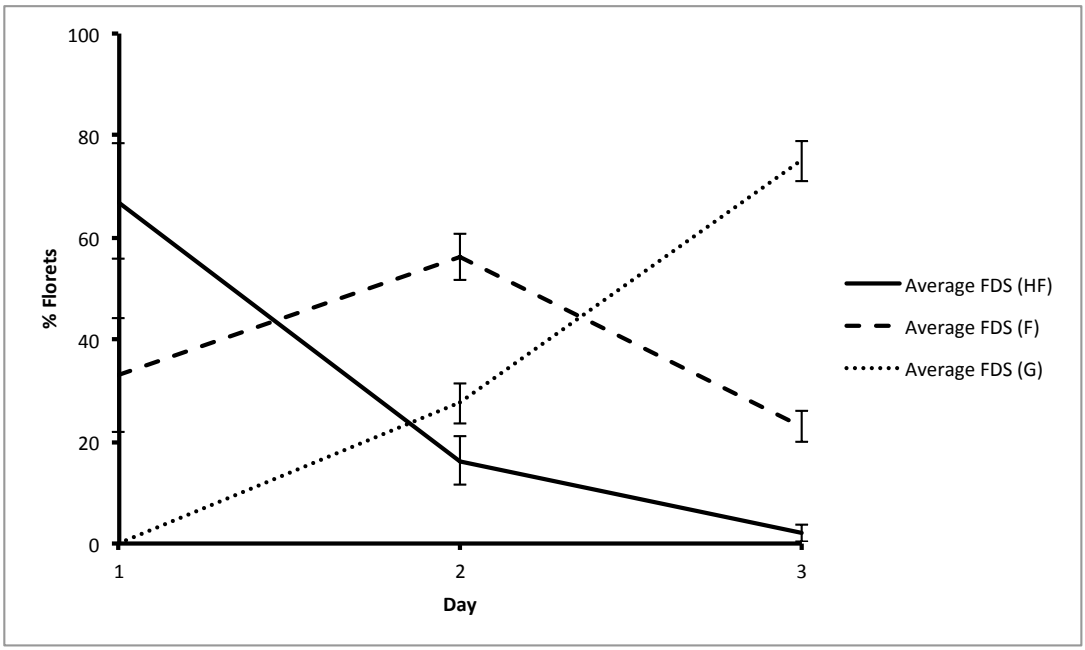

Fig. 2.16 – Daily change in the average percentage of stigmas at each female FDS, pooled for all genotypes and treatments during Experiment 1. Error bars represent standard error.

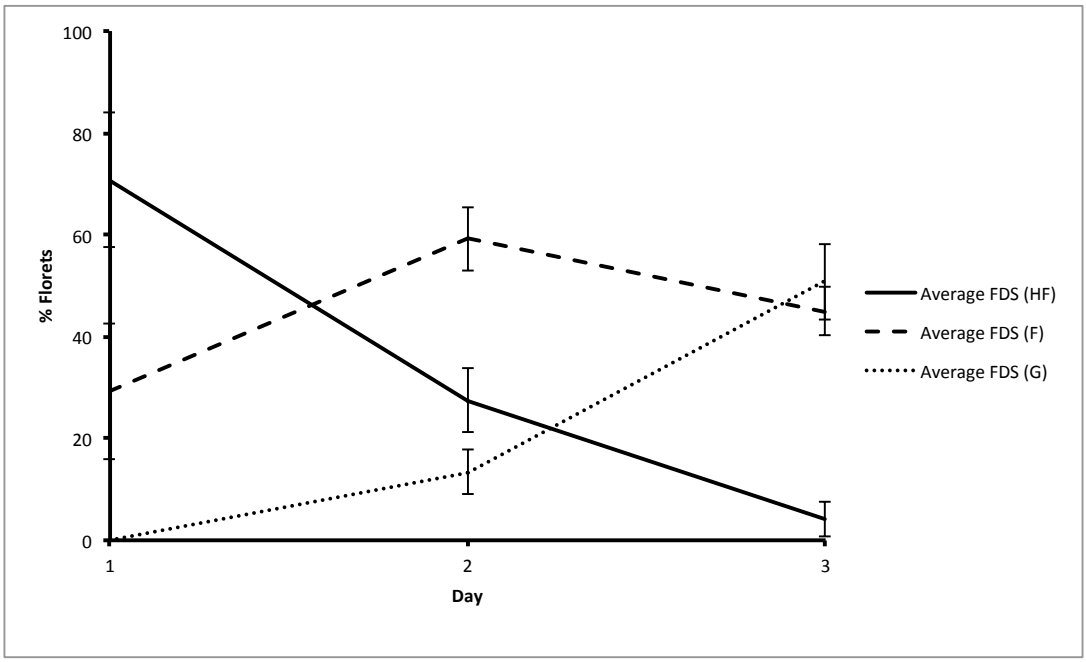

Fig. 2.17 – Daily change in the average percentage of stigmas at each female FDS, pooled for all genotypes in the AT temperature treatment during Experiment 1. Error bars represent standard error.

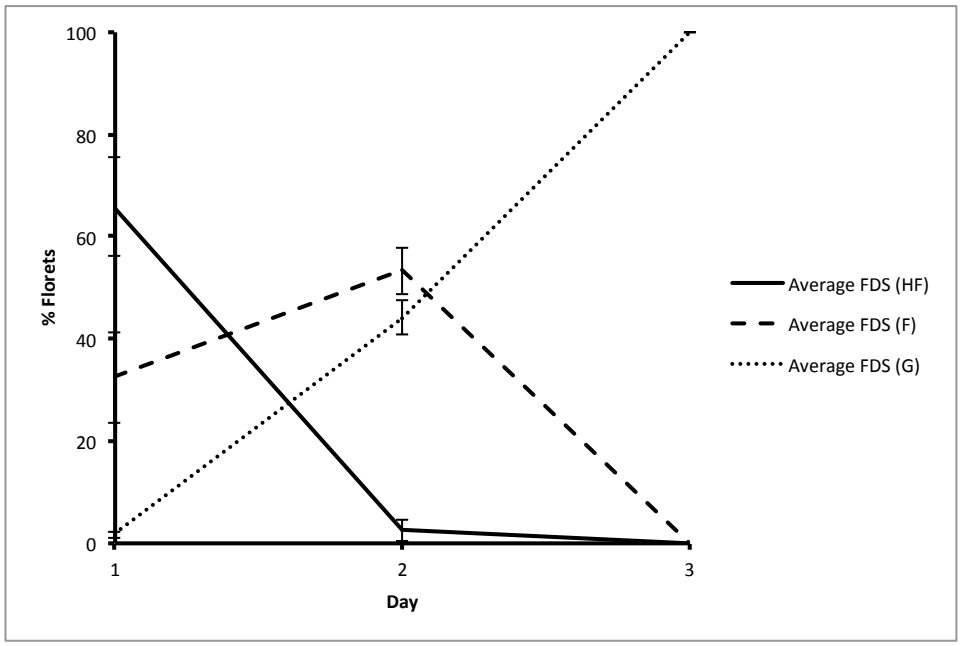

Fig. 2.18 – Daily change in the average percentage of stigmas at each female FDS, pooled for all genotypes in the HT temperature treatment during Experiment 1. Error bars represent standard error.

Similarly to the pattern observed in the change in male floral structures, Fig. 2.17 and 2.18 illustrate that the HT temperature treatment resulted in a significantly faster decline in the percentage of florets at FDS (HF) compared to the AT temperature treatment. The rate at which florets at FDS (G) increased from Day 1 to 3 was greater in the HT treatment than in the AT treatment. The presence of elevated temperatures resulted in a faster progression of female florets from the early to the late stages of stigma development. Table 2.8 and 2.9 (see Appendix) summarize the daily change in the average percentage of female florets recorded at each development stage for each of the five genotypes examined during Experiment 1, in the AT and HT temperature treatment, respectively. In the AT temperature treatment, the proportion of florets at FDS (F) did not significantly differ between genotypes ($p=0.872$) on each day of observation. In the HT treatment, the proportion of florets at FDS (F) differed significantly between genotypes on Day 1 and 2 ($P=0.010$). On Day 1, SB156 had fewer florets at FDS (F) than the other lines, whilst on Day 2, SB019 and SB165 contained significantly more florets at FDS (F) than the other three lines examined. No significant differences in the proportion of florets at FDS (F) on Day 3 were found.

2.4.5.2 Experiment 2

2.4.5.2.1 Flag Leaf Temperature Depression

Table 2.12 – A three-way ANOVA analysis table for FLTD on Day 1 during Experiment 2.

Source of variation	d.f.	*P*-value
Genotype	2	0.915
Heat	1	<.001*
Irrigation	1	0.116
Genotype x Heat	2	0.007*
Genotype x Irrigation	2	0.461
Heat x Irrigation	1	0.872
Genotype x Heat x Irrigation	2	0.977
Residual	60	
Total	71	

Table 2.13 – A three-way ANOVA analysis table for FLTD on Day 2 during Experiment 2.

Source of variation	d.f.	*P*-value
Genotype	2	0.035*
Heat	1	<.001*
Irrigation	1	0.002*
Genotype x Heat	2	0.041*
Genotype x Irrigation	2	0.137
Heat x Irrigation	1	0.153
Genotype x Heat x Irrigation	2	0.546
Residual	60	
Total	71	

Table 2.14 – A three-way ANOVA analysis table for FLTD on Day 3 during Experiment 2.

Source of variation	d.f.	*P*-value
Genotype	2	0.31*
Heat	1	<.001*
Irrigation	1	0.004*
Genotype x Heat	2	0.306
Genotype x Irrigation	2	0.552
Heat x Irrigation	1	0.822
Genotype x Heat x Irrigation	2	0.371
Residual	60	
Total	71	

Table 2.15 – A three-way ANOVA analysis table for FLTD on Day 4 during Experiment 2.

Source of variation	d.f.	*P*-value
Genotype	2	0.247
Heat	1	<.001*
Irrigation	1	<.001*
Genotype x Heat	2	0.547
Genotype x Irrigation	2	0.140
Heat x Irrigation	1	0.859
Genotype x Heat x Irrigation	2	0.484
Residual	60	
Total	71	

Tables 2.12, 2.13, 2.14 and 2.15 summarize the results of a three-way ANOVA analysis looking at the interactions affecting FLTD for each day of observation during Experiment 2. No significant differences in FLTD were identified between the three genotypes examined (P=0.684). FLTD did however differ significantly between the two temperature treatments imposed (P=0.007), with FLTD being significantly greater in the HT treatment (4.2°C) than in the AT treatment (1.0°C). Similarly, the irrigation treatments applied resulted in significant differences in the FLTD (P<0.001). FLTD was found be significantly lower in the WS treatment (2.1°C) compared to the WW treatment (3.1°C). FLTD varied significantly between experimental days (P=0.002), with the lowest values being observed on Day 2 (2.2°C). FLTD was comparable on Day 3 and 4 (2.7 and 2.4°C, respectively) and the highest on Day 1 (3.0°C). When FLTD was categorized by temperature treatments between Days, significant differences were uncovered (P=0.022). On all days, FLTD in the HT treatment was higher than in the AT treatment. There were no significant differences in the FLTD in the AT treatment from Day 1 till Day 4. In the HT treatment however, FLTD was the greatest on Day 3 (4.7°C) and the lowest on Day 2 (3.5°C). No significant difference in FLTD, when categorized by temperature treatment and day, was uncovered between Day 1 and 4 (4.5°C and 4.0°C, respectively). Significant differences in the FLTD were also found as a result of the different temperature treatments between genotypes (P<0.001). In all three genotypes examined, FLTD was greater in the HT treatment than in the AT treatment. FLTD in the HT treatment was however not found to significantly differ between genotypes. In the AT treatment, FLTD was the lowest in SB020 (0.6°C)

and the highest in SB155 (1.5°C). SB020 also exhibited the greatest difference in FLTD between the two temperature treatment categories (3.9°C). This difference was smaller for SB155 and SB165 (2.4 and 3.2°C, respectively). These findings are illustrated by Fig. 2.19.

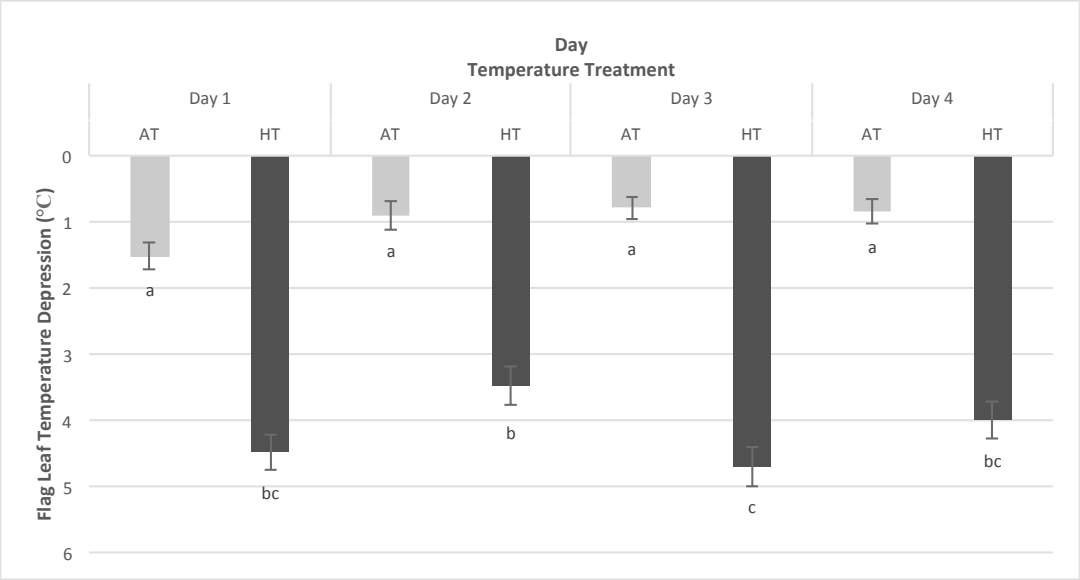

Fig. 2.19 – The average FLTD recorded in each of the two temperature treatments, categorized by observation day in Experiment 2. Error bars represent standard error. Different letters above bars indicate significant differences (*P*<0.05).

2.4.5.2.2. Spike Temperature Depression

Table 2.16 – A three-way ANOVA analysis table for STD on Day 1 during Experiment 2.

Source of variation	d.f.	*P*-value
Genotype	2	0.586
Heat	1	<.001*
Irrigation	1	0.423
Genotype x Heat	2	0.673
Genotype x Irrigation	2	0.358
Heat x Irrigation	1	0.339
Genotype x Heat x Irrigation	2	0.262
Residual	60	
Total	71	

Table 2.17 – A three-way ANOVA analysis table for STD on Day 2 during Experiment 2.

Source of variation	d.f.	*P*-value
Genotype	2	0.041*
Heat	1	<.001*
Irrigation	1	0.026*
Genotype x Heat	2	0.156
Genotype x Irrigation	2	0.671
Heat x Irrigation	1	0.112
Genotype x Heat x Irrigation	2	0.046
Residual	60	
Total	71	

Table 2.18 – A three-way ANOVA analysis table for STD on Day 3 during Experiment 2.

Source of variation	d.f.	*P*-value
Genotype	2	0.305
Heat	1	<.001*
Irrigation	1	<.001*
Genotype x Heat	2	0.924
Genotype x Irrigation	2	0.389
Heat x Irrigation	1	0.796
Genotype x Heat x Irrigation	2	0.019
Residual	60	
Total	71	

Table 2.19 – A three-way ANOVA analysis table for STD on Day 4 during Experiment 2.

Source of variation	d.f.	*P*-value
Genotype	2	0.965
Heat	1	0.002*
Irrigation	1	<.001*
Genotype x Heat	2	0.868
Genotype x Irrigation	2	0.080
Heat x Irrigation	1	0.823
Genotype x Heat x Irrigation	2	0.472
Residual	60	
Total	71	

Tables 2.16, 2.17, 2.18 and 2.19 summarize the results of a three-way ANOVA analysis looking at the interactions affecting STD for each day of observation during Experiment 2. Similarly to the FLTD, no significant differences in STD were

uncovered between the three genotypes examined (P=0.082). However, significant differences in the STD between the two temperature treatments (P=0.014) and the two irrigation treatments imposed (P<0.001) was found. STD was lower in the AT (0.4°C) than the HT (1.9°C) environment. Similarly for the two irrigation treatments, STD was lowest as a result of WS (0.7°C) and highest under WW conditions (1.5°C). STD was significantly different between the four days of observation that took place (P=0.002), with the lowest STD values being observed on Day 4 (0.6°C) and the highest on Day 2 (1.6°C). STD was comparable on Day 1 and 3 (1.2°C). STD was further found to differ significantly between temperature treatments between the four days of observations (P=0.011). No significant differences in STD were identified in the AT treatment on any of the four days but significant variation was uncovered in the HT treatment. STD in the HT treatment was greatest on Day 2 (2.7°C) and lowest on Day 4 (1.1°C). STD on Day1 and 3 was comparable and higher than on Day 4 (1.8 and 2.1°C, respectively). The greatest difference in STD between the two temperature treatments was observed on Day 2 (2.2°C) and the lowest on Day 4 (0.9°C). Fig. 2.20 illustrates these relationships.

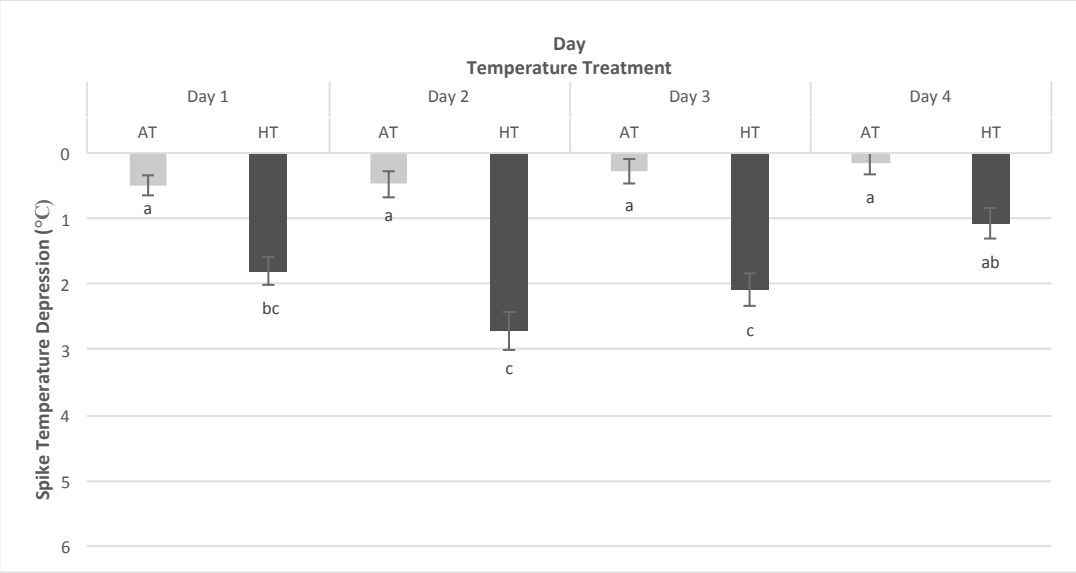

Fig. 2.20 – The average STD recorded in each of the two temperature treatments, categorized by observation day in Experiment 2. Error bars represent standard error. Different letters above bars indicate significant differences (P<0.05).

2.4.5.2.3. Floral Development Observations

2.4.5.1.3.1. Anther

Between Days 1 and 4, a distinct change in average FDS (3) was observed for the anthers in Experiment 2. On Day 1, 30% of florets had advanced to FDS (3). On Day 2, the proportion of florets at FDS (3) rose to 56%. On Day 3, the total proportion of florets at FDS (3) declined slightly to 49% with florets at FDS (3) continuing to decline on Day 4 to 14% of total florets. Fig. 2.21 illustrates the changes observed in the male floral structures on all four days of observation, pooled for all genotypes and treatments. Fig. 2.22 and 2.23 illustrate the pooled genotypic change in male FDS, on all four days of observation in Experiment 2, in the AT and HT temperature treatments, respectively.

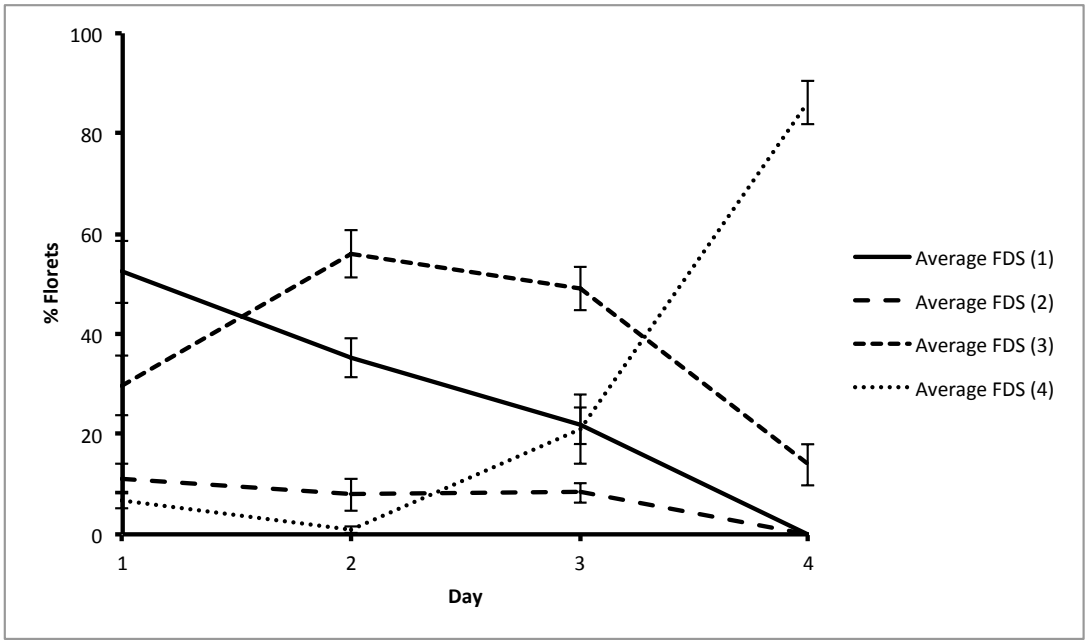

Fig. 2.21 – Daily change in the average percentage of anthers at each male FDS, pooled for all genotypes and treatments during Experiment 2. Error bars represent standard error.

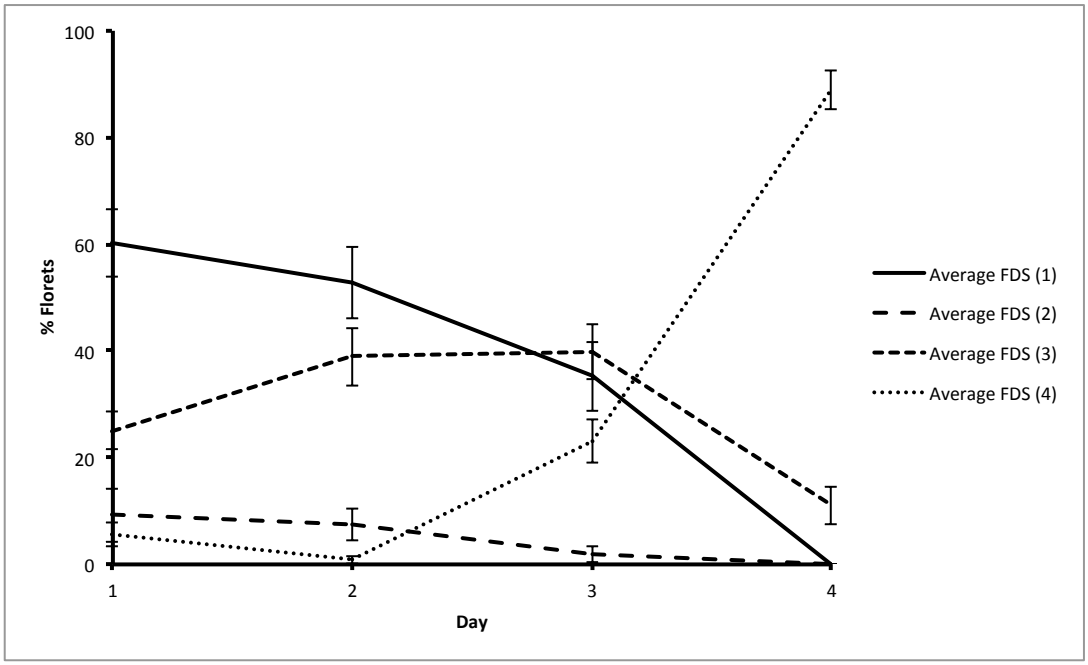

Fig. 2.22 – Daily change in the average percentage of anthers at each male FDS, pooled for all genotypes in the AT temperature treatment during Experiment 2. Error bars represent standard error.

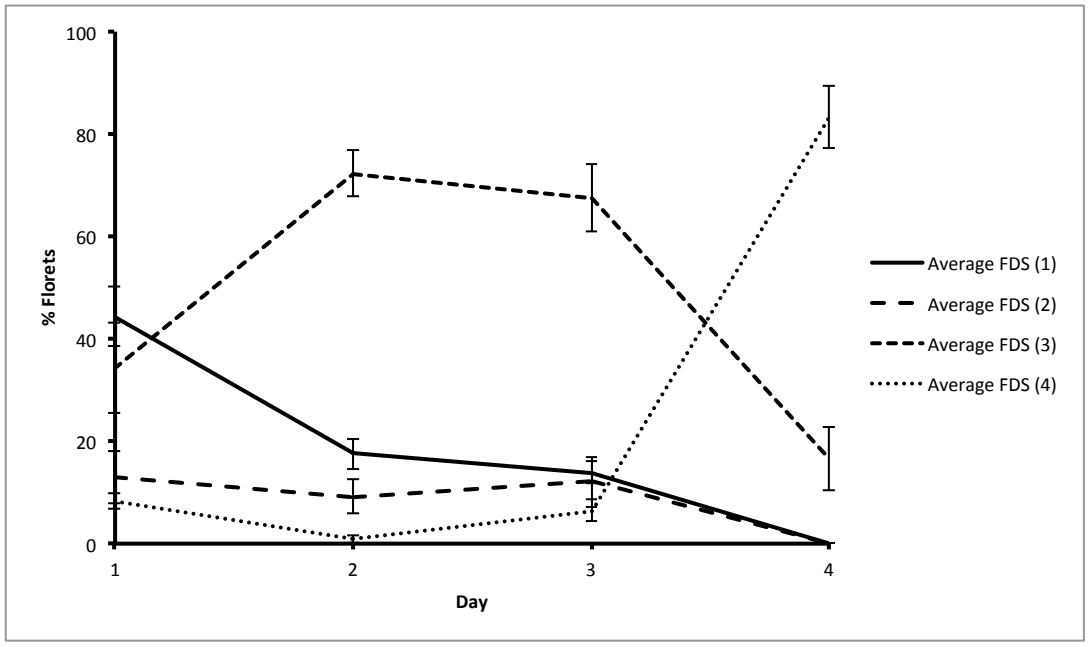

Fig. 2.23 – Change in the pooled genotypic average percentage of male florets observed during anthesis in the HT temperature treatment of Experiment 2. Error bars represent standard error.

Fig. 2.22 and 2.23 illustrate the difference in the progression of male floral development as a result of the two contrasting temperature treatments applied. On Days 2 and 3, a greater proportion of florets were at FDS (3) in the HT treatment compared to the AT treatment. The rate of decline of florets at FDS (1) was greater as a result of elevated temperature. On Day 4, the portion of florets at each male FDS did not differ significantly. Compared to Experiment 1, the difference in the rate of anther development between the two temperature treatments was less evident in Experiment 2.

Table 2.10 and 2.11 (see Appendix) summarize the daily change in the average percentage of male florets recorded at each development stage for each of the three genotypes examined during Experiment 2 in the AT and HT temperature treatment, respectively. On each day of observation in both the AT and HT temperature treatments, the proportion of florets at FDS (3) did not significantly differ between genotypes ($p=0.073$ and $P=0.063$, respectively).

2.4.5.1.3.2. Stigma

Similarly for the stigma, a distinct change in average FDS (F) was observed in Experiment 2. On Day 1, 41% were at FDS (F) with the proportion rising to 53% on Day 2. On Day 3, a decline in florets at FDS (F) to 29% was recorded, with florets at FDS (F) continuing to decline on Day 4 to 2%. Fig. 2.20 illustrates the changes observed in the female floral structures on all four days of observation, pooled for all genotypes and treatments. Fig. 2.21 and 2.22 illustrate the pooled genotypic change in female FDS on all four days of observation in Experiment 2, in the AT and HT temperature treatments, respectively.

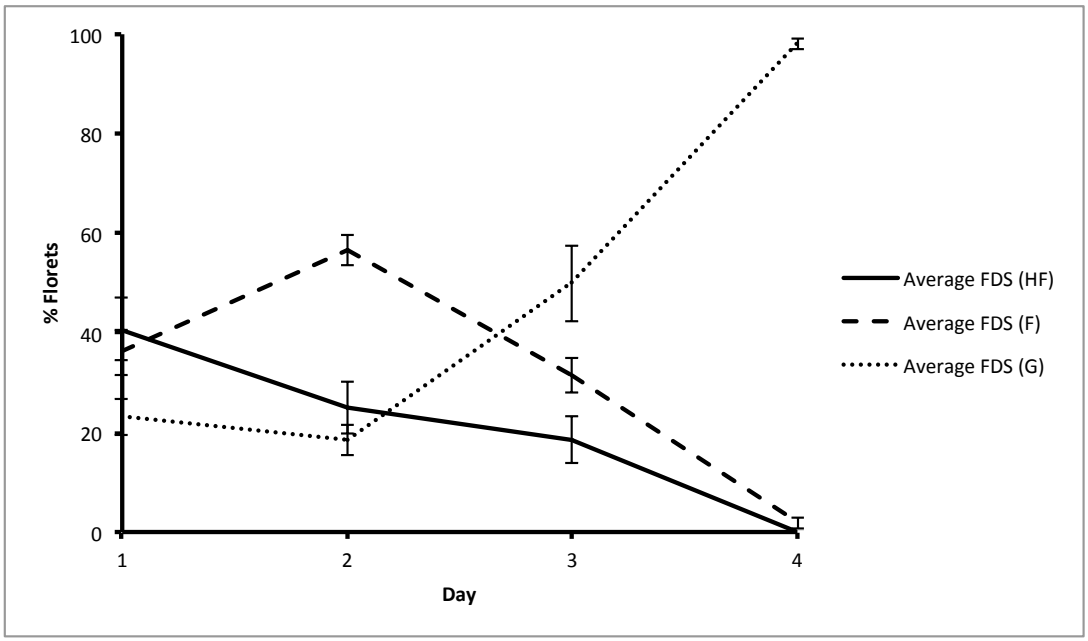

Fig. 2.24 – Daily change in the average percentage of stigmas at each female FDS, pooled for all genotypes and treatments during Experiment 2. Error bars represent standard error.

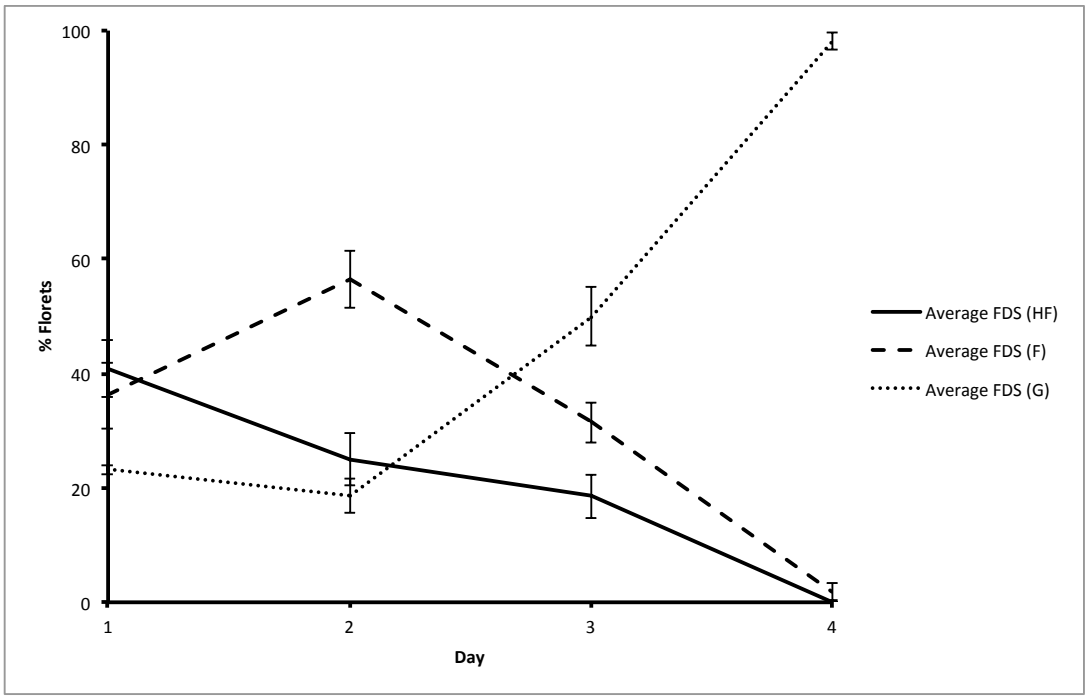

Fig. 2.25 – Daily change in the average percentage of stigmas at each female FDS, pooled for all genotypes in the AT temperature treatment during Experiment 2. Error bars represent standard error.

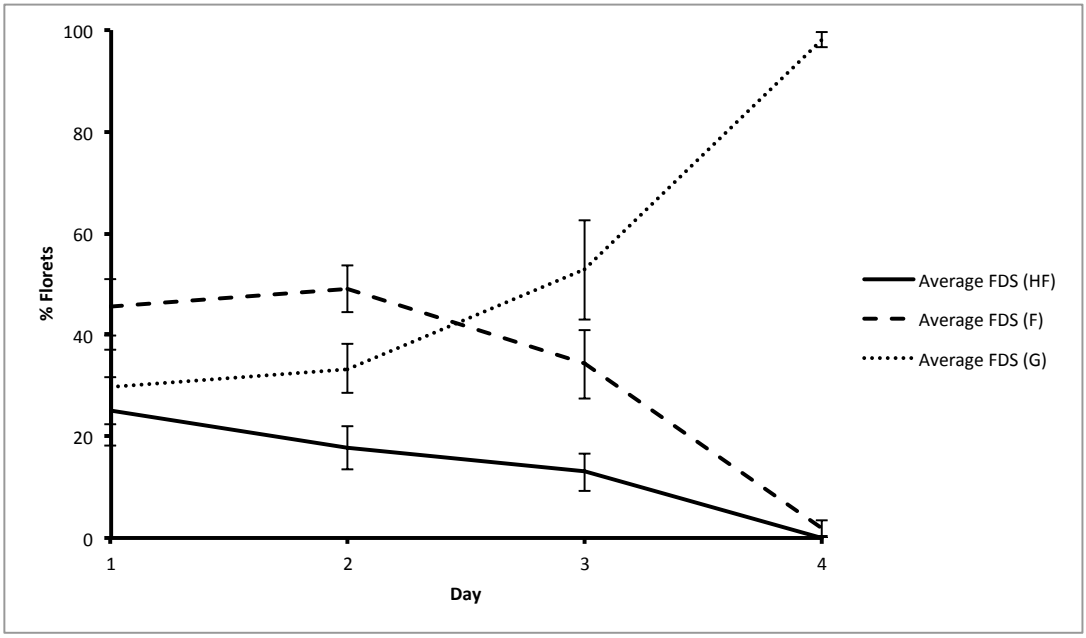

Fig. 2.26 – Daily change in the average percentage of stigmas at each female FDS, pooled for all genotypes in the HT temperature treatment during Experiment 2. Error bars represent standard error.

Fig. 2.25 and 2.26 illustrate the difference in stigma development between the AT and the HT treatments. Elevated temperature resulted in an increase in the presence of florets at FDS (G) on Day 1 in the HT treatment compared to the AT treatment. The difference in the rate of stigma development between the two temperature treatments is less evident in Experiment 2 than in Experiment 1.

Table 2.12 and 2.13 (see Appendix) summarize the daily change in the average percentage of female florets recorded at each development stage for each of the three genotypes examined during Experiment 2 in the AT and HT temperature treatment, respectively. On each day of observation in both the AT and HT temperature treatments, the proportion of florets at FDS (F) did not significantly differ between genotypes (P=0.062 and P=0.572, respectively).

2.4.6. Temperature Depression and Grain Weight

2.4.6.1. Experiment 1

When categorized by genotype, no significant correlations between FLTD or STD at mid-anthesis, and GW were found. Similarly, no significant correlations between the FLTD or STD and GW were found when categorized by the contrasting irrigation treatments applied. Significant correlations were however identified between FLTD in both the AT (P<0.001) and HT (P=0.031) temperature treatments (Fig. 2.27). Greater FLTD and mid-anthesis was associated with higher GW. No such correlations were however identified for STD.

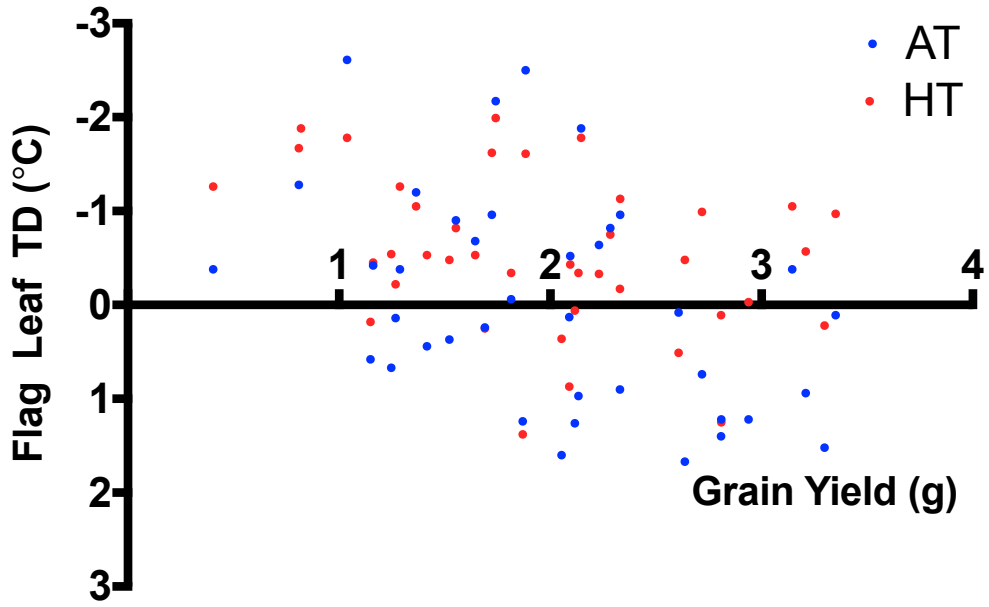

Fig. 2.27 – The pooled genotypic correlation between flag leaf temperature depression at mid-anthesis and grain yield, categorized by the two temperature treatments applied during Experiment 1.

2.4.6.2. Experiment 2

When categorized by genotype, a significant negative correlation between the FLTD of the three genotypes used, SB020, SB155 and SB165, and GW was found (P=0.005, 0.004 and 0.005, respectively). Increased FLTD at mid-anthesis was found to result in lower GW. The highest GW was observed when tissue temperature was in the region of 0-2°C below ambient air temperature. FLTD greater than approximately 4°C resulted in the lowest GW recorded. Whilst a significant correlation between STD and GW was found for SB155 (P=0.024) (Fig. 2.28), the relationship was non-significant in the other two genotypes, SB020 and SB165. Similarly to FLTD, STD was negatively correlated to increased GW. STD close to, or above, ambient were found to result in the highest grain yields.

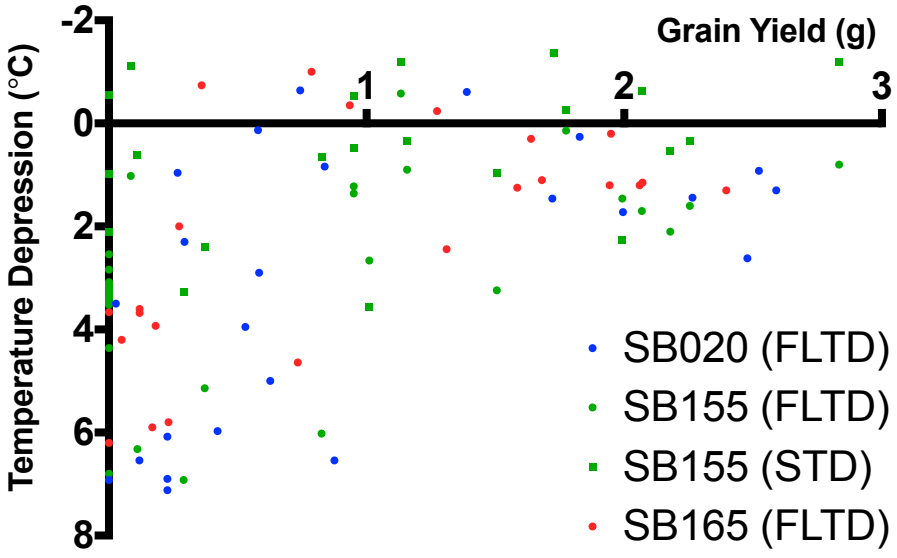

Fig. 2.28 – The correlation between flag leaf temperature depression and grain yield for SB020, SB155 and SB165, as well as the correlation between spike temperature depression of SB155 and grain yield in Experiment 2.

When organ TD was categorized by the temperature treatment applied, a significant positive correlation between FLTD/STD at mid-anthesis and increased GW was found in the AT treatment (P=0.003 and 0.045, respectively) (Fig. 2.29). No significant relationship between organ TD and grain yield was found in the HT treatment. Lower yields were associated with organ TD close to, or above, ambient temperature i.e. negative organ TD values.

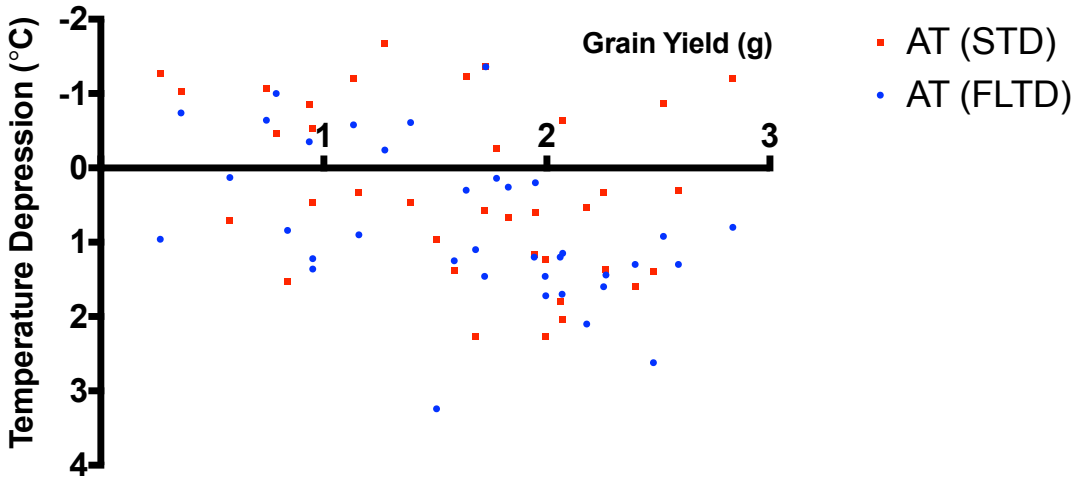

Fig. 2.29 – The pooled genotypic correlation between flag leaf temperature depression and spike temperature depression with grain yield in the cool temperature treatment during Experiment 2.

When categorized by irrigation treatment, FLTD and STD in the WW treatment were both significantly, negatively correlated to GW (P<0.001). Under WS conditions, FLTD and STD were similarly negatively correlated (P=0.001 and 0.027, respectively) (Fig. 2.30).

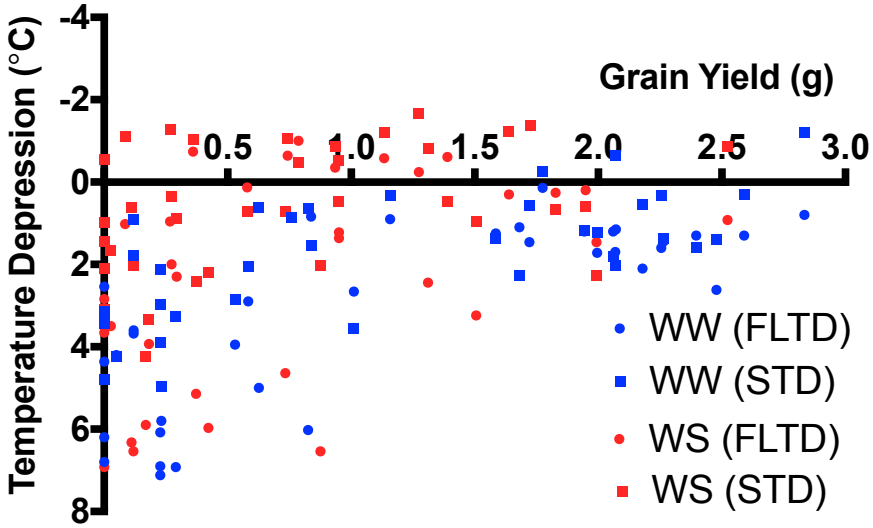

Fig. 2.30 – The pooled genotypic correlation between flag leaf temperature depression and spike temperature depression with grain yield in the two irrigation treatments during Experiment 2.

2.4.7. Temperature Depression and Floret Development Stage

2.4.7.1. Experiment 1

No statistically significant differences in the slope of the relationship between either FLTD or STD, and FDS of the male and female reproductive organs were found amongst the genotypes. The slope of the linear relationship between STD in the AT temperature treatment and the FDS scores of the anther was significant (P=0.007). No further significant relationships were found between organ TD and the male FDS at mid-anthesis. Similarly, no significant relationship was found between TD of the flag leaf or spike, and the female FDS score at mid-anthesis during Experiment 1. Table 2.20 shows the significance, gradient and intercept of the relationship between tissue temperature depression and the male FDS whilst Table 2.21 shows this relationship with regards to the female FDS.

Table 2.20 – Significance, gradient and intercept of the relationship between the pooled genotypic organ temperature depression and male FDS, at mid-anthesis, for the two temperature treatments applied in Experiment 1.

Organ	Temp. Treatment	Significance	ns,*,***	Gradient	Intercept
Flag Leaf	AT	0.260	ns	-0.225	0.268
Flag Leaf	HT	0.141	ns	-0.509	3.146
Spike	AT	0.007	*	-0.352	0.044
Spike	HT	0.245	ns	-0.354	2.113

Table 2.21 – Significance, gradient and intercept of the relationship between the pooled genotypic organ temperature depression and female FDS, at mid-anthesis, for the two temperature treatments applied in Experiment 1.

Organ	Temp. Treatment	Significance	ns,*,***	Gradient	Intercept
Flag Leaf	AT	0.722	ns	-0.129	0.131
Flag Leaf	HT	0.131	ns	-0.369	0.134
Spike	AT	0.896	ns	-0.092	1.841
Spike	HT	0.917	ns	0.069	0.886

At mid-anthesis, a significant relationship was identified between the FLTD under WW conditions and the FDS of both the male and female reproductive organs (P=0.002 and 0.005, respectively). As illustrated by Fig. 2.31A-B, greater FLTD

was observed for florets in the late stages of anthesis (FDS 4/G) compared to the early stages (FDS 1/HF). No further significant relationships were uncovered between organ TD and male or female FDS at mid-anthesis in the irrigation treatments applied during Experiment 1. Table 2.22 and 2.23 summarize these findings.

Table 2.22 – Significance, gradient and intercept of the relationship between the pooled genotypic organ temperature depression and male FDS, at mid-anthesis, for the two irrigation treatments applied in Experiment 1.

Organ	Irr. Treatment	Significance	ns,*,***	Gradient	Intercept
Flag Leaf	WW	0.002	*	0.625	0.084
Flag Leaf	WS	0.536	ns	-0.178	0.526
Spike	WW	0.236	ns	0.245	-0.010
Spike	WS	0.848	ns	0.048	-0.133

Table 2.23 – Significance, gradient and intercept of the relationship between the pooled genotypic organ temperature depression and female FDS, at mid-anthesis, for the two irrigation treatments applied in Experiment 1.

Organ	Irr. Treatment	Significance	ns,*,***	Gradient	Intercept
Flag Leaf	WW	0.005	*	1.357	-1.393
Flag Leaf	WS	0.789	ns	0.129	0.248
Spike	WW	0.525	ns	0.336	-0.635
Spike	WS	0.492	ns	0.316	-0.684

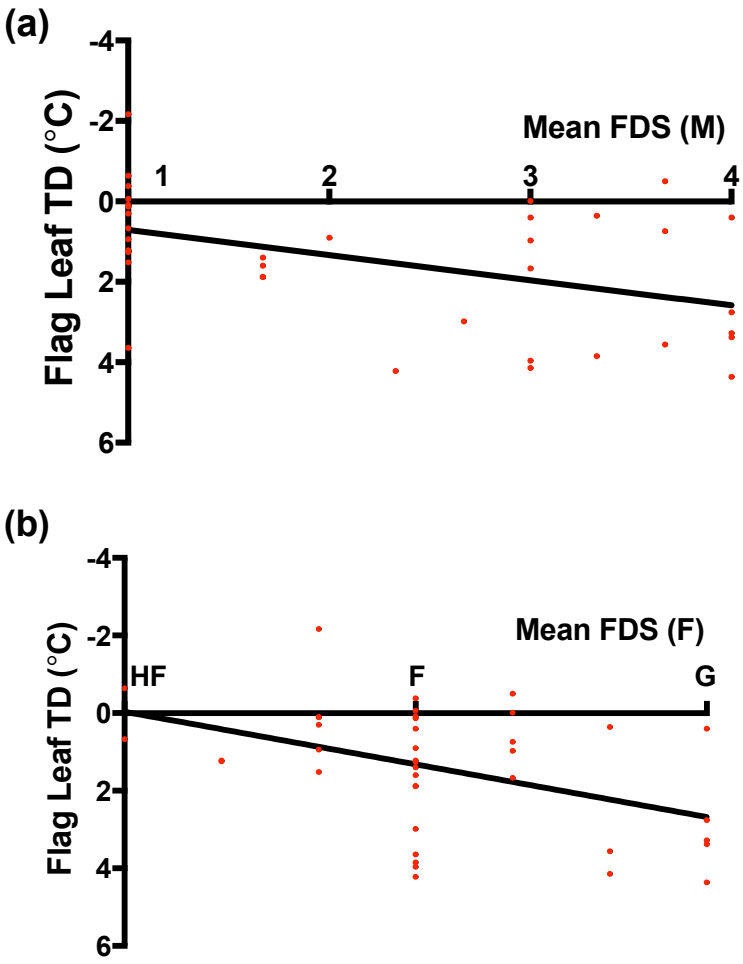

Fig. 2.31A-B – The relationship between mean floret development score of the (a) male florets and the (b) female florets with flag leaf temperature depression under WW conditions in Experiment 1.

When the interaction between the genotypes and two temperature treatments applied was examined at mid-anthesis, a statistically significant relationship between SB019 and the FDS of the male reproductive organ in the AT treatment was identified. Both FLTD and STD showed a significant relationship with the FDS of the anthers in the AT treatment (P=0.042 and 0.034, respectively). As anthesis advanced from the early stages (FDS 1/HF) to the latter stages (FDS 4/G), a reduction in the cooling of both the flag leaf and spike was observed in the AT treatment. Fig. 2.32A-B illustrates these significant relationships. In the HT treatment, no such relationship was observed. No significant relationships were identified for the genotypic interaction between organ TD and female FDS with the temperature treatment. Table 2.24 and 2.25 summarize these findings.

Table 2.24 – Significance, gradient and intercept of the relationship between flag leaf temperature depression and male FDS, for each genotype at mid-anthesis, for the two temperature treatments applied in Experiment 1.

Genotype	Temp. Treatment	Significance	ns,*,***	Gradient	Intercept
SB019	AT	0.042	*	-0.671	0.924
	HT	0.633	ns	-0.225	0.941
SB020	AT	0.751	ns	0.175	-1.573
	HT	0.548	ns	0.558	-1.623
SB155	AT	0.162	ns	0.242	-0.205
	HT	0.583	ns	1.265	-4.049
SB156	AT	0.478	ns	0.825	0.910
	HT	0.404	ns	-0.766	5.152
SB165	AT	0.095	ns	-0.964	2.087
	HT	0.103	ns	-1.534	3.687

Table 2.25 – Significance, gradient and intercept of the relationship between spike temperature depression and female FDS, for each genotype at mid-anthesis, for the two temperature treatments applied in Experiment 1.

Genotype	Temp. Treatment	Significance	ns,*,***	Gradient	Intercept
SB019	AT	0.034	*	-0.566	0.176
	HT	0.937	ns	-0.062	0.516
SB020	AT	0.736	ns	0.068	-1.510
	HT	0.565	ns	0.347	0.949
SB155	AT	0.148	ns	-0.327	0.306
	HT	0.784	ns	-0.339	1.200
SB156	AT	0.816	ns	0.123	-0.416
	HT	0.489	ns	-0.691	3.980
SB165	AT	0.058	ns	-1.000	1.600
	HT	0.282	ns	-0.948	3.380

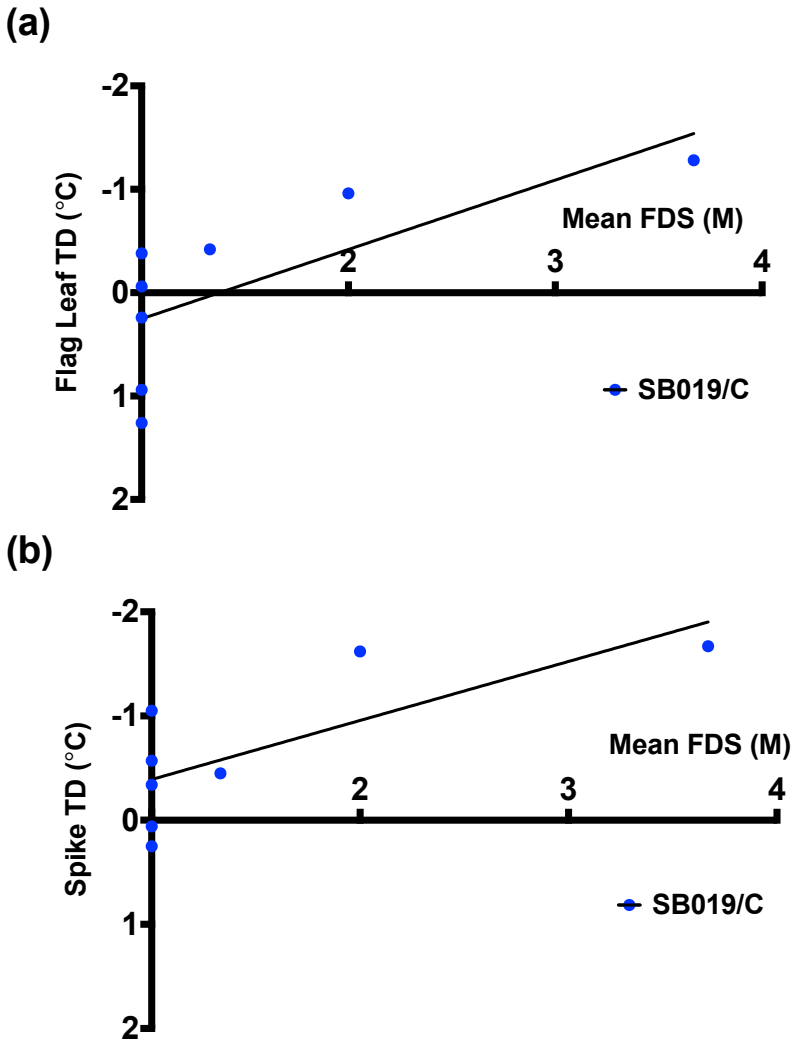

Fig. 2.32A-B – The relationship between mean floret development score of the male florets with (a) flag leaf temperature depression and (b) spike temperature depression in SB019 in the AT treatment during Experiment 1.

When the interaction between the genotypes and two irrigation treatments applied was examined at mid-anthesis, a statistically significant relationship between SB020 and the FDS of the male reproductive organ in the WW treatment was identified. The slope of the relationship between FLTD and both the male and female reproductive organs was statistically significant (P=0.026 and 0.017, respectively). This is illustrated by Fig. 2.33A-B and summarized by Table 2.26 and 2.27. Increased FLTD was observed at mid-anthesis in the latter stages of floral development (FDS 4/G) compared to the early stages (FDS 1). Similarly for STD, a significant relationship was found for both the male and female FDS (P=0.012 and 0.007, respectively). This is illustrated by Fig. 2.34A-B and summarized by Table

2.28 and 2.29. At the latter stages of floral development (FDS 4/G), increased STD was observed at mid-anthesis.

Table 2.26 – Significance, gradient and intercept of the relationship between flag leaf temperature depression and male FDS, for each genotype at mid-anthesis, for the two irrigation treatments applied in Experiment 1.

Genotype	Irr. Treatment	Significance	ns,*,***	Gradient	Intercept
SB019	WW	0.326	ns	0.509	-0.103
	WS	0.536	ns	-0.213	0.192
SB020	WW	0.026	*	1.436	-2.447
	WS	0.679	ns	0.487	-0.599
SB155	WW	0.188	ns	0.599	-0.518
	WS	0.926	ns	-0.035	-0.067
SB156	WW	0.181	ns	0.671	0.189
	WS	0.373	ns	-0.271	0.347
SB165	WW	0.303	ns	1.017	0.608
	WS	0.291	ns	-1.132	3.256

Table 2.27 – Significance, gradient and intercept of the relationship between spike temperature depression and male FDS, for each genotype at mid-anthesis, for the two irrigation treatments applied in Experiment 1.

Genotype	Irr. Treatment	Significance	ns,*,***	Gradient	Intercept
SB019	WW	0.116	ns	0.878	-1.078
	WS	0.946	ns	-0.048	-0.396
SB020	WW	0.012	*	1.179	-2.987
	WS	0.757	ns	0.238	-0.209
SB155	WW	0.427	ns	-0.181	0.443
	WS	0.764	ns	0.104	-0.589
SB156	WW	0.620	ns	0.353	0.012
	WS	0.115	ns	0.603	-0.746
SB165	WW	0.304	ns	1.803	-0.314
	WS	0.419	ns	-0.724	1.599

Table 2.28 – Significance, gradient and intercept of the relationship between flag leaf temperature depression and female FDS, for each genotype at mid-anthesis, for the two irrigation treatments applied in Experiment 1.

Genotype	Irr. Treatment	Significance	ns,*,***	Gradient	Intercept
SB019	WW	0.977	ns	-0.057	0.752
	WS	0.161	ns	-0.938	1.696
SB020	WW	0.017	*	3.517	-6.850
	WS	0.619	ns	0.918	-1.272
SB155	WW	0.084	ns	1.645	-2.553
	WS	0.847	ns	-0.202	0.327
SB156	WW	0.442	ns	0.853	0.034
	WS	0.317	ns	-0.514	0.726
SB165	WW	0.221	ns	1.810	-1.183
	WS	0.168	ns	-3.378	7.700

Table 2.29 – Significance, gradient and intercept of the relationship between spike temperature depression and female FDS, for each genotype at mid-anthesis, for the two irrigation treatments applied in Experiment 1.

Genotype	Irr. Treatment	Significance	ns,*,***	Gradient	Intercept
SB019	WW	0.820	ns	0.512	-0.777
	WS	0.399	ns	-1.224	2.179
SB020	WW	0.007	*	2.877	-6.580
	WS	0.664	ns	0.522	-0.691
SB155	WW	0.690	ns	0.205	-0.493
	WS	0.951	ns	-0.058	-0.152
SB156	WW	0.668	ns	-0.615	2.145
	WS	0.090	ns	1.086	-1.482
SB165	WW	0.997	ns	-0.005	1.322
	WS	0.266	ns	-2.339	4.547

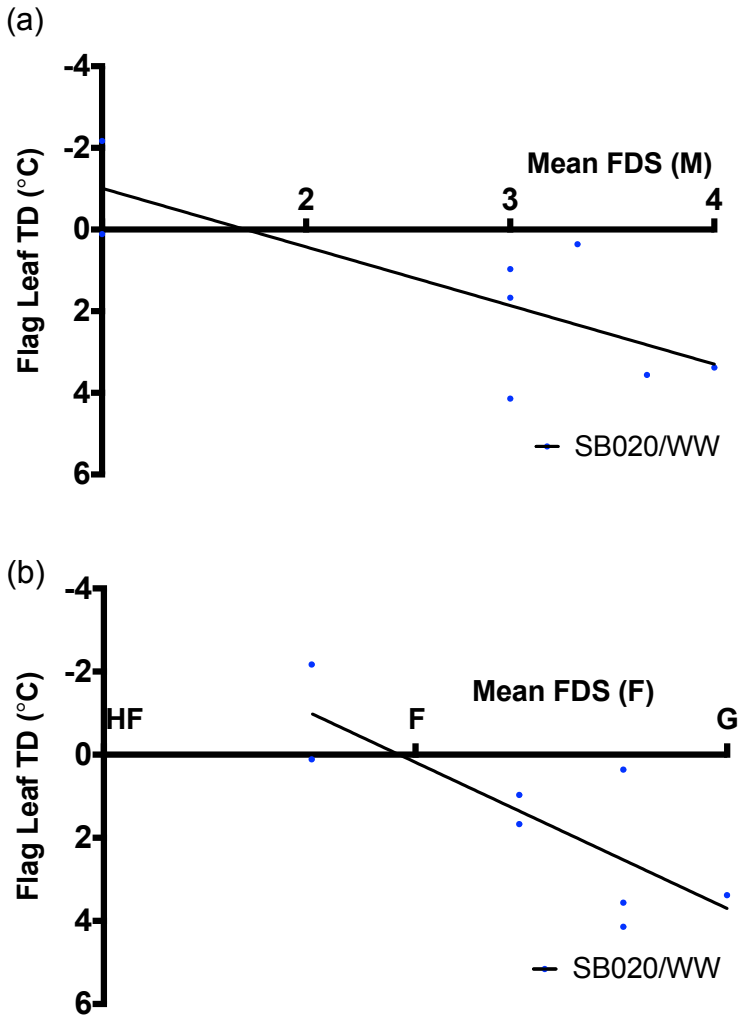

Fig. 2.33A-B – The relationship between flag leaf temperature depression at mid-anthesis and the mean floret development score of the (a) male florets and (b) female florets, in SB020 in the WW treatment during Experiment 1.

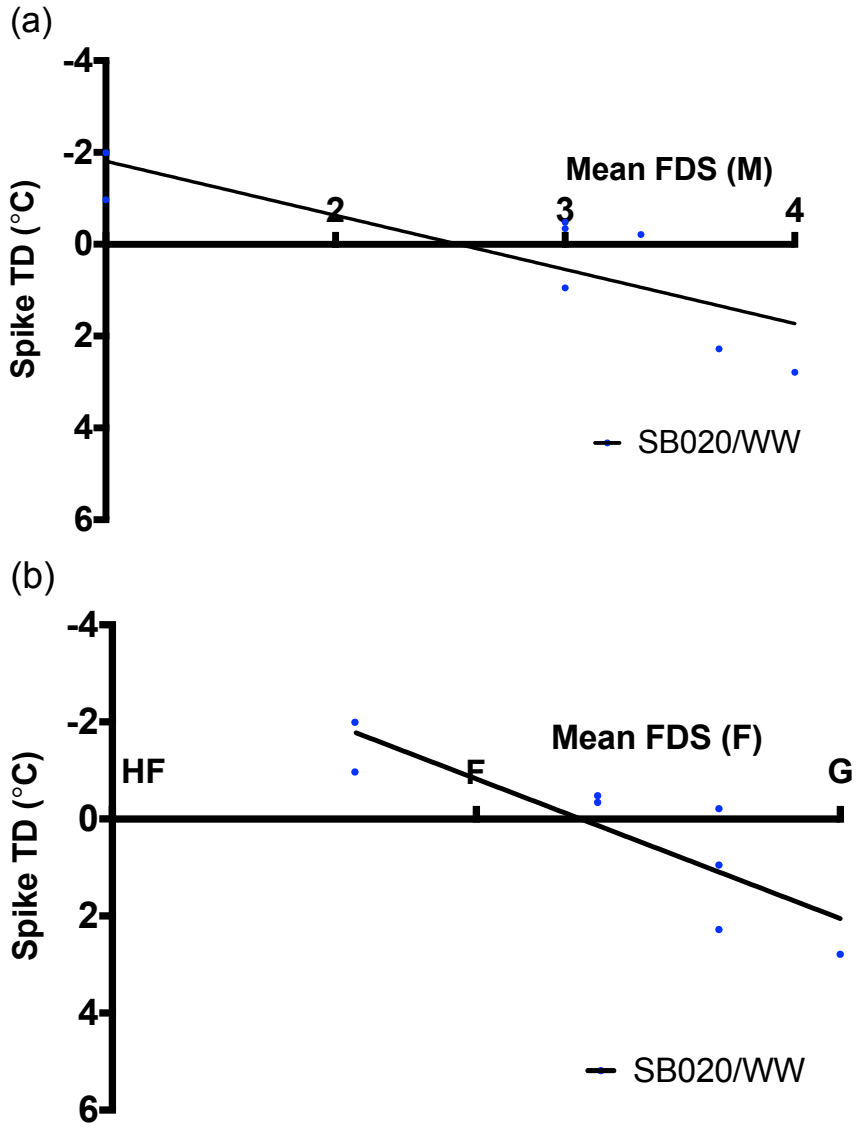

Fig. 2.34A-B – The relationship between spike temperature depression at mid-anthesis and the mean floret development score of the (a) male florets and (b) female florets, in SB020 in the WW treatment during Experiment 1.

2.4.7.2. Experiment 2

Neither the temperature, nor the irrigation treatments applied, resulted in a statistically significant slope between FLTD, or STD, and the FDS of either the male or female reproductive organs.

When categorized by genotype, the slope of the linear relationship between STD of SB165 and the corresponding FDS scores of the stigma was significant (P=0.029) (Fig. 2.35). As the FDS moved from the early stages of reproductive development FDS (HF) towards the latter stages FDS (G), STD was found to increase. No significant relationships between FLTD and the FDS of either the male or female organs was found in any genotype.

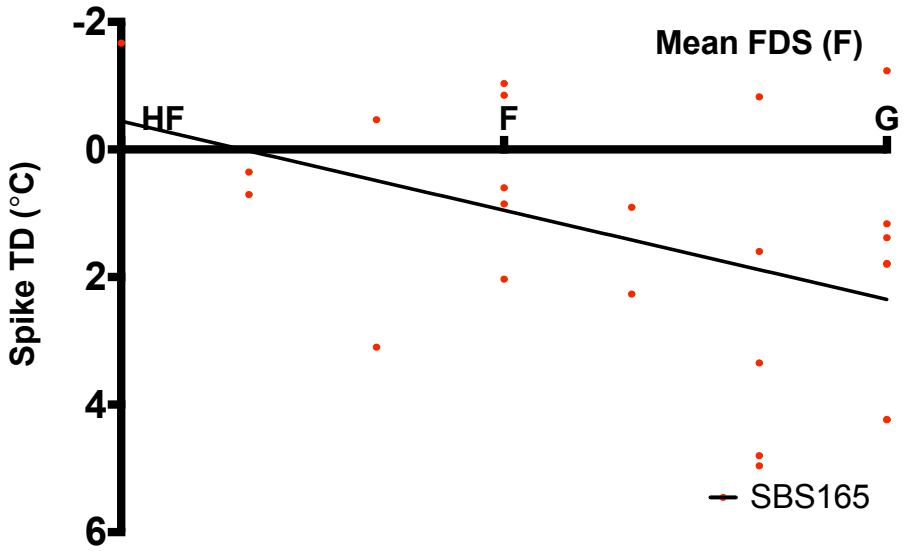

Fig. 2.35 – The pooled treatment relationship between mean floret development score of the female florets and spike temperature depression in SB165 during Experiment 2.

The slope of the linear relationship between FLTD and the FDS of the anthers of SB165 in the AT treatment was significant (P=0.025) (Fig. 2.36). In relation to the stigma, the only statistically significant relationship found was in FLTD of SB165 in the AT treatment (P=0.016) (Fig. 2.37). No significant relationships were found as a result of the interaction between genotypes and the irrigation treatments applied.

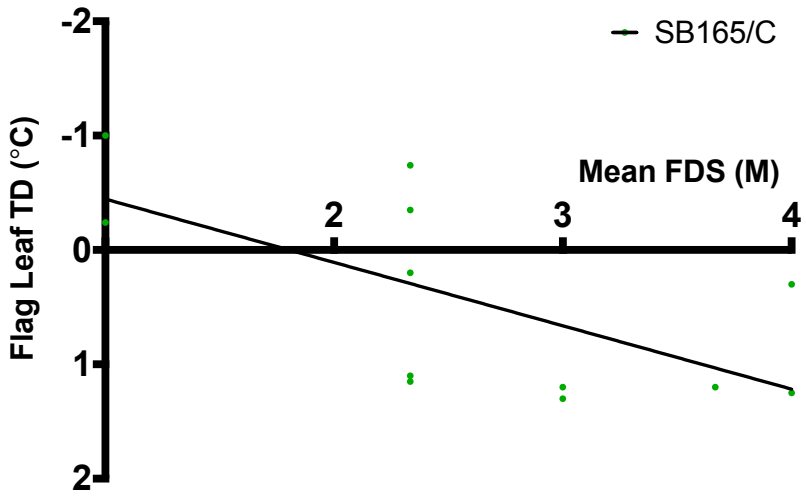

Fig. 2.36 – The relationship between mean floret development score of the male florets and flag leaf temperature depression of SB165 in the cool treatment during Experiment 2.

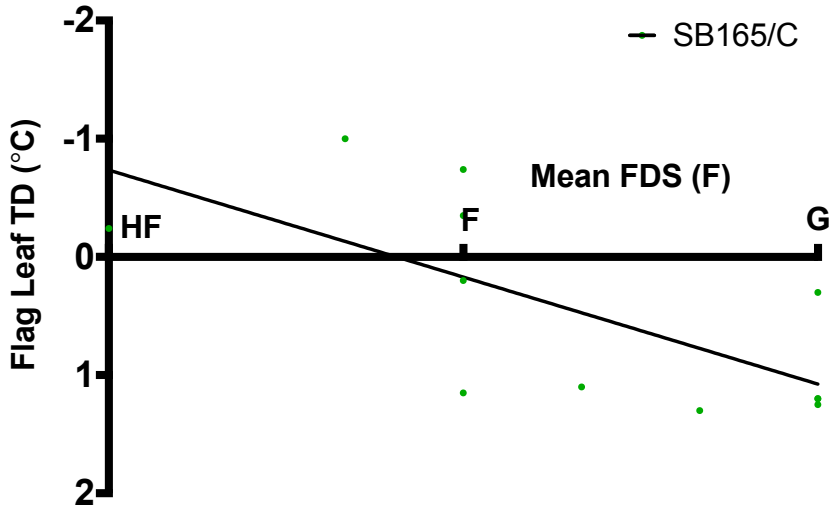

Fig. 2.37 – The relationship between mean floret development score of the female florets and flag leaf temperature depression of SB165 in the cool treatment during Experiment 2.

2.5. DISCUSSION

It was hypothesized that once ambient air temperature exceeds a critical thermal threshold during anthesis, a genotype with the ability to maintain lower tissue temperatures will limit the damage to the male and female reproductive organs and the processes contained therein. As a consequence, improved grain set and therefore yield may be expected. Previous work by Steinmeyer *et al*. (2013) hypothesized that the greatest STD coincides with FDS (3), the period of anthesis at which both the stigma and anthers are at their peak of flowering. Their results however did not support this and consequently, they suggested that it is the early stages of anthesis that are more likely to exhibit greater TD than the latter stages.

In both Experiments 1 and 2, a significant variation in FLTD between genotypes was found. With the difference in average FLTD measurements between the AT and the HT treatments ranging from less than 1°C to over 3°C, a significant, and differing cooling capacity of the plant tissue grown in a CE was demonstrated. The differing responses to the temperature treatments applied may have been due to the differences in pot density and distribution between Experiment 1 and 2. This may have impacted light penetration through the canopy as well as airflow, both of which could have altered the microclimate within the canopy. In line with the findings made by Steinmeyer *et al*. (2013), STD was found to significantly differ between genotypes in Experiment 1. The cooling capacity of the spike at anthesis was lower than that of the flag leaf in both experiments. The frequent observation of negative TD in Experiment 1, but not in Experiment 2, suggests that the physiological response to the treatments applied differed between the two years of experimentation. Similarly to the flag leaf, TD of the spike showed a decline from the start to the end of the observation period that may have been as a consequence of sustained growth in the CE chambers.

The use of CTD, a scaled-up form of FLTD that incorporates STD, has been well documented as a selection tool for heat and drought tolerance in wheat (e.g. Amani *et al.,* 1996; Pinto and Reynolds, 2015). Of similar significance was the finding that greater FLTD was recorded in the WW treatment compared to the WS treatment. Although STD was lower than FLTD, STD was similarly greater in the WW

treatment compared to the WS treatment. These findings are in line with those of other authors who have reported TD as an indicator of overall plant water status (Idso, *et al.*1981; Jackson *et al.,* 1981; Blum *et al.,* 1982).

Negative TD values occur when tissue temperature exceeds the ambient air temperature. The occurrence of negative TD values in wheat grown in growth chambers has previously been reported by Steinmeyer *et al*. (2013). The results from this experiment suggest that the tissue of both the flag leaf and spike are prone to reaching temperatures above that of ambient air temperature. The occurrence of negative STD was more frequent than negative FLTD. Three possible theories may be offered which explain the occurrence of negative TD. Firstly, TD in plants is a function of water availability for transpiration (Amani *et al.,* 1996). Siddique *et al*. (2000) hypothesized that increased leaf temperature may be due to increased respiration and decreased transpiration as a consequence of stomatal closing. The same authors also concluded that drought stress results in reduced RWC in wheat. Irrigation has been reported to reduce canopy temperature by over 10°C in field-scale experiments (Purushothaman and Krishnamurthy, 2014). Consequently, it can be inferred that the explanation of negative TD observations is insufficient transpiration from leaf tissue to match, or exceed, the heating of the plant tissue by the surrounding air. If the transpiration-pull exerted by the above ground biomass, in particular the spike, is insufficient to translocate enough water from the roots to the transpiring organ, said organ will be prone to warming. Radiative heating from the lights and the sides of the growth cabinet, along with conductive heating from the air, can then raise the temperature of the drying tissue to above ambient, thus effectively converting the tissue into a heat sink. Due to the spikes position relative to the canopy, water must travel a significant distance up the stem before it can be utilized by the spike. The issue of vertical and horizontal temperature gradients within growth cabinets has been raised by several authors in the past (Downs and Krizek, 1997; Chen *et al.,* 2013). This vertical temperature gradient has been reported to range from 0.1-0.4°C between the bottom and the middle of a growth cabinet (Hamasaki and Okada, 2000), although the extent of this gradient varies between growth chamber designs. No three-dimensional temperature monitoring took place during either of the experiments so it is not clear whether a temperature gradient existed within any of the growth cabinets. Data relating to root zone

heating was not collected in either Experiment 1 or 2. However, the pots were frequently handled and in the process it was found that pots receiving the WS treatment in both the AT and HT treatments were warmer than those receiving the WW treatment. In order to reduce heating of the root zone, a reflective cover could have been placed around each pot. It is advised that monitoring of root zone temperatures in each pot is performed in the future so that this data can be included as a covariate in analysis.

Secondly, the physiological evolution of the spike has taken place to cope with both abiotic and physical stresses. The period after anthesis, grain filling, often coincides with periods of high temperature and drought stress (Wardlaw, 2002; Kong *et al.,* 2013). The glumes of the spike have a greater ability to resist abiotic stress compared to the flag leaf by (1) maintaining a higher RWC under stressed conditions (Wardlaw, 2002), (2) displaying a greater WUE (Bort *et al.,* 1996) and (3) senescing at a slower rate than other organs (Bort *et al.,* 1996; Lopes *et al.,* 2006). The risk of late-season, high temperature stress (day-time temperatures exceeding 40°C) occurring during grain filling is predicted to increase (Kong *et al.,* 2013). The hypothesis tested, assumed that greater TD of both the spike and flag leaf was associated with higher grain yield. The results from both experiments however demonstrate that although average STD was often negative, grain still successfully formed. Successful seed set may however have been due to pollination occurring early on during the first day of observation, which would mean that stress experienced later may not affect the process of fertilization at all. It is also possible that the base temperature of the spike may operate at a higher level than that of the flag leaf. Negative STD may therefore be linked to differences in tissue composition as well as photosynthetic and respiratory differences between the spike and the leaf tissue. The complex geometry of the spike compared to the comparatively simple structure of the flag leaf may be partially responsible for higher tissue temperature observations as well.

Thirdly, the observation of negative TD values may be linked to the natural limitations posed by the design of the growth chamber. The temperature of plant tissue is governed by the transfer of thermal energy between the plant and its surrounding environment. In theory, this constant exchange of energy tends towards

a steady-state equilibrium, although in most real-world scenarios this rarely occurs. Most plant species do not absorb incoming radiation in the range of 700-1,500nm efficiently so the thermal energy transfer to the plant from this spectrum is low (Mellor *et al.,* 1964). Field-grown crops for example are exposed to very little radiation above 3,000nm due to the Earth's atmosphere absorbing most of the incoming far-infrared radiation (1,500-30,000nm). With the cool white fluorescent lights used in the growth chamber, minimal radiation is emitted outside of the visible spectral range so that the lights themselves essentially do not contribute to thermal loading of the plant tissue. A drawback of using growth cabinets in physiological experiments is the lack of turbulent air-flow to dissipate the heat in the boundary layer which exists between the leaf and the surrounding air. McCree (1984) found that when sorghum was grown in a growth cabinet with insufficient air flow (<0.1 m s^{-1}), tissue temperature was up to 16°C hotter than the air temperature. STD may be exacerbated due to the position of the spike on the plant relative to the flag leaf in the growth cabinet. The increased proximity to the light source at the top of the growth cabinets may result in unintended and unavoidable radiative heating of the spike versus the flag leaf. Similar to the temperature gradients discussed previously, a vertical light gradient exists in all growth chambers, the extent of which is dependent on the chambers design (CSS, 2002). In a field experiment, the difference in organ proximity is generally ignored e.g. it is accepted that when determining CTD, the spike tissue is unavoidably included in measurements as well.

During senescence, the plant will re-allocate phloem-mobile nutrients from source organs (stem and canopy) to the primary sink organs, the developing grains in the spike (Davies and Gan, 2012). The regulation of terminal plant senescence is a complex process of the interplay between numerous plant signalling phytohormones (Thomas and Stoddart, 1980) and is not initiated by the occurrence a single, short-lived elevated temperature stress event. There is currently no data that supports a theory of preferential water allocation, during a periodic high temperature stress event at anthesis to the spike over the flag leaf. It is therefore likely that water moving from the root zone into the above-ground portion of the plant, will be utilized by the organs it first reaches. In the case of wheat, this would be the canopy. It is only once signalling for terminal canopy senescence has begun, that the mass

movement of phloem-mobile nutrients towards the developing grains occurs en masse (Distelfeld *et al.,* 2014). Water that is now moving from the root zone through the plant cannot be utilized to the same extent by the closing canopy, which results in increased allocation of water-based phloem to the spike. This is supported by the results from both Experiments 1 and 2, in which increased TD is observed during the later stages of floret development and into the early stages of grain set.

The observation of significant correlations between organ TD and FDS was not consistent between years, or amongst genotypes and treatments. Several inherent limitations prevented more data from being collected during anthesis. For example, it was not possible to image individual plants continuously during reproductive development due to a lack of thermal imaging cameras with this capability. Increasing floret development stage assessments from once daily to hourly would have resulted in significant damage to the glumes which could have affected transpiration and yield parameters. Furthermore, hourly opening of the growth cabinet doors to perform additional assessments would have resulted in severe fluctuations of the air temperature within the growth chambers.

Increased organ TD was negatively correlated to grain yield in both the WW and WS irrigation treatments. When categorized by temperature treatment however, both experiments showed that increased TD results in increased grain yield. Experiment 2 however showed no significant correlation between increased STD/FLTD and increased grain yield in the HT treatment. From this work, it remains unclear whether increased STD in wheat during anthesis is in fact positive. Field-scale experimentation has repeatedly demonstrated that CTD, both under elevated temperature and water-deficit stress, is associated closely with increased yield (Amani *et al.,* 1996; Ayeneh *et al.,* 2002; Lopes and Reynolds, 2010; Pinto *et al.,* 2010; Karimizadeh and Mohammadi, 2011). However, as both temperature depression and yield in wheat has been closely linked to deep rooting (Lopes and Reynolds, 2010), it is likely that the inherent nature of a CE experiment may be masking the relationship between TD and yield in this case. To be valuable to the commercial wheat breeding community, a secondary screening trait has to be consistently reliable and demonstrate a yield benefit to justify the financial outlay associated with trait screening. The absence of a clear and consistent correlation

between TD and grain yield at mid-anthesis suggests that the current methodology of TD examination in a CE is inconsistent and lacks the strong correlations associated with yield which are found in field-scale CTD assessment.

Several authors have reported yields from CE experiments that are several times greater than yields commonly encountered under field conditions (Polinskii and Lisovskii, 1980; Tollenaar and Migus, 1984). The reporting of high dry matter accumulation in a CE may be attributed to several factors including low replication due to a small sample size, optimal water and nutrient supply, a highly uniform distribution of diffuse radiation through the canopy as well as an absence of biotic and abiotic stress. Concurrently, very little quantitative data is available detailing the yield penalty associated with the addition of abiotic stress to a crop grown in a CE.

Although significant differences in RWC were identified between the genotypes as well as the irrigation treatments applied, the lack of any significant correlation between RWC and grain yield suggests that RWC may not be a suitable screening tool in a CE. Contrary to the findings of others (Larbi, 2004), the results do not support the conclusion that RWC, as measured in a CE experiment, is a valuable analytical selection tool for grain yield in wheat under drought stress. Although rapid and relatively inexpensive to perform, the lack of correlation between RWC and grain yield in this experiment leads to the conclusion that the assessment of RWC should be limited to field-scale experiments.

Although high chlorophyll content may have provided an evolutionary advantage to a wild plant by capturing as much sunlight as possible, high chlorophyll content in plant tissue may result in photo-chemical damage (Zhu *et al.,* 2010). In a cropping situation where productivity per unit area instead of per plant is important, a high tissue chlorophyll content is undesirable (Donald and Hamblin, 1976). This is in part due to the resource investment required to produce chlorophyll. Due to the overlapping structure of the canopy, individual wheat leaves operate at levels well below light saturation which negates the need for high chlorophyll content. Reduced chlorophyll content in the upper canopy allows for increased light transmission to the lower leaves, which results in more efficient light capture by the plant. Reduced

light capture in the upper portions of the plant will reduce heat stress on the upper organs, so less water is required for evapotranspirational cooling and more can be allocated to grain filling. Finally, as chloroplasts are extremely nutrient dense, there is a trade-off between the synthesis of chlorophyll and the availability of photoassimilates for grain filling.

The flag leaf's significantly greater chlorophyll concentration over the glumes is in line with previously reported findings (Lu and Lu, 2004). With approximately half the chlorophyll concentration of the flag leaf, it can be inferred that the photosynthetic activity of the glumes is considerably lower than the flag leaf. This confirms reported findings by others (Khanna-Chopra and Sinah, 1981). The potential to increase spike photosynthesis is large (Tambussi *et al.,* 2007; Parry *et al.,* 2011) and these results indicate that the photosynthetic potential of the spike may currently be underestimated. Photosynthetic rate of the whole spike was not known as data from the awns was not collected. Chlorophyll concentration of the flag leaf and glume varied between Experiment 1 and 2. A possible explanation for this is that due to the reduction in overall pot numbers used in the growth cabinets during Experiment 2, the effect of changes in temperature gradients, airflow and light saturation within the canopy may have impacted the pot temperature as well.

2.6. CONCLUSION

FLTD was found to be consistently greater than STD in Experiment 1 and 2. The inconsistent relationships identified between organ TD and the FDS of the anther and stigma did not provide concrete elucidation of how they are related.

The frequent observation of negative TD was most probably a result of growing plants at high temperatures in closed growth chambers. Similarly, RWC has been widely used as an indicative trait of grain yields in the past. The absence of a correlation between high RWC of the flag leaf and grain yield suggests that the plants may have encountered abiotic stress conditions which deviate significantly from those traditionally encountered in field-grown wheat. The data infers that the proxy photosynthetic potential of the glumes was lower than that of the flag leaf. Consequently, the glumes represent a large potential site at which photosynthesis can be improved.

Although improved grain yields have been widely reported to be associated with cooler canopies under heat and drought stress, the data from this work provided conflicting views of this relationship. When breeders examine the merits of a potentially new and novel breeding trait, the association of the trait to improved yields is paramount in considering its adoption into an early generation selection breeding program. Previous work, which has examined the relationship between CTD and yield has taken place exclusively in the field. The numerous conflicting relationships identified between the two years of experimentation question the practical applicability of conducting these types of physiological assessments in a CE growth chamber.

In order for a novel screening tool to be considered viable to cereal breeders, the trait examined must have high heredity, the assessment of the trait must provide a clear yield benefit, be both reliable and replicable, as well as inexpensive and rapid to use. To the authors best knowledge, commercial wheat breeders are not publicly pursuing STD as a phenotyping tool. CTD is the closest comparable trait used in commercial breeding programs. If STD fails to demonstrate a yield benefit in field-grown wheat, it is likely that it will not be adopted in crop breeding programs in the

future. It appears that STD, similar to FLTD, at anthesis is an artefact of evapotranspirational cooling. However, only a very limited number of genotypes have been investigated. Along with the wild progenitors of wheat, it is possible that other modern genotypes may produce alternative results. The spike is an extremely complex organ, vital to the production of grain. It has however historically received less attention than other organs, such as the flag leaf and peduncle for instance, and therefore warrants further, detailed examination.

CHAPTER 3

TEMPERATURE DEPRESSION IN THE FIELD AND IN RHIZOBOXES

3.1. CHAPTER SUMMARY

The development of novel screening tools for heat and drought tolerance at anthesis may assist in the development of stress resilient wheat varieties. A significant cooling capacity of the wheat spike has been reported at anthesis. Spike temperature depression (STD), as a potentially useful screening tool, has recently been examined in controlled environments (CE). However it is unclear whether the STD potential of freely rooting plants is associated with the temperature-sensitive floret development stage (FDS) of the reproductive organs and grain yield (GY). Two experiments were conducted to explore these relationships. A field experiment was conducted between 2013-2014 at Sonning Farm (UoR, UK) in which winter wheat (*cvs*. Cadenza) was grown in plots covered by polytunnels (Experiment 3). A second experiment utilized two lines of spring wheat (*cvs*. Seri-Babax) in rhizoboxes to observe root behaviour (Experiment 4). In both experiments, two levels of temperature and drought stress were applied to coincide with anthesis. In Experiment 3, FDS was assessed using a binary scoring system that categorized the complex process of anthesis as either 'active flowering' or 'not flowering'. A detailed assessment of FDS was made in Experiment 4. STD and flag leaf temperature depression (FLTD) were measured using a hand-held infrared camera. Canopy temperature depression (CTD) was assessed using a hand-held infrared thermometer. FDS and organ TD were recorded daily in a three-hour window around solar noon. The polytunnels used in Experiment 3 failed to significantly stress the plants for a sustained period at anthesis and no GY differences were detected between treatments. The rhizoboxes used in Experiment 4 were significantly better at applying stress. No significant differences in rooting depth were found in Experiment 3 but differences in rooting at depth were identified in Experiment 4. No relationship between STD/CTD and active flowering was identified in Experiment 3. No significant relationship between organ TD and GY was identified in either Experiment 3 or 4. Conducting field-scale evaluation of tissue temperature depression is possible in the UK but it comes with an inherent

risk of failure. The rhizoboxes used were a suitable hybrid solution to assessing root growth in a controlled environment, although the financial cost associated with them is considerable versus a field-scale experiment.

3.2. CHAPTER INTRODUCTION

3.2.1. Justification of Study

In recent years, changes in global and regional weather phenomena have caused concern for the long-term stability of our climate (IPCC, 2014). Climate models are predicting an increase in both the frequency and severity of previously rare climatic phenomenon, such as flooding, drought, cold and heat (IPCC, 2014). Although studies have identified that some northern regions above 55°N may in fact benefit from climate change (Ewert *et al.,* 2005), areas that are currently marginal for crop production will suffer disproportionately from the combined effects of climate change (Parry *et al.,* 2004; Gregory *et al.,* 2005; Sivakumar *et al.,* 2005). As 40% of the Earth's land surface is used for dry land agriculture, the impacts from climate change are likely to be detrimental to many societies in the future.

Several authors have successfully linked deep rooting to canopy temperature depression (CTD) in wheat (Reynolds *et al.,* 2007; Lopes and Reynolds, 2010; Pinto and Reynolds, 2015). However, no work has previously examined the relationship between deep rooting in wheat and spike temperature depression (STD) at anthesis. The potential to accurately quantify STD in growth cabinets has been demonstrated by Steinmeyer *et al*. (2013). The relevance of results obtained from controlled environment (CE) experiments to field-grown crops has been questioned. The ability to examine highly adaptive and heritable traits that are likely to form a significant part in future wheat breeding efforts, such as organ temperature depression (TD) in a CE, but without constraining the below ground plant biomass, would be significant. The relationship between CTD and STD must also be examined in a field setting. A two pronged approach, consisting of novel CE and field-scale experiments, is likely to be the most productive when attempting to unravel the complexities of rooting morphology and organ TD in wheat.

3.2.2. The Root System of Wheat

The wheat root system is a fibrous network of roots, consisting of both primary and secondary roots. Primary roots (also called seminal or seed-borne roots) are the first roots to appear after germination, whereas the formation of secondary roots (known as adventitious or crown roots) is associated with the onset of tillering. Gregory *et al*. (1978) found that winter wheat cultivars have on average six seminal roots. There are typically three to five seminal roots that emerge at the epiblast and scutellar nodes of the germinating caryopsis of spring wheat (O'Brien, 1979; Manschadi *et al.,* 2006), which then grow laterally through the soil profile, to form the seminal roots system. The adventitious root system develops subsequently to the seminal root system, by emerging from the basal nodes of the main tillers (Klepper, 1984). The average root radius in wheat plants is between 0.07 and 0.15cm. Previous work looking into roots found that those with a smaller radius have higher respiration rates, suggesting that different types of root may perform different functions for the plant (Makita *et al.,* 2009). As the above ground leaf area expands during vegetative growth so does the root system in parallel below ground which, typically, ceases at the point of anthesis (Gregory, 2005; Palta and Watt, 2009). A significant amount of variation in root architecture has been reported among species (Kutschera, 1960; Fitter and Stickland, 1992), and also within species (McPhee, 2005; Manschadi *et al.,* 2006).

The depth of wheat roots varies greatly between soil types and varieties cultivated. Insufficient water at depth as a result of little rainfall, along with any chemical and physical limitations of the soil, can place restrictions on rooting depth (Barraclough and Weir, 1988; Bengough and Mullins, 1990; Pierret *et al.,* 1999). Typically, the horizontal distribution of the roots occurs between 30-60cm (Weaver, 1926; Entz *et al.,* 1992; Zhang *et al.,* 2004; Manschadi *et al.,* 2006). For spring wheat, maximum rooting depths at maturity have been reported between 1.0-1.3m (Entz *et al.,* 1992). In cases where soil moisture is inadequate to meet the plants requirements, the root system may well grow into the lower soil horizons (Mishra *et al.,* 1999). Deep rooting in wheat has been reported by several authors, with the roots system reaching over 2.0m in depth (Zhang *et al.,* 2004; Kirkegaard and Lilley, 2007).

These deep roots typically are packed into biopores and have a far lower density than roots higher up in the soil structure (Passioura, 1991).

As a result of the interaction between the large spatial and temporal variability of moisture and nutrients within the soil, variation in the abiotic environment and the genetic characteristics of wheat, there is a high degree of phenoplasticity in wheat roots (Malamy, 2005). In response to changing environmental conditions, roots exhibit both morphological and phenological plasticity by altering their distribution through the soil (Drew *et al.,* 1973; Feddes and Raats, 2004; Benlloch-Gonzalez *et al.,* 2014a; Benlloch-Gonzalez *et al.,* 2014b). In order to adapt crops to a changing environment in the future, it is crucial to understand how the structure and dynamics of the root system affect key physiological plant processes.

3.2.3. Rooting Depth and Organ Temperature Depression

In order to meet the challenges posed by climate change, breeding programmes have examined adaptive traits that can mitigate the threats posed by high temperatures and drought. Wheat genotypes that invest a significant amount of resources into the root network are able to extract residual soil moisture at depth, even under surface drought conditions (Reynolds *et al.,* 2007). In heat stressed environments, a high vapour pressure deficit increases evaporative transpiration in plants, which in turn results in the cooling of plant tissue through increased stomatal conductance (Amani *et al.,* 1996). Previous work by Lopes and Reynolds (2010) found the cool canopies are associated with increased water availability as a result of deep rooting. Genotypic variation in the transpiration rates at differing VPDs has however been reported in soybean (Sadok and Sinclair, 2009). Genotypes exhibiting this response may therefore conserve soil water during periods of drought, in anticipation of a water-deficit event, which in turn results in sustained plant productivity (Sinclair *et al.* 2010).

With specific focus on CE experiments, many artefacts associated with CE and pot-grown plants do not allow for reliable extrapolation to the field level. Shoot and root growth are highly dependent on one another and a reduced uptake capacity from the roots leads to a reduction in shoot biomass accumulation (Hammer *et al.,* 2009). Pot

size has been found to impact plants both physically and morphologically. Carmi *et al*. (1983) found that restricting root growth by using small pots resulted in depressed shoot growth. Fiscus *et al.* (2007) identified lower yields in pot grown soybean versus soybean grown in the field. The differences observed between plants grown in small pots and plants grown in the field is as a result of the restrictions placed on the root environment affecting, in particular, the function, growth and morphology of the roots (NeSmith and Duval, 1998). Pooter *et al*. (2012) found that doubling the pot size a plant was grown in resulted in a 43% increase in yield and linked this to a reduction in the photosynthetic rate of plants grown in smaller pots. The differing responses observed varied been crops but may also vary between cultivars of the same crop. Passioura (2006) concluded that in addition to the volume limitations of a pot, hypoxia and inadequate soil-water relationships are commonly found. Furthermore, several authors have identified extreme temperature variation in the region of 2-6°C between the air temperature and the temperature in the centre of the pot (Passioura, 2006; Fiscus *et al.,* 2007).

CE experiments are essential to developing and understanding novel physiological traits for identifying novel phenotypes and consequently unlocking the genetic potential of a crop. The ease of experimentation and the ability to analyze key physiological traits in a non-destructive manor is of great benefit. As stressed by Passioura (2006), the limitations of a CE need to be considered during the interpretation of results and any extrapolation of these findings to the field.

Although field-based experimentation results in a conditions most similar to those experienced by commercial crops, the limitations of using this approach over a CE protocol include a lack of control of the environment (temperature, airflow, irrigation), irregular soil structure/composition across plots, competition from non-crop species (weeds, pest, disease) and a significant financial outlay in order to conduct an experiment. Rhizoboxes provide a cost effective, simple and accurate alternative opportunity to observe below ground processes, whilst conducting a non-destructive, CE experiment.

3.2.4. Chapter Objectives

The objectives of this chapter were therefore to:

a) Successfully measure spike/canopy temperature depression at anthesis of field-grown wheat in the UK;
b) Assess the relationship between spike/canopy temperature depression at anthesis and deep rooting in field-grown wheat in the UK;
c) Assess the relationship between spike/flag leaf temperature depression at anthesis and rooting depth in rhizobox grown wheat;
d) Examine the relationship between spike/flag leaf /canopy temperature depression at anthesis and the grain yield of field- and rhizobox-grown wheat.

3.3. MATERIALS AND METHODOLOGY

3.3.1. Experiment 3

3.3.1.1. Crop Husbandry

In order to evaluate the relationship between CTD and STD at anthesis, rooting depth and floret development stage, a field-based, polytunnel experiment was conducted at the University of Readings' Sonning Farm during 2013-2014. The plant material used was Cadenza winter wheat (*Triticum aestivum* L.).

The soil in the polytunnels was classified as loamy, coarse and stone less sand, over non-calcareous river terrace gravel deposits. The free-draining soil had a pH of 5.6-6.2 and a low organic matter content of 1.4% in the top 30cm of soil. Available phosphorus, potassium and magnesium in the soil were 86.4, 207 and 66mg/L, respectively. In order to prepare the soil for the experiment, 69kg/ha of KCl was applied to the plots on September 13th 2013, and then ploughed to a depth of 30cm before being power harrowed. Sowing took place on October 2nd 2013 in plots measuring 1.9m in width and 18.0 m in length. A Hege 80 plot drill mounted on a Hege 76 tool carrier was used to sow the plots at a seed rate of 250 seeds/m^2. A discard border of dwarf perennial ryegrass was sown around the plots at a rate of 24kg/ha. Emergence counts were conducted 44 DAS and a 76% emergence rate was found (190 plants/m^2). A standard crop protection schedule was in place with a full rate application (4L/ha) of pendimethalin/flufenacet taking place 56 DAS as an autumn herbicide. This was followed by an application of a broadleaf herbicide at 162 DAS in the form of a metsulfron-methyl/thifensulfuron-methyl mix (125g/ha). A fungicide consisting of epoxiconazole and chlorothalonil, at 1L and 2L mixed in 220L of water respectively, was then applied at 169 DAS. Finally, an application of nitrogen (100kg/ha) and ammonium nitrate (40kg/ha) was applied as a Double Top dressing 168 DAS. A polytunnel frame was constructed around each plot, over which the polytunnel plastic could be stretched. Each polytunnel measured 20mx3m. This in turn meant that each irrigation regime plot measured 5.5m in length. With 1.75m at each end of the irrigation regime plots serving as a discard zone, the total size of the plot remaining measured 2.0x1.9m. Of this 3.8m^2 plot,

only $1m^2$ in the centre was used for crop measurements. This creation of large discard zones between plots ensured that crop measurements taken of the plants within the $1m^2$ plots were not affected by treatments applied to neighbouring plots. Between 178 and 184 DAS, a polythene cover was attached to the frame to complete the polytunnels.

3.3.1.2. Temperature and Irrigation Treatments

The experiment was designed to be a complete randomized design with a two-way factorial on whole plots with three replicates. Each polytunnel acted as a whole plot, which had two possible treatment levels:

i: Wheat cultivar
ii: Temperature treatment
    a) Ambient temperature (AT)
    b) Elevated temperature (HT)

Each polytunnel was further split into sections relating to soil moisture content:

iii: Irrigation treatment
    a) Full irrigation supplied throughout the lifecycle (WW)
    b) Restricted irrigation simulate drought at anthesis (WS)
    c) A hybrid intermediate between WS and WW at anthesis (Hybrid)

The application of the temperature treatment to the polytunnels could take place within a few hours of the desired stress period, as all that this required was turning on the heating units that supplied the hot air to the tunnels. The irrigation treatments however required soil moisture management several weeks in advance in order to create conditions of water-deficit stress that coincided with anthesis. Since the polytunnel acted as a rainout shelter, the three treatment levels outlined above were created by adjusting the amount of irrigation water available to each split polytunnel section. For the WS plots, irrigation was withheld approximately one month before anticipated anthesis (197 DAS). For the plots receiving the WS treatment, irrigation was withheld for a four week period before the start of anthesis so that the available

water content during this period fell to 25% of field capacity (FC) at anthesis. For the WW plots, irrigation was maintained at the full irrigation rate so that the available water content was at 75% of FC. From the end of anthesis to harvest, full irrigation was supplied to all treatment plots so that the available water was at 75% FC. Authors have previously examined the effect of drought stress on crops and have used 25% and 75% of the FC as benchmarks for drought stress (Emam *et al.* 2010; Medeiros *et al*. 2012).

A timeline illustrating when, in Experiment 3, the elevated temperature and water-deficit stress treatments around the period of anthesis were applied, can be found in the Appendix (see Fig. 3.26).

Elevated temperature stress was applied during anthesis (231-234 DAS) by using a single 72kW indirect flue heater for each polytunnel receiving the elevated temperature treatment. The heating units were located outside the polytunnels in order to avoid the heaters affecting the composition of the air inside the polytunnels. Because of both financial, labour and time restrictions, the only wheat cultivar used in this experiment was Cadenza. Both AT and HT treatments were used but the 'Hybrid' irrigation treatment was excluded. The two irrigation treatments that were used were WW and WS. Consequently, plants were exposed to four distinct treatments combinations at anthesis, namely [AT+WW], [AT+WS], [HT+WW] and [HT+WS].

3.3.1.3. Spike Temperature Determination Protocol

Prior to anthesis, twenty spikes in each treatment block were tagged for IR imaging during anthesis in order to measure STD. Care was taken not to select damaged spikes, spikes that stood out for any reason compared to the others in the plot or spikes that were located within 20cm of the plot margins. Infrared (IR) imaging took place using a hand-held FLIR infrared camera (FLIR T350), the same used for tissue temperature determination in Chapter 2.3.2. Images were recorded daily during anthesis between 12.00h and 14.00h. Although it was accepted that airflow within the tunnel would be more turbulent than in the growth cabinets used in

Experiment 1 and 2, care was taken to ensure that IR image capture did not take place if strong gusts of wind entered the polytunnel.

3.3.1.4. Canopy Temperature Determination Protocol

During anthesis, CTD was assessed daily between 12.00h and 14.00h for each of the plots utilized. CTD was measured using a hand-held infrared thermometer (IRT) (GM900, Benetech GMC, Shenzhen, China) according to the guidelines outlined in Pask *et al.,* (2012).

3.3.1.5. Active Flowering Determination Protocol

After IR imaging of the tagged spikes in each plot had been completed, the spikes were scored to determine the stage of floret development they were in. The scoring system utilized was a simplified adaptation of the one outlined in Chapter 2.3.3. The advantages of utilizing this simplified scoring method over the more detailed methods outlined in Chapter 2.3.3 were manifold and included considerations relating to timing of the measurements, damaging the sensitive glumes of the wheat cultivar used, reducing the time the polytunnels were exposed to the cold outside air and minimizing damage to the experimental plots. The system used was a binary system whose aim was to determine whether or not the centrally located spikelets were actively flowering. When the examined florets were found to be in the FDS F3 phases (as outlined in Chapter 2.3.3) spikes were considered to be actively flowering and assigned the scoring code ‘Y’. When the florets of a spike were found to be in a developmental phase prior to, or post, FDS F3, they were assigned the scoring code ‘N’.

3.3.1.6. Root Depth Assessment

Root coring took place at 240 DAS. A 80.0mm diameter root corer was used to extract a single core from the centre of each plot used. The corer removed vertical cores of soil in 15.0cm sections, down to a depth of 60.0cm. The cores were washed and sieved using a 1.4mm mesh before being stored in sealed plastic bags ready for analysis. Roots were stored in a dark room at 5.0°C for no more than 48h prior to

analysis. Washed roots were laid out in a thin film of water in a clear plastic tray before being scanned using a flatbed scanner (Epson Perfection V500, Shinjuku, Tokyo). The scanned images were then analyzed for root length, root surface area and root volume using WinRhizo (Regent Instruments Inc., Canada).

3.3.1.7. Morphological Trait Assessment

The above ground biomass of all tagged spikes was harvested for yield analysis at 292 DAS. The plants were harvested by cutting the shoots at the crown and transferring them into a sealed cooler box in an attempt to remove the field heat as quickly as possible. Once in a clean laboratory environment, a range of morphological data relating to the plants was recorded. This included flag leaf length, spike length, stem height and pith diameter.

3.3.1.8. Yield Assessment

After morphological trait analysis had been completed, the plants were individually packed in paper bags and placed in an 80°C oven for 72 hours. Once dry, yield variables were recorded. These included spike weight, grain weight, and chaff weight and stem biomass.

3.3.1.9. Statistical Analysis

Analysis of the IR images was carried out using FLIR Quick Report 1.2 SP1 (FLIR Systems, Oregon, USA). REML, ANOVA and correlation analysis was performed using Genstat Version 16 (VSN International, Hemel Hempstead, UK). Separate plots within polytunnels were considered independent replicates. Effects were considered to be significant at $P<0.05$.

3.3.2. Experiment 4

3.3.2.1. Crop Husbandry

Two recombinant inbred lines of Mexican spring wheat were studied in controlled environment (CE) conditions at the Plant Environment Laboratory (UoR, UK). The plant material originated from a reciprocal crossing of two related parent lines, namely 'Seri M82' (IWIS CODE (Fox *et al* 1996), selection history: M31 IBWSN S-1 MXI96-97) and 'Babax' (IWIS CODE (Fox *et al* 1996), selection history: CM92066-J-0Y-0M-0Y-4M-0Y-0MEX-48BBB-0Y). Both are considered to be highly adapted semi-dwarf lines (Waddington, 1986), with Babax being highly tolerant to severe drought whereas Seri M82 is moderately susceptible to severe drought (Pfeiffer, 1988). Known as SB, this cross is used widely for phenotyping studies in heat and drought stress environments. SB has a relatively short period of flowering between 10 and 15 days, making it ideal for this type of work (Olivares-Villegas *et al.,* 2007). The SB lines used in this study were SB020 and SB165. The selection was based on differing flowering phenology and contrasting yield performance from Experiment 1 and 2.

3.3.2.2. Rhizobox Design

The rhizoboxes used were of similar design to those outlined by Liao *et al*. (2006) and Palta *et al*. (2007). They were constructed out of 5mm thick polyvinyl chloride and were 1.0m tall, 0.3m wide and 0.15m deep, with the front wall of the rhizobox being made out of 5mm thick clear-as-glass acrylic. The soil used to fill the boxes was a well-homogenized sandy loam used for all-purpose garden landscaping and was representative of many commonly encountered agricultural soils in the UK. The soil was sieved through a fine 2mm mesh, before 2kg of Osmocote slow release granules (The Scotts Company, Marysville, OH) containing $N:P_20_5:K_2O$:MgO (15:11:13:2 ratio) was added and thoroughly mixed in. The osmocote content averaged 83g per rhizobox. Three holes were drilled into the base of the boxes to allow for excess irrigation to escape and prevent waterlogging of the box. A 2cm layer of gravel was placed at the base of all boxes before they were filled with soil in order to facilitate easier drainage and prevent waterlogging. The soil was then

packed into the boxes at a bulk density of 1.2g/cm^3. The boxes were thoroughly watered until saturation and left to drain freely for 24h before sowing. Five seeds were sown at uniform intervals against the glass wall of the rhizobox at a depth of approximately 2cm. Once successfully germinated, the plants were thinned out to leave two plants in each box. There were three replicates per genotype x environment (GxE) interaction resulting in a total of 24 rhizoboxes. The rhizoboxes were placed in a glasshouse located at the Plant Environment Laboratory (UoR, UK) and held up on steel supports at a 30° angle, with 0.1m between the boxes. In order to protect the soil from adverse temperature exposure from the sun, the rhizoboxes were wrapped in an adjustable reflective sleeve made of a high temperature resistant insulation material. The covered clear-acrylic section of the boxes was oriented towards the north side of the glasshouse in order to minimize the effect of the southern aspect (prolonged sunlight exposure and higher temperatures).

3.3.2.3. Temperature and Irrigation Treatments

The glasshouse in which the plants were grown was naturally lit and all air-vents were left wide open. This ensured that the internal temperature of the glasshouse would stay close to the ambient external temperature and minimize the effect of the glasshouse on daily internal temperature fluctuations. Thermistors inside the glasshouse reported day/night temperatures averaging 19°C/18°C during vegetative growth. The daily photoperiod during the growth cycle was 14h. Soil water content of each box was not monitored during the experiment but a weekly visual inspection of the soil profile was made to assess moisture distribution within the box and identify visual discrepancies in soil moisture between the rhizoboxes. Plants were exposed to two possible temperature and irrigation treatments at anthesis:

i. Temperature treatment
    a) Ambient glasshouse temperature (AT)
    b) Elevated temperature of 35°C between 11.00h and 14.00h (HT)

ii. Irrigation treatment
    a) Full irrigation supplied throughout the lifecycle (WW)
    b) Restricted irrigation to simulate water-deficit stress at anthesis (WS)

Consequently, plants were exposed to four distinct treatments combinations at anthesis, namely [AT+WW], [AT+WS], [HT+WW] and [HT+WS].

The heat stress tents (HST) used was specially constructed within the glasshouse in order to apply the HT treatment at anthesis. It was constructed from the same polythene cover material used to construct the polytunnels in Experiment 3 and measured 8 x 3 x 2.2m. An incorporation of two sub dividers within the tent created three separate chambers within the HST. These were labelled HST 1, HST 2 and HST 3. A perforated, expandable polythene tube ran along the top of the HST in order to allow hot air to be pumped into each subdivided unit. An indirect fuel heater then fed hot, dry and clean air into the HST when required. Fig. 3.1 (see Appendix) is an illustration of the glasshouse and heat stress tents used during Experiment 4. Fig. 3.2 and 3.3 (see Appendix) show what the heat stress tents looked like in the glasshouse.

Up to 10 days before the estimated anthesis date, all rhizoboxes received identical irrigation consisting of careful hand watering every third day. 0.5L of water was supplied to each rhizobox during each of these irrigation applications. This ensured that the water content of the soil in the boxes was close to field capacity and it avoided overwatering. 10 days prior to the estimated onset of anthesis, rhizoboxes in the WS treatment stopped receiving water. This gradual drying out period, allowed the soil water content to decrease via evaporative, transpirational and gravitational water loss. Rhizoboxes in the WW treatment continued to receive irrigation every third day up to anthesis. At the onset of anthesis, the top 20cm of soil in the WS treatment boxes was visibly drier than those receiving the WW treatment. During anthesis, irrigation was withheld to all boxes so that the drying out process of the boxes between, and within, the two treatments was uniform and that no sudden increase in water availability took place between individual boxes. 24 hours after the end of anthesis full irrigation resumed to all the rhizoboxes. During the three weeks prior to harvest, all boxes received 50% reduced irrigation rates until irrigation was withheld entirely one week before harvest.

A timeline illustrating when, in Experiment 4, the elevated temperature and water-deficit stress treatments around the period of anthesis were applied, can be found in the Appendix (see Fig. 3.27).

3.3.2.4. Thermal Imaging Assessment Protocol

A detailed description of the methodology used to capture thermal images can be found in Chapter 2.3.2.

3.3.2.5 Floret Development Stage Determination Protocol

A detailed description of the methodology used to assess floret development stage can be found in Chapter 2.3.3.

3.3.2.6. Root Mapping

At seven-day intervals, starting at the one-leaf stage and ending one day before the onset of anthesis, the growth of the root system was traced through the clear-acrylic wall of the rhizoboxes. At each tracing, the reflective sleeve covering the clear wall was removed and replaced with a transparent acetate film. The visible weekly root growth was then traced onto the transparent film using a fine, waterproof permanent marker pen. A different colour marker pen was used every week to distinguish between the newly grown and pre-existing roots. After the roots had been traced, the transparent film was removed and the reflective sleeve was replaced. Once the final root tracing was completed, the transparent film used was cut into 0.1m horizontal sections and scanned using the Cannon LiDE 220 (Tokyo, Japan). The scanned images were subsequently analyzed using WinRhizo image analysis software (Regent Instruments Inc., Canada).

3.3.2.7. Tissue Sample Collection Protocol

Tissue samples of the glumes, flag leaf and peduncle were harvested at mid-anthesis (GS65) within three hours of the onset of daylight. The samples were flash frozen in liquid nitrogen and placed in 15ml Falcon tubes before being stored at -80˚C in a

freezer until analysis. The samples were analyzed at the Lancaster Environment Centre (LU, UK). In order to ensure standardized sample collection, the same guidelines for sample collection from Chapter 2.3.6 were adhered to.

3.3.2.8. Yield Assessment

Harvest of the above ground biomass took place once grain filling was completed and the kernel was sufficiently hard (GS92). All samples were oven dried at 80°C for 72h prior to assessments being made. The yield variables recorded included spike weight, grain weight, grain number, spike chaff weight and stem weight.

3.3.2.9. Statistical Analysis

Analysis of the IR images was carried out using FLIR Quick Report 1.2 SP1 (FLIR Systems, Oregon, USA). REML, ANOVA, correlation and regression analysis were performed using Genstat version 16 (VSN International, Hemel Hempstead, UK). Separate boxes within HST were considered independent replicates. Effects were considered to be significant at $P<0.05$.

3.4. **RESULTS**

3.4.1. Experiment 3

3.4.1.1. Polytunnel Temperature

Both the external polytunnel temperature (EPT) and internal polytunnel temperature (IPT) was recorded using iButtons (Maxim Integrated, California, USA) during anthesis. The external relative humidity (ERH) was recorded by the same methodology. The results below summarize the average temperature and relative humidity recorded over a three-hour period at solar noon (between 11.00 and 14.00), the period during which IR imaging took place.

3.4.1.1.1. External Polytunnel Temperature and Relative Humidity

Significant differences in the average EPT were identified between Days 1 and 4 (P=0.001). The highest average EPT recorded was on Day 2 (23.1°C), with Day 1 being slightly cooler (21.1°C) and Day 3 and 4 being the coolest days (15.88 and 14.9°C, respectively) (Table 3.1). The change in EPT of approximately 8.7°C from Day 2 to Day 4 was due to a weather front moving over the polytunnels which brought wind, rain and hail with it. The ERH was comparably high on Day 1 and Day 3 (94.1 and 94.0%, respectively) and comparably low on Day 2 and Day 4 (59.4 and 65.8%, respectively). On Day 3, heavy rain and winds increased the ERH significantly from the day before (an increase of 34.6%). Fig. 3.4 illustrates the average ERT and ERH between 11.00h and 14.00 on each day.

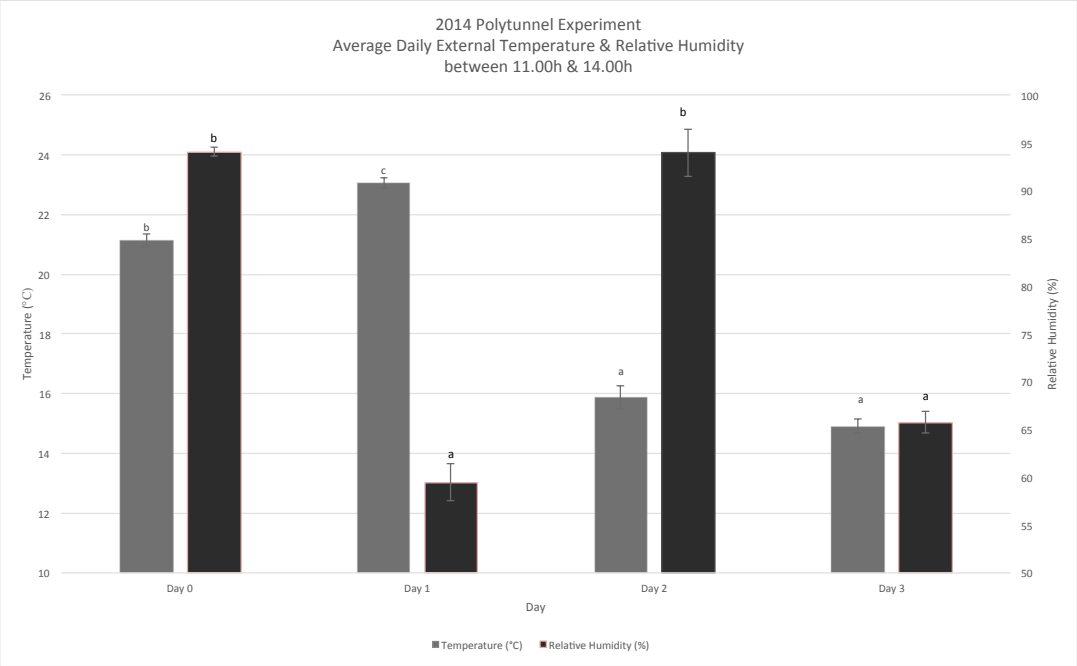

Figure 3.4 – The average external air temperature (light) and relative humidity (dark), recorded between the hours of 11.00h and 14.00h for Days 0 to 3. Bars represent standard error. Different letters above bars indicate significant differences (*P*<0.05).

Table 3.1 – Average external temperature and relative humidity recorded between 11.00h and 14.00h on all four days of observation during Experiment 3. Values in brackets represent standard error.

Day	Temperature (°C)	Relative Humidity (%)
1	21.1 (0.2)	94.1 (0.5)
2	23.1 (0.2)	59.4 (1.9)
3	15.9 (0.4)	94.0 (2.5)
4	14.9 (0.3)	65.8 (1.2)

3.4.1.1.2. Internal Polytunnel Temperature

Significant differences in the average IPT between the AT and HT was identified on all four days (*P*=0.014). The IPT recorded in the AT treatment was lower than that of the HT treatment on each day. The greatest difference daily IPT between the two temperature treatments was on Day 2 (7.6°C) and the smallest on Day 4 (3.7°C). The average IPT of the AT (15.5°C) and HT (19.2°C) treatments on Day 4, was

significantly lower than those on any other day over the four-day observation period (Table 3.2).

Table 3.2 – Average internal polytunnel temperature for the AT and HT treatments, recorded between 11.00h and 14.00h on all four days of observation during Experiment 3. Values in brackets represent standard error.

Day	Temp. Treatment	Temperature (°C)
1	AT	17.7 (0.2)
	HT	21.5 (0.2)
2	AT	24.6 (0.2)
	HT	32.2 (0.3)
3	AT	17.0 (0.3)
	HT	22.0 (0.5)
4	AT	15.5 (0.2)
	HT	19.2 (0.4)

Fig 3.5 illustrates the average IPT for each polytunnel used, on each day of observation. Despite the presence of heaters in the HT polytunnel, the large volume of cold, humid air on Day 3 and 4 prevented the heaters from successfully raising the IPT of the tunnels to above 25.0°C. The inclement weather on Day 3 and 4 reduced the heating capacity to the point that the average internal temperature of the HT polytunnel fell from its peak on Day 2 (32.2°C) to 19.2°C, a drop of approximately 13.0°C. The inability of the heaters in the HT polytunnels to maintain an IPT of above 25.0°C meant that there was only one day of true elevated temperature stress applied to the plants during anthesis (Day 2).

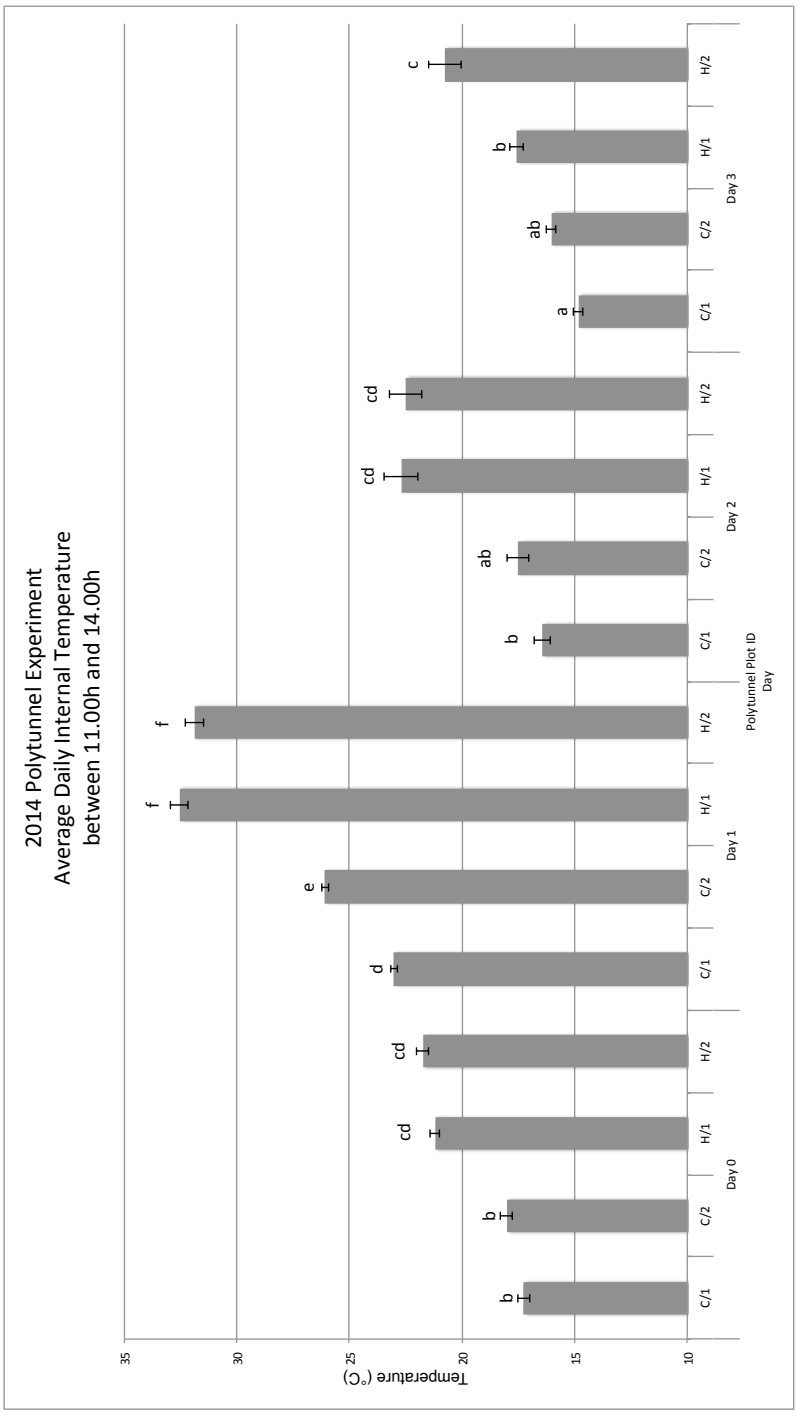

Figure 3.5 – The internal polytunnel temperature of each plot, recorded between the hours of 11.00h and 14.00h for Days 0 to 3. Bars represent standard error. Different letters above bars indicate significant differences (*P*<0.05).

3.4.1.2. Yield Data

No significant differences in the average spike weight were identified between the WW and WS irrigation treatments (P=0.479) or the AT and HT temperature treatments (P=0.867). No significant differences in the grain weight/spike were identified between the WW and WS irrigation treatments (P=0.448) or the AT and HT temperature treatments (P=0.851). No significant differences in the stem weight/plant were identified between the WW and WS irrigation treatments (P=0.662) or the AT and HT temperature treatments (P=0.944). Fig. 3.6 illustrates these results.

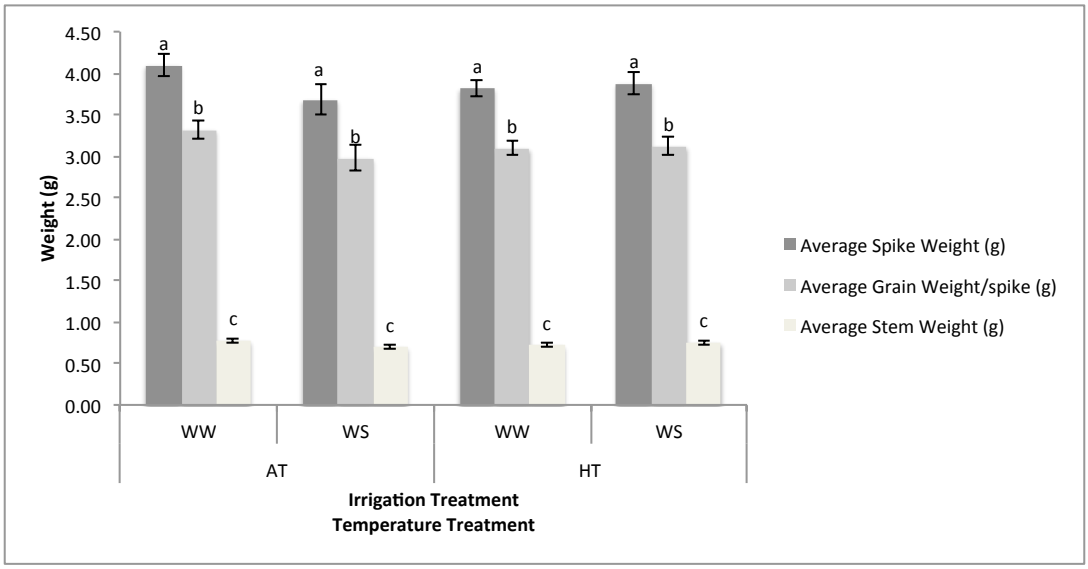

Fig. 3.6 – Average spike, grain and stem weight recorded in each of the four treatment combinations applied during Experiment 3. Error bars indicate standard error.

3.4.1.3. Morphological Data

No significant differences in flag leaf length were identified as a result of the two temperature (P=0.604) or irrigation treatments (P=0.959) applied. No interaction between temperature and irrigation on flag leaf length was found (P=0.915). No significant differences in flag leaf length were discovered between trial plots (P=0.098). No significant differences in spike length were identified as a result of the two temperature (P=0.448) or irrigation treatments (P=0.478) applied. No

interaction between temperature and irrigation on spike length was found (P=0.308). No significant differences were discovered in spike length between trial plots (P=0.104). No significant differences in stem height were identified as a result of the two temperature (P=0.433) or irrigation treatments (P=0.972) applied. No interaction between temperature and irrigation on stem height was found (P=0.304). Significant differences in stem height were only identified between plots (P<0.001) 8 (88.2cm) and 2 (74.5cm). No significant differences in pith diameter were identified as a result of the two temperature (P=0.672) or irrigation treatments (P=0.212) applied. No interaction between temperature and irrigation on pith diameter was found (P=0.506). No significant differences in pith diameter were discovered between trial plots (P=0.108).

3.4.1.4. Root Data

3.4.1.4.1. Root Dry Weight

Fig. 3.7 shows the average root dry weight (RDW) from the plots of the four treatment combinations applied. Significant differences in the RDW existed between the horizons (P<0.001) but no differences were found between the treatment plots (P=0.425). In the 0-15cm horizon, the only significant difference in RDW was for the HT+WS treatment, in which the RDW was significantly lower than that of the other three treatments (0.08g). In the 15-30, 30-45 and the 45-60cm horizon, no significant differences in the RDW were identified. A decline in the RDW from the 0-15cm horizon to the 45-60cm horizon was found.

3.4.1.4.2. Root Length, Surface Area and Volume

No significant differences in the total root length, total root surface area and the total root volume were identified within each soil horizon of each plot (P=0.241). There was a negative trend in all the variables relating to root phenotype from the 0-15cm horizon to the 45-60cm horizon. No single set of trial plots differed significantly enough so that one might conclude that rooting depth varied significantly between the trial plots. Fig. 3.7 illustrates the change in average root dry weight for each of the four temperature/irrigation treatment combination.

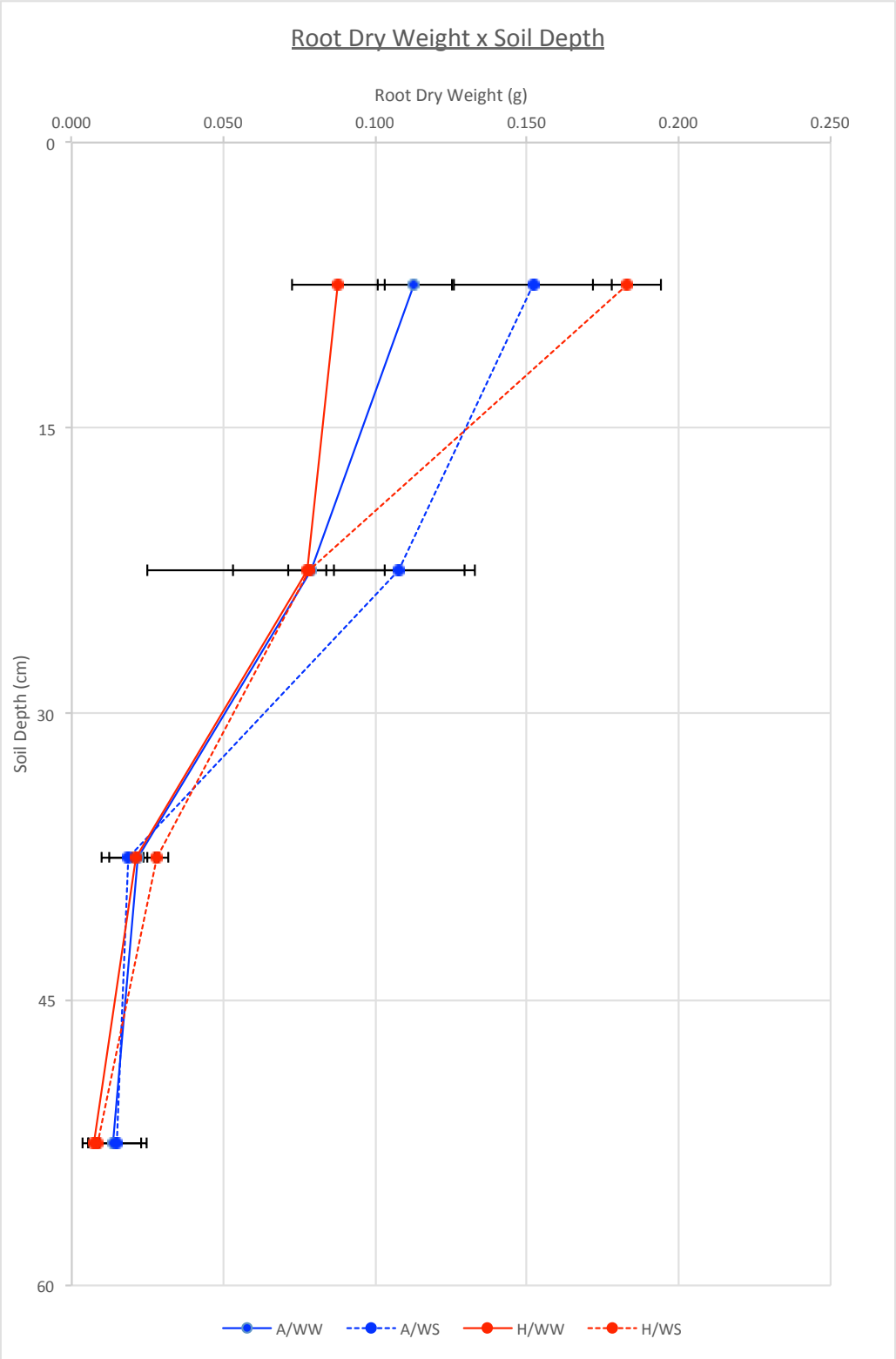

Fig. 3.7 – The average root dry weight at 0-15cm, 15-30cm, 30-45cm and 45-60cm depth, for each of the temperature and irrigation treatment combinations applied during Experiment 3. Error bars represent standard error.

3.4.1.5. Canopy Temperature Depression

Fig. 3.8 illustrates the daily average CTD for the two temperature treatments applied at anthesis. When categorized by temperature treatment, significant daily differences in CTD between the AT and HT treatments were identified ($P<0.001$). CTD was significantly greater for the HT treatment than the AT treatment on all days ($P<0.001$). The largest canopy cooling capacity was observed in the HT treatment on Day 2 (7.9°C). The difference in CTD between the two temperature treatments was greatest on Day 2 (5.5°C) and smallest on Day 4 (0.9°C). At no point was CTD found to be negative i.e. tissue temperature remained below ambient air temperature during all four days of observation.

Fig. 3.9 illustrates the daily average CTD for the two irrigation treatments applied at anthesis. When CTD was categorized by irrigation treatments on individual days, a weaker contrast between the two treatments was found than when categorized by temperature treatment. The two differing irrigation treatments were not found to significantly affect CTD over the four days of observation ($P=0.725$), although on individual days CTD differed significantly between the irrigation treatments ($P<0.001$). On Days 1, 2 and 4, CTD was greater in the WW treatment compared to the WS treatment. On Day 3 CTD was comparable for both irrigation treatments applied. Similarly to when categorized by temperature treatment, it was on Day 2 that the largest difference in CTD between the WW and the WS treatments was observed (5.7 and 4.8°C, respectively).

Table 3.3 summarizes the daily average CTD for each temperature and irrigation treatment combination. CTD differed significantly depending between days ($P<0.001$), with the largest differences between combined treatments being observed on Day 2. On all four days, CTD in the HT treatment was significantly greater than in the AT treatment ($P<0.001$). The greatest CTD was recorded on Day 2 in the HT treatment, for both the WW and the WS irrigation treatments (8.5 and 7.4°C, respectively). The lowest CTD was recorded on Day 1 in the AT+WW and the AT+WS treatment combinations (0.3 and 0.2°C, respectively) and on Day 3 in the AT+WS treatment combination (0.2°C).

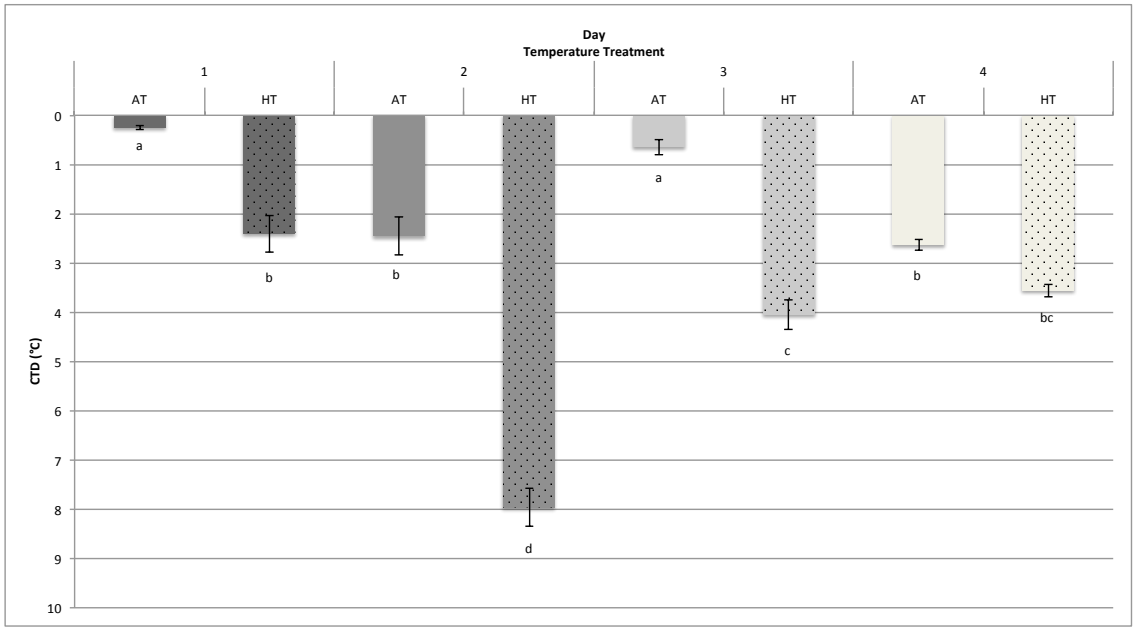

Fig. 3.8 – Average CTD on each day of observation, categorized for the two temperature treatments applied in Experiment 3. Error bars represent standard error. Letters indicate significant difference at *P*<0.05.

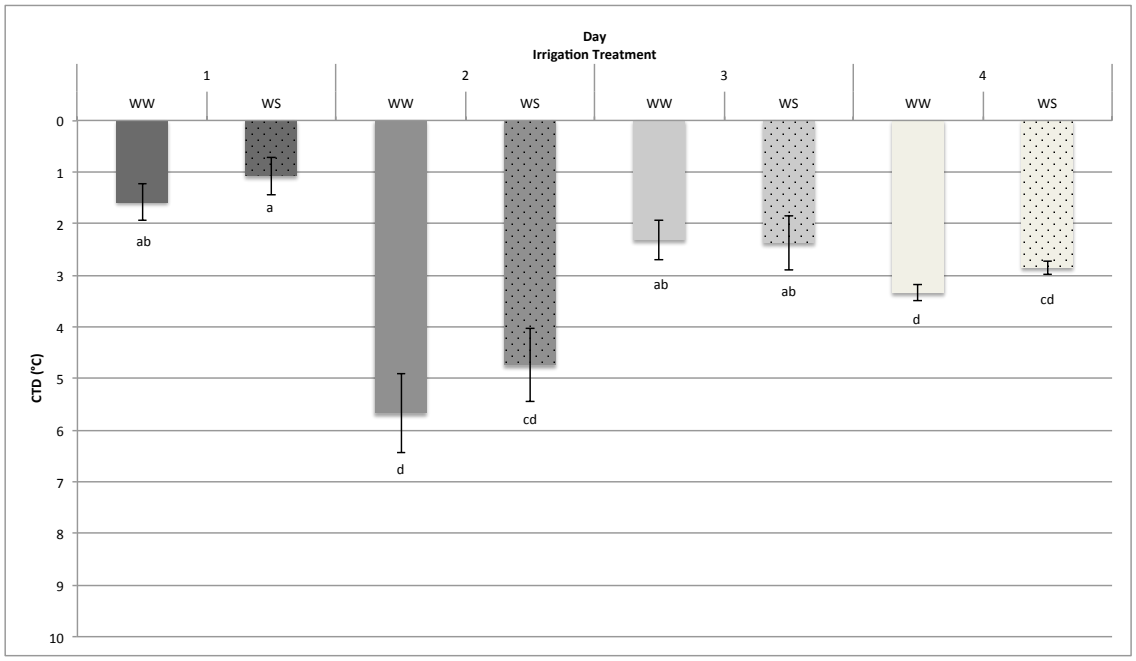

Fig. 3.9 – Average CTD on each day of observation, categorized for the two irrigation treatments applied in Experiment 3. Error bars represent standard error. Letters indicate significant difference at *P*<0.05.

Table 3.3 – Average CTD on all four days of observation, categorized for all four temperature and irrigation treatment combinations in Experiment 3. Values in brackets represent standard error.

Day	Temperature Treatment	Irrigation Treatment	CTD (°C) (±se)
1	AT	WW	0.3 (0.1)
		WS	0.2 (0.0)
	HT	WW	2.9 (0.4)
		WS	1.9 (0.6)
2	AT	WW	2.9 (0.7)
		WS	2.1 (0.4)
	HT	WW	8.5 (0.5)
		WS	7.4 (0.6)
3	AT	WW	1.1 (0.1)
		WS	0.2 (0.2)
	HT	WW	3.6 (0.5)
		WS	4.5 (0.3)
4	AT	WW	2.9 (0.1)
		WS	2.4 (0.1)
	HT	WW	3.8 (0.2)
		WS	3.4 (0.1)

3.4.1.6. Spike Temperature Depression

Fig. 3.10 illustrates the daily average STD for the two temperature treatments applied at anthesis. STD was not significantly different between the two temperature treatments applied (P=0.191). Significant differences in STD were however recorded between days (P<0.001) with the average STD being greater on Day 2 (2.9°C) compared to on Day 1 (1.5°C), Day 3 (2.2°C) or Day 4 (1.3°C). The largest difference in STD between the two temperature treatments, 2.19°C, was observed on Day 3. When contrasted to CTD, the cooling capacity of the spike was significantly smaller than the canopy. In the HT temperature treatment on Day 2, CTD was approximately 5.0°C greater than the spike. On Day 4, the final day of anthesis, the average cooling capacity of the spike for both the AT and HT treatments was 1.3°C. In contrast, the cooling capacity of the canopy was found to be 3.1°C.

Fig. 3.11 illustrates the daily average STD for the two irrigation treatments applied at anthesis. No significant differences in STD were identified as a result of the two

irrigation treatments applied (P=0.965). Significant differences in STD were however identified between irrigation treatments on Day 2 and 3 (P=0.040). On Day 2, STD was greater in the WW treatment (3.3°C) compared to the WS treatment (2.6°C). The following day however, on Day 3, STD was greater in the WS treatment (2.5°C) than in the WW treatment (1.9°C). On the final day of observation, STD was comparably low when categorized into the two irrigation treatments.

Table 3.4 summarizes the daily average STD for each temperature and irrigation treatment combination. In contrast to CTD, the combined effect of HT+WS did not result in reduced STD values on all days. On Day 3 for instance, the combination of HT+WS resulted in greater STD (3.9°C) than the HT+WW treatment combination (2.6°C).

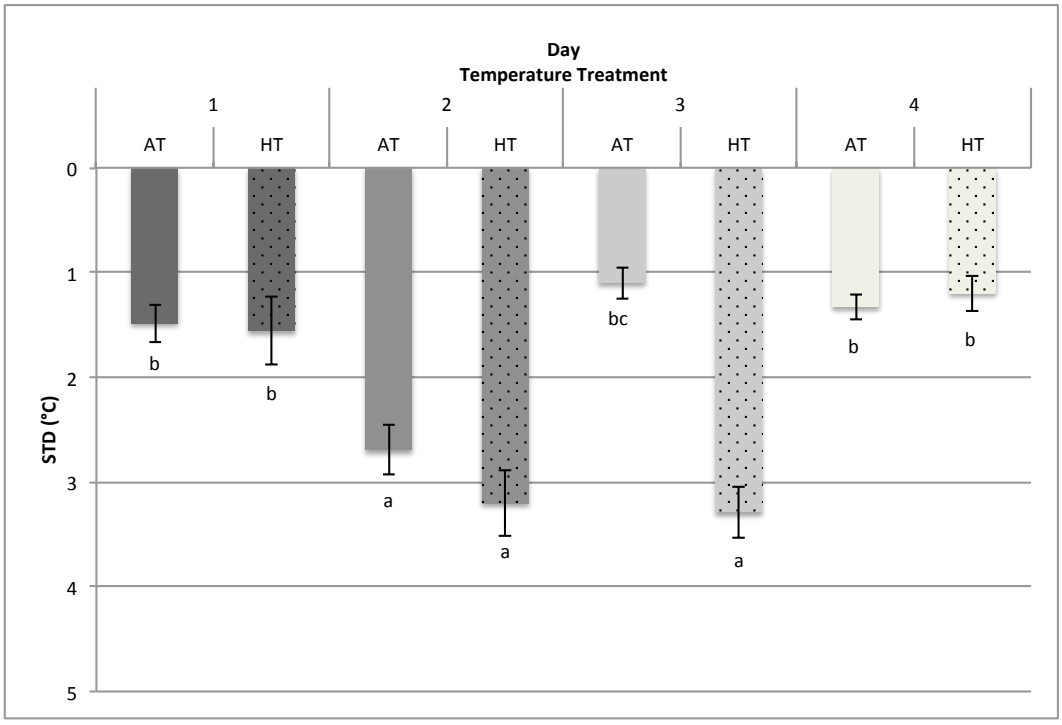

Fig. 3.10 – Average STD on each day of observation, categorized for the two temperature treatments applied in Experiment 3. Error bars represent standard error. Letters indicate significant difference at P<0.05.

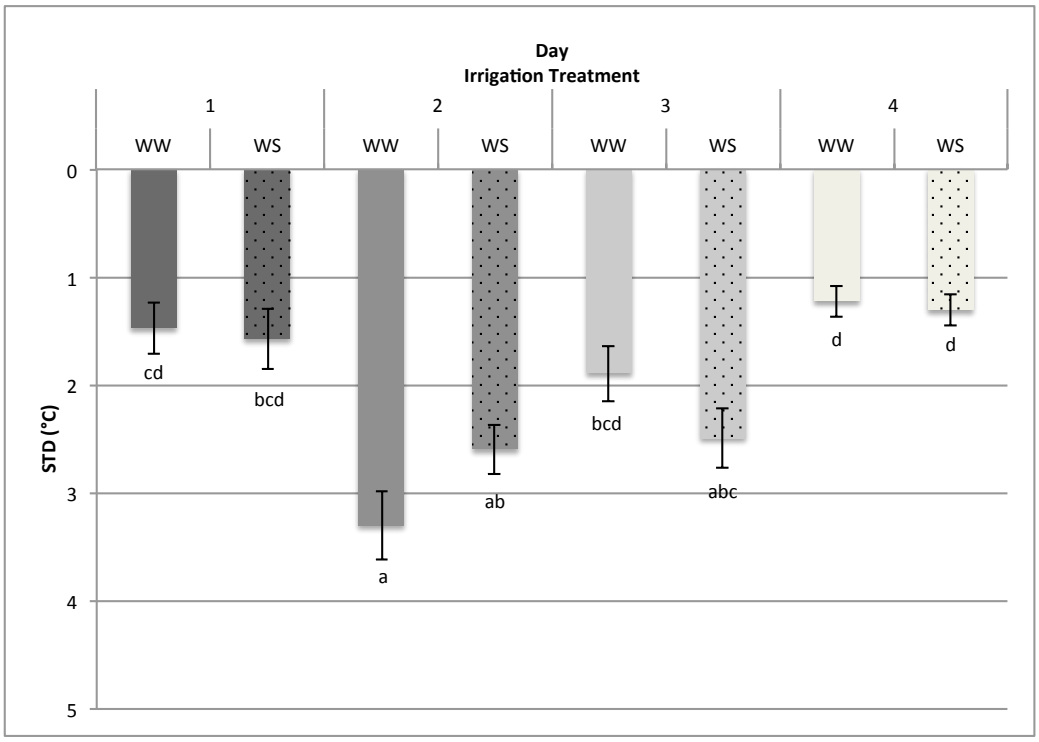

Fig. 3.11 – Average STD on each day of observation, categorized for the two irrigation treatments applied in Experiment 3. Error bars represent standard error. Letters indicate significant difference at *P*<0.05.

Table 3.4 – Average STD on all four days of observation, categorized for all four temperature and irrigation treatment combinations in Experiment 3. Values in brackets represent standard error.

Day	Temperature Treatment	Irrigation Treatment	STD (°C) (±se)
1	AT	WW	1.3 (0.3)
		WS	1.7 (0.3)
	HT	WW	1.6 (0.4)
		WS	1.5 (0.5)
2	AT	WW	3.1 (0.4)
		WS	2.3 (0.2)
	HT	WW	3.5 (0.5)
		WS	2.9 (0.4)
3	AT	WW	1.2 (0.3)
		WS	1.0 (0.2)
	HT	WW	2.6 (0.0)
		WS	3.9 (0.4)
4	AT	WW	1.3 (0.2)
		WS	1.4 (0.1)
	HT	WW	1.2 (0.2)
		WS	1.2 (0.3)

3.4.1.7. Temperature Depression and Active Flowering

There was no significant effect on the STD as a result of whether the spike was actively flowering ('Y') or not actively flowering ('N') (P=0.111). Similarly, combined temperature and irrigation treatments did not impact the relationship between FDS of the spikes observed and FLTD (P=0.365).

3.4.1.8. Temperature Depression and Grain Yield

No significant correlations were found between average CTD/STD during anthesis and grain yield, as a result of the temperature and irrigation treatments applied, or as a result of the combined treatment effects (Table 3.5, see Appendix). Similarly, no significant correlations between CTD/STD on Day 2 and grain yield were identified (Table 3.6, see Appendix).

3.4.2. Experiment 4

3.4.2.1. Glasshouse And Heat Stress Tent Temperature

Table 3.7 summarizes the average temperature recorded in the AT and HT treatments on each of the five days of observation. Significant differences in the temperature of the AT and HT treatments were recorded over the five-day observation period (P<0.001). On all five days, the temperature in the AT treatment was significantly lower than in the HT treatment. The temperature in the AT treatment differed significantly over the five days (P<0.001), with the temperature on Day 5 (32.1°C) being significantly greater than on the previous four days. In the HT treatment, the target temperature for the stress period between 11.00h and 14.00h was 35.0°C. Table 3.8 (see Appendix) details the average temperature recorded between 11.00h and 14.00h in each of the three HST, on all five days of observation. In HST 2 and HST 3, the temperature during this period was significantly greater than the target temperature on Days 4 and 5. The highest temperature recorded was 41.9°C. Table 3.9 (see Appendix) summarizes the night-time temperature recorded in the glasshouse on all five days of anthesis.

Table 3.7 – Average temperature of the AT and HT treatments, recorded between 11.00h and 14.00h on all five days of observation during Experiment 4. Values in brackets represent standard error.

Day	Treatment	Temperture (°C) (±se)
1	AT	21.3 (0.3)
	HT	34.4 (0.6)
2	AT	22.5 (0.3)
	HT	35.3 (0.7)
3	AT	26.4 (0.6)
	HT	36.6 (0.9)
4	AT	25.9 (0.3)
	HT	38.8 (0.4)
5	AT	32.1 (0.3)
	HT	38.4 (0.5)

3.4.2.2. Yield Data

3.4.2.2.1. Spike and Grain Weight

No significant differences in the spike weight were identified between the genotypes examined (P=0.514) or as a result of the two temperature treatments applied (P=0.057). Irrigation was found to significantly affect spike weight (P=0.003), as was the interaction between the temperature and irrigation treatments (P=0.003). The restriction of irrigation during anthesis in the WS treatment resulted in a lower spike weight (1.48g) than was recorded in the WW treatment (1.98g). The combined effect of HT+WS resulted in a lower average spike weight (0.97g) than HT+WW (1.95g). No significant differences in grain weight were identified between the genotypes (P=0.483) or between the two irrigation treatments applied (P=0.121). The two temperature treatments applied at anthesis however resulted in significant differences in grain weight (P=0.024) with the HT treatment (0.96g) significantly reducing grain weight compared to the AT treatment (1.41g). Similarly to spike weight, grain weight was significantly affected by the combined effect of HT+WS (P=0.031). The combined effect of HT+WS also resulted in a lower average grain weight (0.59g) than HT+WW (1.32g).

3.4.2.2.2. Chaff and Stem Weight

No significant differences in chaff weight were identified between genotypes (P=0.608), temperature treatments (P=0.291) or irrigation treatments (P=0.566). Stem biomass was significantly affected by the HT treatment (P=0.035) but no significant effect was detected between the stem biomass of the two irrigation treatments applied (P=0.747). Stem weight was greater in the AT treatment (1.31g) than in the HT treatment (1.07g). The combined stress effect on stem weight was found to be significant (P=0.012), with elevated temperature and restriction of irrigation at anthesis (HT+WS) significantly reducing stem weight (1.01g) compared to the AT+WW treatment (1.12g).

3.4.2.2.3. Grain Number

No genotypic variation was identified for grain number per spike (P=0.616). Similarly, irrigation was not found to significantly affect grain number (P=0.078). The HT treatment at anthesis was however found to negatively affect grain number (P=0.047). Mean grain number in the HT treatment (26.2) was significantly lower than grain number recorded in the AT treatment (43.3). The compounding effects of HT+WS had a negative impact of grain number as well (P=0.023), significantly reducing it compared to in the HT+WW treatment (17.3 and 35, respectively). When grain number was categorized by genotype and combined temperature and irrigation treatments, no significant differences were identified (P=0.933). Fig. 3.12 illustrates the combined effect of the two temperature and irrigation treatments on grain number.

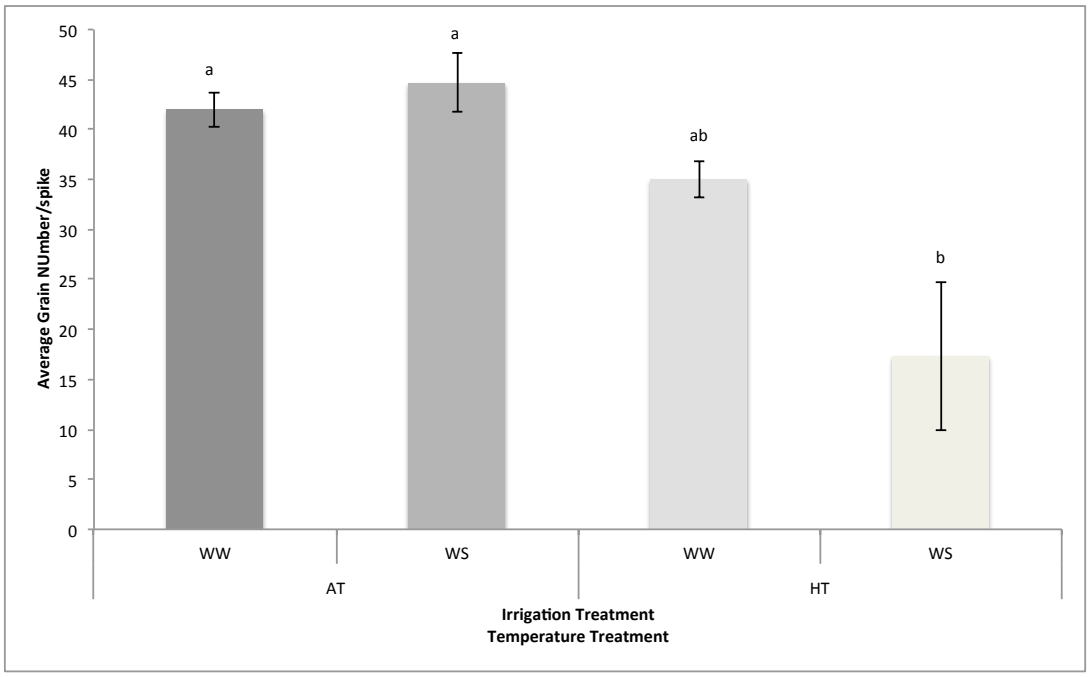

Fig. 3.12 – Average grain number per spike, categorized by each of the temperature and irrigation treatment combinations applied in Experiment 4. Error bars represent standard error. Letters indicate significant difference at *P*<0.05.

3.4.2.3. Root Data

As only a portion of the roots in the rhizoboxes were visible through the glass wall, the root length density (RLD) of each section was calculated using the following formula:

$$RLD = RL_S \times VSV$$

where RL_S is the root length of a given section and VSV is the visual soil volume of each section. The VSV was assumed to be 5mm (Hurd, 1968; Hurd and Spratt, 1975; Liao *et al.,* 2006).

For both SB020 and SB165, RLD was lower at the 90-100cm than in the uppermost soil horizon ($P<0.001$ and $P=0.008$, respectively). SB020 maintained a more consistent RLD throughout the rhizobox, whereas SB165 showed a significant reduction in RLD towards the base of the box ($P<0.001$). At the base of the

rhizobox, the RLD for SB165 was just above 0.002cm/cm^3 whereas in SB020 the RLD at the base of the box was just over 0.05cm/cm^3. Except at the 70-80cm (P=0.014), where the RLD for SB020 was greater than for SB165, no other significant difference in rooting depth between the two genotypes was identified in the other soil horizons. Fig. 3.13 illustrates these findings.

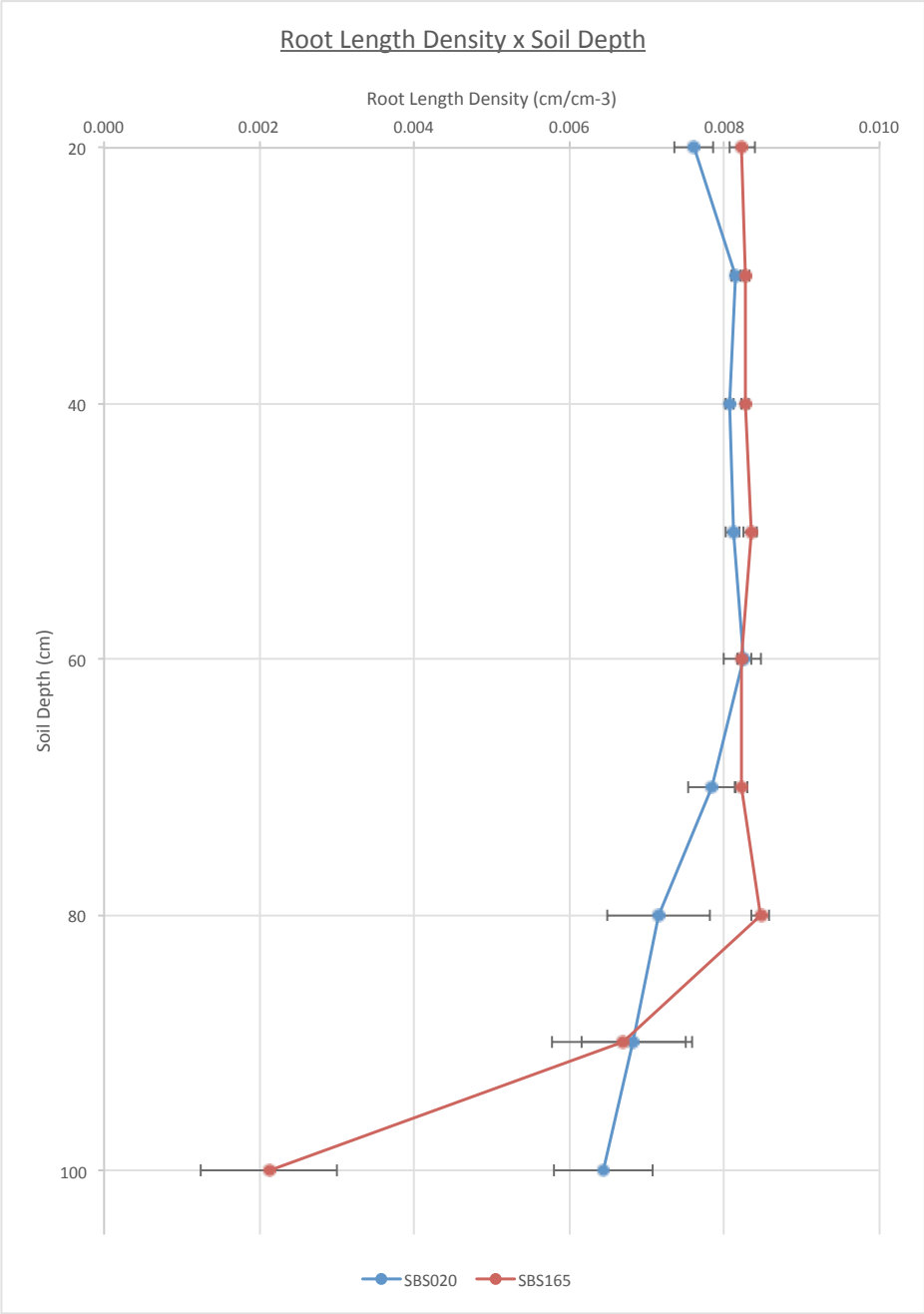

Figure 3.13 – The root length density recorded in the rhizoboxes from 20cm to 100cm depth, for both genotypes used. Bars indicate standard error.

3.4.2.4. Flag Leaf Temperature Depression

No significant differences in FLTD were identified between the two genotypes (P=0.701), or as a result of the temperature (P=0.995) and irrigation treatments (P=0.118) applied. Significant differences in FLTD between days were however found (P=0.003). FLTD was significantly lower on Day 3 (3.1°C) whilst on Day 5 the highest FLTD was recorded (5.7°C). Significant differences in FLTD between temperature treatments on individual days (P<0.001) were found. Fig. 3.14 illustrates these results.

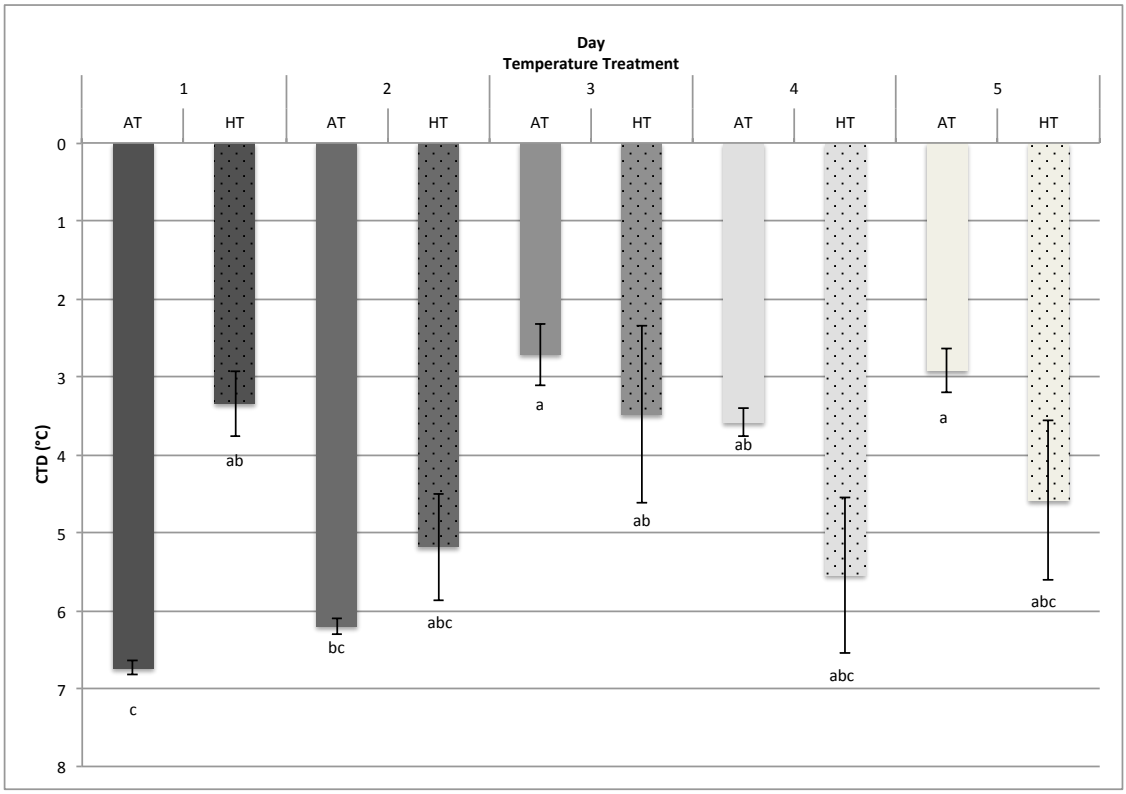

Fig. 3.14 – Average FLTD on each day of observation, categorized for the two temperature treatments applied in Experiment 4. Error bars represent standard error. Letters indicate significant difference at P<0.05.

3.4.2.5. Spike Temperature Depression

No significant differences in STD were identified between the two genotypes examined (P=0.702) or as a result of the two irrigation treatments applied (P=0.602). Significant differences in STD at anthesis were identified as a result of

the two temperature treatments applied (P=0.010), with STD being greater in the HT treatment (5.3°C) compared to the AT (2.4°C) treatment. Significant differences in the STD were also identified between the temperature treatments applied on individual days of observation (P=0.034). The largest cooling capacity of the spike was recorded on Day 4 and 5 in the HT treatment (6.6 and 6.4°C, respectively). STD in the AT treatment was consistently lower than that in the HT on all days, with the greatest difference in STD between the two temperature treatments being observed on Day 3 (3.9°C). An increase in the STD recorded in the HT treatment was observed from the start to the end of the observation period whilst STD in the AT treatment remained significantly lower on all five days.

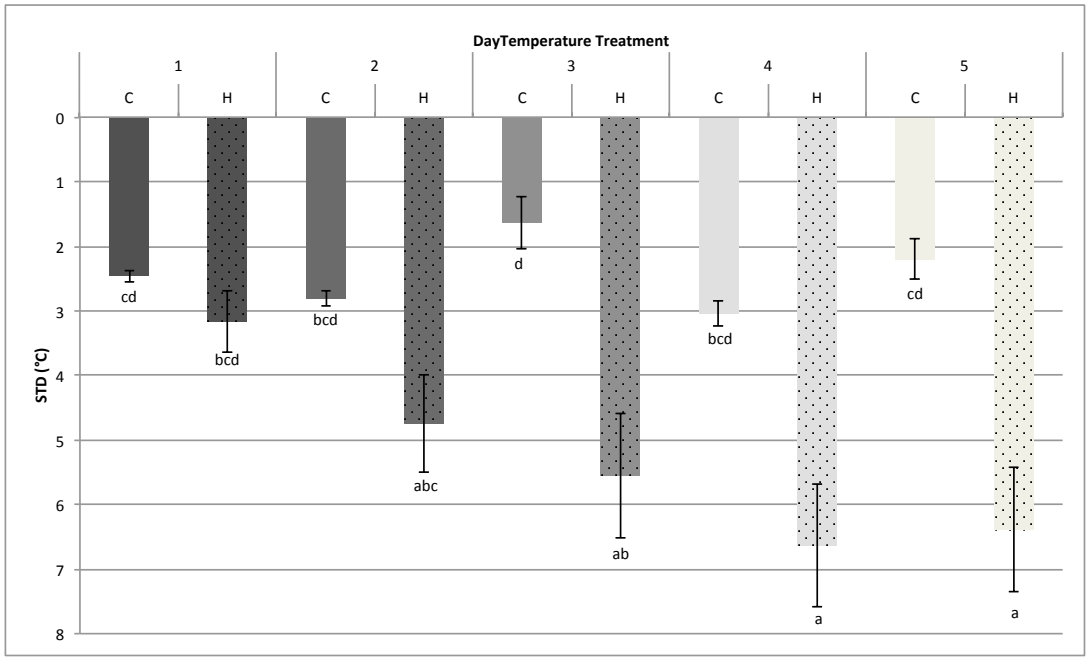

Fig. 3.15 – Average STD on each day of observation, categorized for the two temperature treatments applied in Experiment 4. Error bars represent standard error. Letters indicate significant difference at P<0.05.

3.4.2.6. Floral Development Observations

3.4.2.6.1. Anther

Between Days 1 and 3, a moderate rise in the percentage of florets at FDS (3) was recorded, with 19% of florets being at FDS (3) on Day 3. On the following day, the percentage of florets at FDS (3) rose to just over 50% of total florets. From Day 4 to Day 5, the proportion of florets at FDS (3) decreased rapidly to only 4% of total florets. Fig. 3.16 illustrates the changes observed in the male floral structures on all five days of observation, pooled for all genotypes and treatments. Figs. 3.17 and 3.18 illustrate the pooled genotypic change in male FDS, on all five days of observation in Experiment 4, in the AT and HT temperature treatments, respectively.

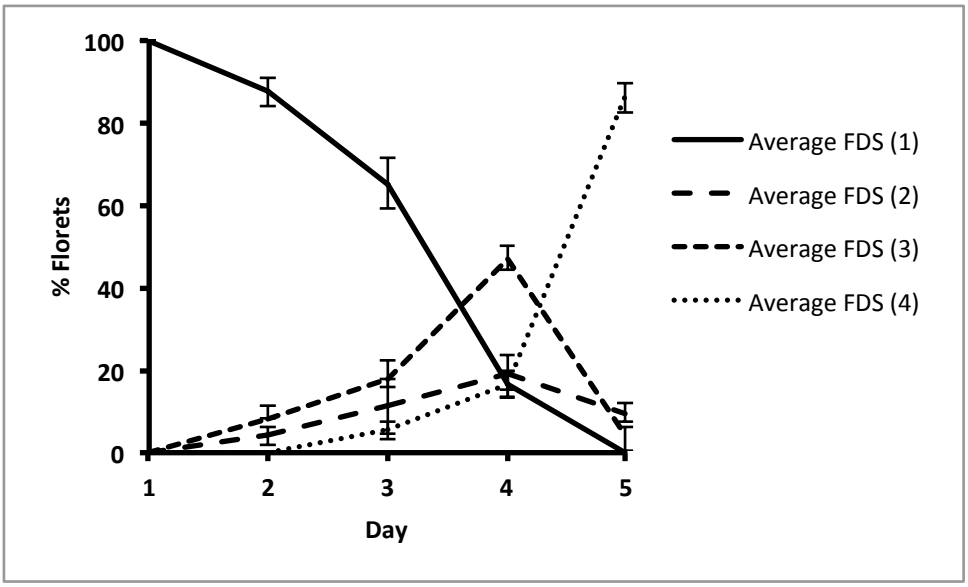

Fig. 3.16 – Daily change in the average percentage of anthers at each male FDS, pooled for all genotypes and treatments during Experiment 4. Error bars represent standard error.

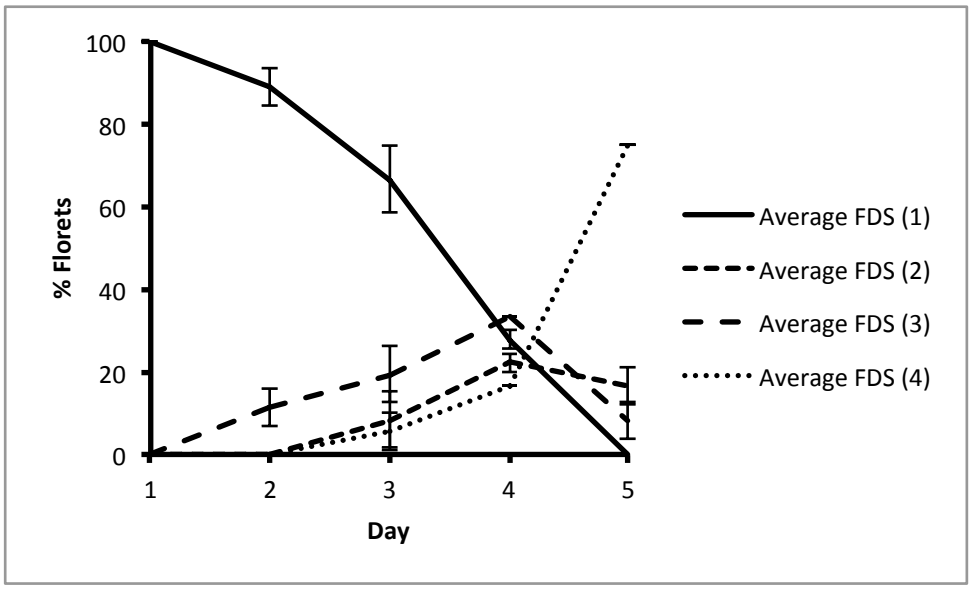

Fig. 3.17 – Daily change in the average percentage of anthers at each male FDS, pooled for all genotypes in the AT temperature treatment during Experiment 4. Error bars represent standard error.

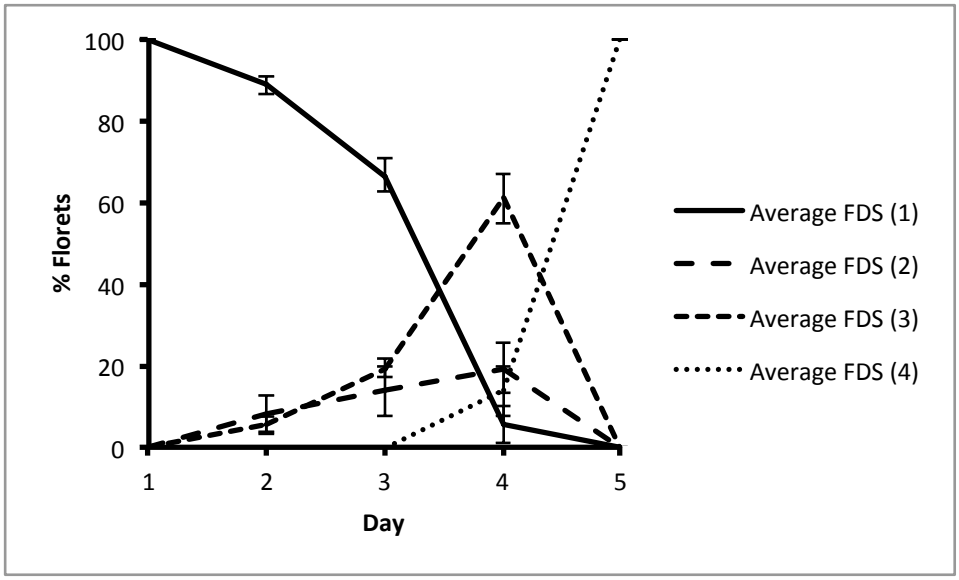

Fig. 3.18 – Daily change in the average percentage of anthers at each male FDS, pooled for all genotypes in the HT temperature treatment during Experiment 4. Error bars represent standard error.

Fig. 3.17 and 3.18 illustrate that as a result of elevated temperature in the HT treatment, the rate of decline in florets at FDS (1) from Day 3 to 4 was faster than in the AT treatment. Although peak occurrence of florets at FDS (3) took place on Day 4, a greater proportion were at FSD (4) in the HT treatment compared to the AT

treatment. Similarly, a greater proportion of florets remained at FDS (2) and FDS (3) on Day 5 in the AT treatment than in the HT treatment. 100% of florets were at FDS (4) on Day 5 as a result of the HT treatment. Consequently, heat stress was found to accelerate the progression of anthesis.

Table 3.10 and 3.11 (see Appendix) summarize the daily change in the average percentage of male florets recorded at each development stage for the two genotypes examined during Experiment 4, in the AT and HT temperature treatments, respectively. On each day of observation, in both the AT and HT temperature treatments, the proportion of florets at FDS (3) did not significantly differ between the SB020 and SB165 (P=0.156 and P=0.644, respectively).

3.4.2.6.2. Stigma

On Day 1, 22% of florets had already progressed to FDS (F). The proportion of florets at this floral stage proceeded to increase until Day 3. From Day 3 to Day 4, the proportion of florets at FDS (F) continued to increase although at a far slower rate. From Day 4 to Day 5, a sharp decline in the presence of florets at FDS (F) was observed with approximately 25% of florets remaining at FDS (F) on Day 5. Fig. 3.19 illustrates the changes observed in the female floral structures on all five days of observation, pooled for all genotypes and treatments. Fig. 3.20 and 3.21 illustrate the pooled genotypic change in female FDS, on all five days of observation in Experiment 4, in the AT and HT temperature treatments, respectively.

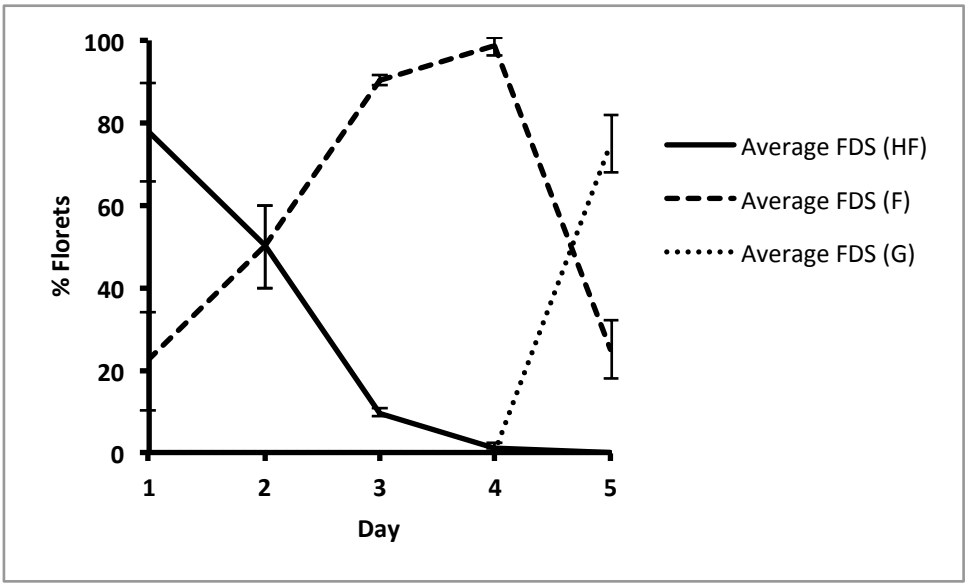

Fig. 3.19 – Daily change in the average percentage of anthers at each female FDS, pooled for all genotypes and treatments during Experiment 4. Error bars represent standard error.

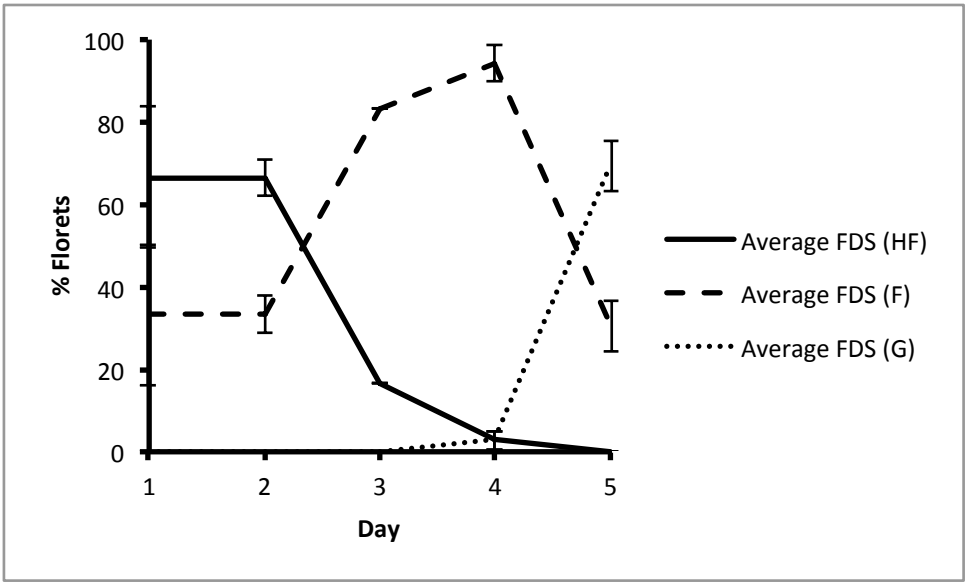

Fig. 3.20 – Change in the average percentage of female florets in the AT treatment on each day of observation, during Experiment 4. Error bars represent standard error.

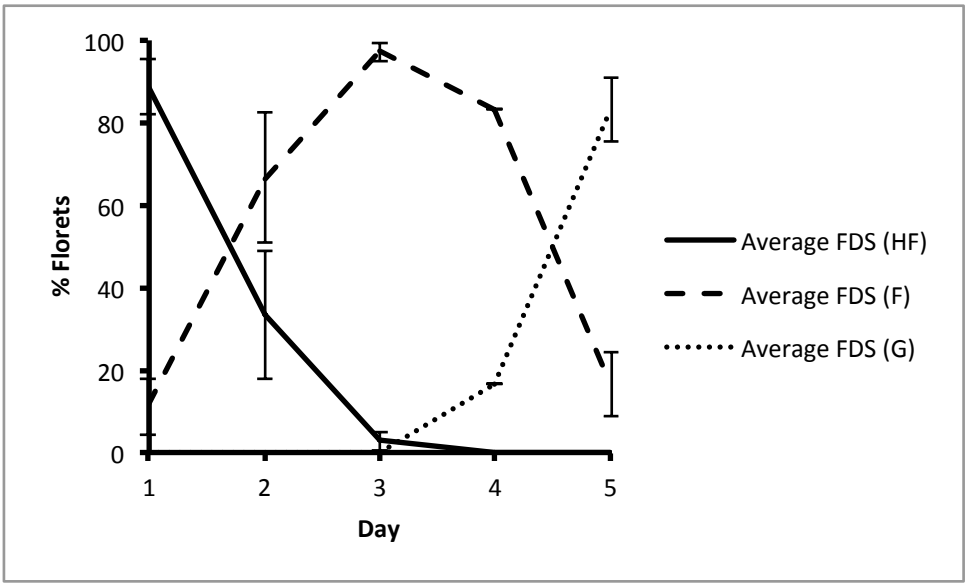

Fig. 3.21 – Change in the average percentage of female florets in the HT treatment on each day of observation, during Experiment 4. Error bars represent standard error.

Fig. 3.20 and 3.21 illustrate the differences in floral progression as a result of the presence of temperature stress. From Day 1 to Day 2, the proportion of florets remained constant at 36% of total florets in the AT treatment before increasing to 84% of total florets on Day 3. The proportion of florets at FDS (F) continued to increase from Day 3 to Day 4 before declining to 28% of total florets on Day 5. In the HT treatment on the other hand, the proportion of florets on Day 1 was at 14% before rising sharply to 96% of total florets on Day 3. A gradual decline was then observed from Day 3 to 4 before a sharp decline in florets at this stage on Day 5.

Table 3.12 and 3.13 (see Appendix) summarize the daily change in the average percentage of female florets recorded at each development stage for the two genotypes examined during Experiment 4, in the AT and HT temperature treatment, respectively. On each day of observation, in both the AT and HT temperature treatments, the proportion of florets at FDS (F) did not significantly differ between SB020 and SB165 the (P=0.369 and P=0.995, respectively).

3.4.2.7. Temperature Depression and Grain Yield

No significant correlations were found between average FLTD/STD during anthesis and grain yield, as a result of the temperature and irrigation treatments applied, or as a result of the combined treatment effects. No genotypic interaction with the temperature treatment correlated significantly to grain yield (Table 3.14). Similarly, no significant correlations between CTD/STD on Day 3 and grain yield were identified (Table 3.15).

Table 3.14 – *P*-values generated from correlation analysis examining average FLTD/STD during anthesis and grain yield, under a range of treatments, pooled for genotype, in Experiment 4. Effects were considered significant at *P*<0.05.

Organ TD	Treatment/Treatment Combinations	P-value	Significant?
FLTD	AT	0.210	ns
	HT	0.776	ns
	WW	0.726	ns
	WS	0.699	ns
	AT+WW	0.277	ns
	AT+WS	0.227	ns
	HT+WW	0.855	ns
	HT+WS	0.554	ns
	SB020+AT	0.256	ns
	SB020+HT	0.801	ns
	SB165+AT	0.745	ns
	SB165+HT	0.418	ns
STD	AT	0.202	ns
	HT	0.456	ns
	WW	0.304	ns
	WS	0.809	ns
	AT+WW	0.141	ns
	AT+WS	0.178	ns
	HT+WW	0.288	ns
	HT+WS	0.309	ns
	SB020+AT	0.145	ns
	SB020+HT	0.144	ns
	SB165+AT	0.981	ns
	SB165+HT	0.608	ns

Table 3.15 – *P*-values generated from correlation analysis examining average FLTD/STD on Day 3 and grain yield, under a range of treatments, pooled for genotype, in Experiment 4. Effects were considered significant at *P*<0.05.

Organ TD	Treatment/Treatment Combinations	P-value	Significant?
FLTD	AT+WW	0.654	ns
	AT+WS	0.267	ns
	HT+WW	0.088	ns
	HT+WS	0.975	ns
STD	AT+WW	0.569	ns
	AT+WS	0.277	ns
	HT+WW	0.175	ns
	HT+WS	0.878	ns

3.4.2.8. Temperature Depression and Floret Development Stage

When FLTD was plotted against mean male FDS, a significant relationship was found for SB020 and SB165 in the AT treatment (*P*=0.002 and *P*=0.001, respectively). For both genotypes, a reduction in the FLTD was observed from FDS (1) to FDS (4). Similarly for STD of SB020 and SB165 in the AT treatment, a significant relationship to mean male FDS was found (*P*=0.031 and *P*=0.008, respectively) in which a reduction in the STD was observed from the early stages of anthesis towards the latter stages. When FLTD was plotted against mean female FDS, significant relationships were also identified for SB020 and SB165 in the AT treatment (P=0.016) in which a similar reduction in FLTD was observed from the early to the late stages of anthesis. STD for SB020 and SB165 in the AT treatment was significantly affected by the mean female FDS (P=0.023), in which the same pattern of reduced organ TD as described above was found to occur in the latter stages of floral development, FDS (G). The two irrigation treatments applied did not result in any significant interactions between FDS and organ TD.

For both SB020 and SB165, the average organ TD at the start of anthesis (FDS HF/1) ranged between 4°C-6°C and was similar for both the flag leaf and the spike (Fig. 3.22-3.25). Organ TD for both genotypes was not found to fall below 0°C i.e. tissue temperature did not exceed ambient temperature during the period of observation. As anthesis progressed and moved from the early stages or reproductive development (FDS HF/1) towards the final floret development stages

(FDS G/4), the observed organ TD decreased significantly. A significant warming of tissue temperature from the start until the end of anthesis was documented. With both FLTD and STD being comparable at the start of anthesis, a divergence in the genotypes examined was observed. The FLTD and STD of SB020 were marginally lower than that of SB165 in the latter stages of anthesis.

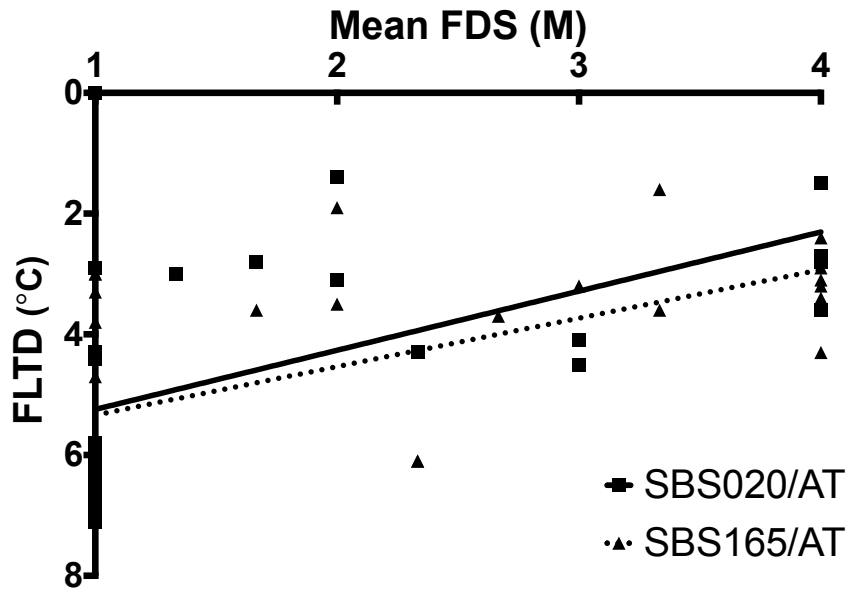

Fig. 3.22 – The relationship between flag leaf temperature depression and mean male floret development stage of SB020 and SB165 in the AT treatment.

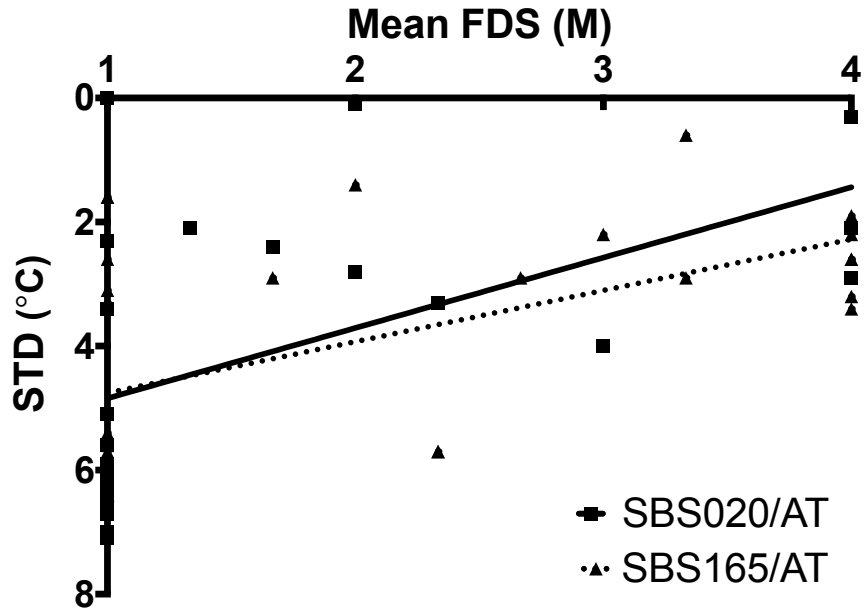

Fig. 3.23 – The relationship between STD and mean male FDS of SB020 and SB165 in the AT treatment.

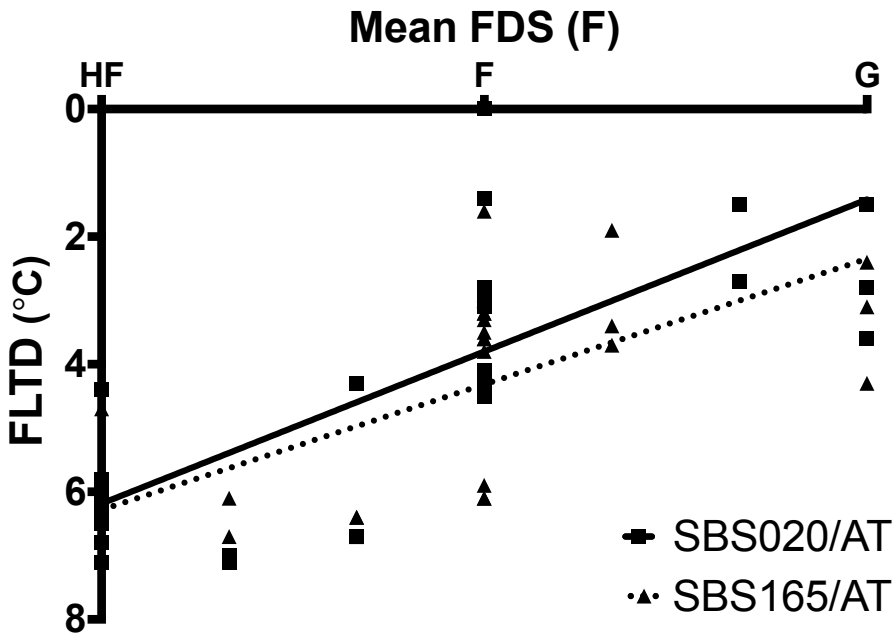

Fig. 3.24 – The relationship between FLTD and mean female FDS of SB020 and SB165 in the AT treatment.

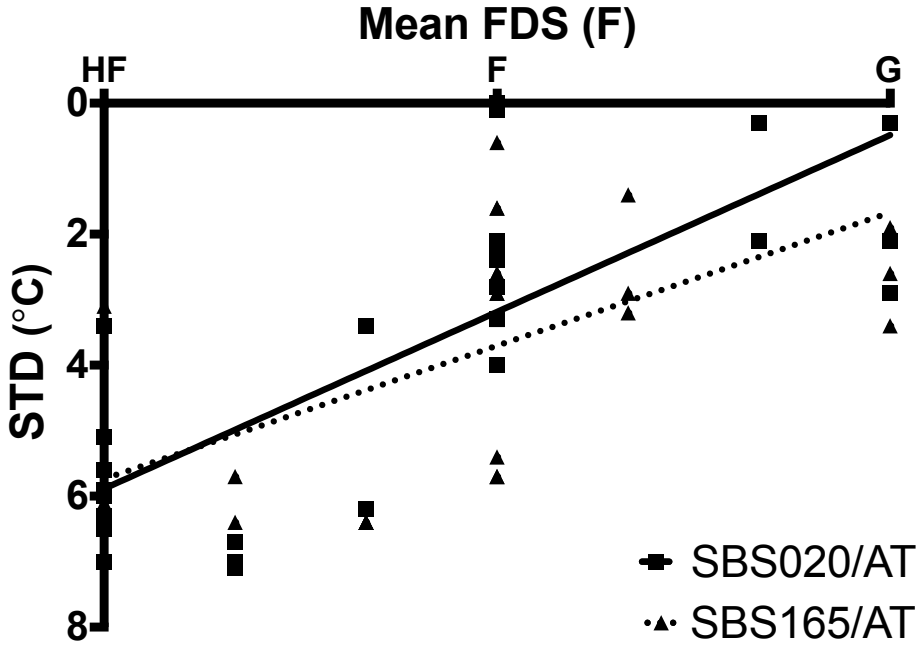

Fig. 3.25 – The relationship between STD and mean female FDS of SB020 and SB165 in the AT treatment.

3.5. DISCUSSION

As traditional field-scale experimentation often lacks the precise and tightly controlled conditions required when applying abiotic stress within narrow limits, researchers often attempt root studies in CE conditions. However, soil volume and depth available for rooting in pot-based experiments, such as the ones detailed in Chapter 2, means that growing conditions in many CE experiments deviate significantly from those encountered in traditional field grown conditions (Anderson, 1986). Due to the restrictions placed on the root system by the pots used in Experiments 1 and 2, it was not feasible to consider examining the belowground biomass in these two experiments.

The SB populations used during Experiments 1, 2 and 4 are traditionally grown in Mexico, West and South Asia and North Africa, regions where heat and drought stress occur frequently during the growing cycle (Lopes *et al.,* 2013). Due to physiological, financial and logistical limitations, it was not possible to conduct full field-scale trials of SB at Sonning Farm. Autumn sown winter wheat, of the variety Cadenza, was used instead. The aim of using a single cultivar in a range of environments was to demonstrate the validity of CTD and STD assessments in a semi-controlled, field-grown crop in the UK.

Experiment 3 raised a number of important issues related to conducting field-scale phenotyping experiments. In the UK, there is a high probability of having unexpectedly cool, wet summers (Met Office, 2016a). These have the potential to disrupt field-scale experiments. This was demonstrated by the cold weather front arriving at mid-anthesis during Experiment 3. The supplementary heating units supplied to each tunnel failed to raise and maintain the internal polytunnel temperature adequately, which resulted in an insufficiently long stress period being applied to the crop during anthesis. Alternative polytunnel designs have been suggested in the past (Hadley *et al.,* 1995) although the cost of constructing these was prohibitive for this experimentation. Other authors have previously used polytunnels at the same site as Experiment 3 was conducted in to assess the effects of elevated temperature on crop development, although the average IPT in this case did not exceed 20°C (Wheeler *et al.,* 1996a).

At anthesis, CTD was found to be significantly greater than STD in field-grown wheat. The absence of a significant correlation between organ TD and active flowering does not support a theory of preferential cooling of the spike over the canopy at anthesis. It is unclear whether there was a significant change in the cooling capacity of the spike and the flag leaf as floral development advanced from the early to the latter stages. Furthermore, increased CTD or STD at anthesis failed to correlate to higher grain yields in the field-grown wheat. The failure of STD to differentiate between a crop exposed to elevated temperature and water-deficient stress at anthesis, suggests that STD is a more complex indicator of plant tissue temperature than CTD.

Despite efforts to limit water availability to the crop, the absence of a yield difference between irrigation treatments at anthesis suggested that the crop maintained its ability to extract moisture from the soil throughout. This is likely to have been as a result of a combination of factors: (i) insufficient soil drying in the drought treatment prior to anthesis, (ii) roots accessing moisture from the high water table in the field, and (iii) horizontal water flow from outside the area covered by the polytunnels and into the drought treatment plots because of the rain events which took place during anthesis. Without expanding the cropped area under polytunnel cover significantly, the negative effect of a large precipitation event during anthesis could not have been managed differently.

The absence of a significant correlation between CTD/STD at anthesis and grain yield in Experiment 3, should not be interpreted as conclusive. The lack of significant yield differences between the abiotic stress treatments as a consequence of the shortened period over which stress could be applied at anthesis is likely to have impacted the possible relationship between organ TD at anthesis and grain yield. It is not possible to predict the effect of extended stress at anthesis from the data present but it can be assumed from previous work that significantly greater and longer stress at anthesis may have resulted in differences in grain yields. In particular, stress at the very early stages of anthesis has the potential to disrupt pollination and successful seed set. With these limitations in mind, the relationship between CTD/STD and grain yield in field-grown wheat in the UK remains unanswered.

The absence of significant differences in rooting depth between treatments was an inherent risk of conducting a trial with a single genotype. The presence of gravel beds at depth throughout the field trial did not affect deep rooting in the treatment plots. Due to the high soil moisture content and high water table of the experimental site, even if differences in rooting depth had been detected it is likely that the root biomass present in shallow rooting cultivars would have had sufficient soil water available to meet the canopy's evaporative demands at anthesis.

Using only CTD as a differentiator it was possible on all four days to successfully distinguish a wheat crop grown in the AT treatment from one grown in the HT treatment. Similarly, although with the exception of Day 4, the CTD data successfully distinguish a WW canopy from a WS canopy. Although these findings are in line with those reported by others (Tuberosa, 2012), it is likely that a greater difference between the two irrigation treatments would have resulted in larger differences between the treatment observations.

When STD was categorized by temperature treatment on each day, an unclear picture of STD emerged. With the exception of Day 2, STD was found to be comparable between the two temperature treatments on all days of observation. On Day 2, the spike temperature was approximately 2°C greater in the HT treatment compared to the AT treatment. STD and CTD were comparable in both temperature treatments on Day 2. When contrasting CTD and STD in the HT treatment on Day 1, CTD averaged 8°C whilst STD averaged a mere 3°C. This significant 5°C difference between the canopy and the spike does not support a theory of preferential cooling of the spike over the canopy during anthesis. When STD was categorized by irrigation treatment on each day of observation, the pattern that emerged was equally unclear. Day 1 and 2 were the only days of observation on which STD differed significantly between irrigation treatments. On Day 1, STD was greater in the WW treatment than in the WS treatment. However, on the following day, STD was found to be greater in the WS treatment. This apparent shift in STD may be explained by the significant reduction in internal polytunnel temperature between the Day 1 and 2.

As the temperature stress applied to the crop varied significantly between days, there was cause to examine just Day 2, the day on which the temperature stress was the greatest, in isolation. On Day 2, CTD was significantly greater than STD. This is in agreement with the findings made in Chapter 2.5. As in Chapter 2.5, there was no evidence from Experiment 3 to suggest a greater level of cooling of the spike compared to the canopy during anthesis. The internal polytunnel temperature on Day 3 and Day 4 did not exceed 25°C. Consequently, over the four days of anthesis, the plants only experienced a short period of high temperature stress above 30°C on Day 2. On Day 2, CTD and STD were greater in the HT treatment than in the AT treatment. When categorized by irrigation treatment however, CTD was comparable for both the WW and WS treatments whereas STD in the WS treatment compared to the WW treatment. It is possible that a complex photosynthetic interplay between temperature stress and water availability took place in the spike. Water-deficit stress in the canopy may have resulted in hormonal signalling to the spike that resulted in differing photosynthetic rates as well as closure of the stomata in the canopy but not in the spike.

Had the duration and intensity of the HT treatment been greater during anthesis, it is hypothesized that a significant reduction in the grain number per spike would have been observed which would consequently have impacted the average grain weight. Higher air temperatures would have resulted in an increase in the evaporative demands of the crop and therefore an increase in the cooling capacity of both the spike and the canopy. Had a significant gradient between the WW and the WS treatment been established, the combined effect of HT+WW would have resulted in hotter tissue temperature observations as well as a reduction in the cooling capacity of the canopy at mid-anthesis and a concurrent rise in STD towards the start of grain filling. Further field-scale examination of this hypothesis is required.

Changes in the rate of floral development due to abiotic stress may take place on a faster time scale in field-grown wheat than changes in assimilate portioning to the spike do. However, the effects of temperature and drought stress during the subsequent period of grain filling may result in increased STD. As discussed previously, the observation that STD is lower than CTD may be an artefact of the spikes adaptation to function at higher temperatures than the canopy and as a result,

the spike may consistently operate at a lower cooling capacity. In this case, selection for STD as a screening trait may be complex. CTD can be measured over a longer timescale than STD, which only becomes available for assessment at anthesis. Furthermore, CTD is likely to remain stable up to anthesis so that early generation selection can take place without having to wait for spike development to occur. STD has successfully differentiated between plants grown under conditions of temperature and drought stress in the past (see Chapter 2 and Experiment 4). The absence of this observation in Experiment 3 does not mean that STD is a less reliable indicator of plant tissue temperature but rather that under field-grown conditions, the strength of STD at anthesis is lower and less evident than CTD. The absence of a significant relationship between STD at anthesis and grain yield reflects the similar findings made in Experiment 1, 2 and 4. Similarly, CTD at anthesis did not correlate with a significantly greater yield. The inability to detect a significant relationship was likely impacted further by the failure to maintain a significant gradient between the two temperature and irrigation treatments applied.

CTD can be assessed rapidly and accurately using hand-held infrared thermometers (Ayeneh *et al.,* 2002) and autonomous remote-sensing helicopters (Chapman *et al.,* 2014) to multispectral cameras attached to helium filled blimps (Goth, 2014). The resolution of the field-of-view for these assessment techniques however does not capture sufficient detail so that individual spikes could be detected. The only device currently capable of accurately imaging a spike in sufficient details is a hand-held IR camera. The assessment of wheat STD in the field was the first of its kind in the UK. Consequently, there was no previous experimental protocol that could easily be modified or replicated. The methodology used has successfully demonstrated that CTD and STD can be measured using hand-held IR devices in field-grown wheat in the UK.

In order to develop the UK's capability to phenotype wheat for TD traits in non-canopy organs in the future, a significant investment in experimental site design, image capture technology and automation of data analysis is required. It is unlikely that phenotyping experiments aimed at selecting for increased TD can take place in the UK without the assistance of some form of an environmental control structure. Novel technologies, such as ground penetrating radar, may provide a rapid, non-

destructive and semi-automated approach to assessing the below-ground biomass (Thompson *et al.,* 2011; Thompson *et al.,* 2012). Automation of STD assessment and image analysis must take place in order to reduce the labour demand currently required.

In Experiment 3 and 4 there was the lack of negative TD values for both the canopy and the spike, which was observed in the growth chamber experiments of Chapter 2. This finding is likely to be the result of four interacting factors. Firstly, the difference in experimental design between the experiments meant that unlike in Experiment 1 and 2, no supplementary lighting was used in Experiment 3 and 4. The plastic sheeting used to construct the polytunnels in Experiment 3 and the HST in Experiment 4 was neither reflective nor acted as strong insulator of heat. It is likely that the plants grown in Experiment 3 and 4 were not exposed to the same thermal load as the plants grown in Experiments 1 and 2. It was assumed that this is the primary reason for the absence of negative organ TD observations. Secondly, increased turbulent airflow through the crop in Experiment 3, compared to Experiments 1 and 2, contributed to tissue temperature remaining below ambient conditions. In Experiment 4, the vertical airflow possible due to the design of the HST meant that the air was constantly in motion compared to in the growth chambers used in Chapter 2. Thirdly, the vertical and horizontal expansion of the roots in the field and rhizobox experiments was not restricted as they were in Experiments 1 and 2. This ability to freely root is likely to have provided the crop with the capacity to extract soil moisture throughout its growing cycle so that when stomatal conductance was increased due to elevated temperature stress, sufficient water was available for evapo-transpirational cooling. Finally, the warming of the root zone found in pot-based, CE experiments was absent in Experiment 3 and 4. Similarly to in Experiment 1 and 2, root zone temperature data was not collected in Experiment 3 but to the touch, the rhizoboxes felt significantly cooler than the pots used in the growth chambers. As demonstrated by the sustained capacity to produce grain under the artificially created, above-ambient tissue temperatures recorded in Experiment 1 and 2, wheat is able to cope with these supra-optimal temperatures. However, the results gathered from field- and rhizobox-grown wheat suggest that the above-ambient tissue temperatures recorded in the growth chamber are likely to

be an artifact of conducting experimentation under CE conditions and unlikely to occur in the field.

Rhizoboxes have previously been used to assess the effects of waterlogging (Palta *et al.,* 2010), temperature stress with elevated CO_2 concentration (Benlloch-Gonzalez *et al.,* 2014b) and nitrogen uptake (Palta *et al.,* 2007) on root growth. No work relating to assessing TD of rhizobox grown wheat has been published previously. As a potentially useful intermediary solution between the pot grown wheat used in Experiment 1 and 2 and field-grown wheat used in Experiment 3, rhizoboxes have the potential to allow researchers to replicate conditions close to those found in a field-grown crop whilst maintaining effective control of the plants environment.

The design of the rhizobox experiment was successful in creating conditions of high-temperature and water-deficit stress that were greater than those encountered by the plants in Experiment 3. Grain yield was significantly different as a result of the two temperature treatments applied at anthesis. This type of short-lived heat stress at anthesis has been identified in the past as being particularly damaging to yields (Porter and Semenov, 2005). The data reiterates the sensitivity of anthesis to elevated temperature stress and the effect of supra-optimal temperatures on grain number (Prasad *et al.,* 2006a; Prasad *et al.,* 2006b). The two irrigation treatments however failed to result in a significant difference in grain yield, highlighting the complexity associated with creating sufficiently large differences in soil moisture so that water-deficit stress occurs in plants. The combined effect of high temperature and water-deficit stress reduced grain number, confirming the negative effect of multiple abiotic stresses during anthesis on successful grain set. The absence of significant grain yield and number differences between SB020 and SB165 concludes that the previously published differences in stress tolerance cannot be attributed to genotypic differences in TD in the time frame assessed within this chapter.

Despite the ability to freely root down to a depth of 1.0m, FLTD and STD did not correlate with grain yield in any of the treatment combinations applied to the rhizoboxes during anthesis. From the data available, there is no evidence to suggest

that that increased organ TD at anthesis is associated with improved grain yields in wheat. The absence of a correlation between grain yield and either FLTD or STD in the rhizobox grown wheat, mirrors the results reported in Experiments 3. There is no evidence to suggest that increased TD of either organ at anthesis is a valuable indicator of future grain yield of the plant. In field-grown wheat however, CTD has repeatedly been found to be a strong indicator of crop yield potential (Reynolds, 1997; Reynolds *et al.,* 2000; Karimizadeh and Mohammadi, 2011). However, the relationship of FLTD and STD for both genotypes in the AT treatment was significantly related to the FDS of both the anther and stigma, a finding in agreement with the data from Experiment 1. Here too, a decrease in organ TD was observed from the early to the late stages of anthesis i.e. the tissue warmed up from the start to the end of anthesis.

The consistent pattern of root growth through the rhizobox observed in SB020 compared to the reduced root density at depth in SB165, did not result in a significantly greater yield. Once the roots reached the bottom of the rhizobox, they began curling which indicates that the root network in SB reaches down well below 1.0m. The growth of wheat roots has been reported to reach depths of up to 1.8m (Gregory *et al.,* 1978; Sauer *et al.,* 2002; Kirkegaard and Lilley, 2007). Winter wheat has been found to reach depth up to 2.2m, twice that found in spring wheat (Thorup-Kristensen, 2009). In order to examine the fully expanded root system of rhizobox grown Spring wheat, a significantly deeper rhizobox would have to be constructed up to a depth of between 1.5-2.0m. The complications associated with constructing a rhizobox this deep are however manifold and considerations include (i) improving the structural integrity of the box to avoid warping due to the increased height, (ii) improving the structural integrity of the box to compensate for the increase in internal pressure associated with the elevated soil and water volume, and (iii) adapting the support structures of the box to make them safe and accessible. A significant investment is required in order to develop a rhizobox that is deeper yet both mobile and cost-effective to construct.

Anthesis in the rhizobox grown wheat of Experiment 4 lasted for a total of five days. This was two days longer than in Experiment 1 and one day longer than in Experiment 2. Heat stress accelerates physiological processes in plants rather than

delaying them. Even moderate heat stress at anthesis will reduce the flowering period considerably. Conversely, cooler temperatures will delay physiological processes. Olivares-Villegas *et al.* (2007) reported the flowering period of SB to range from 10 to 15 days. The results obtained from Experiment 1, 2 and 4 however suggest that under certain CE conditions, the flowering duration may be significantly shortened as a result of prolonged high temperature stress at anthesis. In Experiment 1 and 2, elevated temperature in the HT treatment was applied for a 16h period whereas in Experiment 4, the period of elevated temperature stress during anthesis lasted for three hours at mid-day. The results suggest that CE experiments, in particular ones using pots, have the potential to significantly impact physiological processes in wheat.

The findings made in Porter and Gawith (1999) remain pivotal in our understanding of thermal thresholds in cereals today (see Table 2.1). The results of Experiment 3 however suggest that the T_{max} estimated by them may in fact be higher than the 31°C reported. A blanket T_{max} value for wheat is impossible to obtain as tolerance to heat stress is highly variable between cultivars and the timing of stress at a particular phenophase. The number of literature sources available to Porter and Gawith (1999), 1 to calculate T_{max} is indicative of the historic lack of focus on the regulation of high-temperature stress in wheat at anthesis. In comparison, 10 literature sources were available to calculate the T_{max} during sowing-emergence, 7 at vernalization and 5 at grain-filling.

It was not possible to use FLTD as a tool to make a distinction between the genotypes used or between plants grown under contrasting temperature treatments. STD similarly failed to show any significant differences between the genotypes used or the irrigation treatments applied. However, STD was demonstrated to successfully distinguish spikes grown under contrasting temperature treatments. Although no significant correlation to FDS was established, STD of plants grown in the HT treatment increased from Day 1 to Day 4, with STD remaining high and comparable to the following day. STD on the final two days of observation was greater than FLTD. An increase in the STD of approximately 4°C between the start and end of the observation period was observed, suggesting that this increase in cooling capacity is relayed to changes in photoassimilate translocation from the

canopy to the spike as a result of the onset of senescence. It is hypothesised that a complex interplay in the sink-source-balance is responsible for this increased cooling observed during late anthesis.

3.6. CONCLUSION

The results do not conclusively elucidate the relationship between STD at anthesis and grain yield in field-grown wheat. The recommendation is however made that STD is assessed further using field-scale experimentation techniques. In order to do this, a system of automation is necessary. It is not feasible to conduct large-scale field trials with hundreds of genotypes by the methodology described here. No significant differences in the rooting depth existed between the treatment plots in the polytunnels and consequently the data is unable to quantify the relationship between deep rooting and spike temperature depression at anthesis.

The contrasting findings of negative organ TD between pot grown and field/rhizobox-grown wheat, indicates a differing physiological response to abiotic stress as a result of conducting TD assessments using different experimental designs. The data from the polytunnel and rhizobox experiments were valuable in understanding the occurrence of negative TD observations detailed in Chapter 2. In Experiment 3, it is reported that under conditions of elevated temperature stress at anthesis, STD increased from the start to the end of the observation period. SB020 maintained a greater root dry weight at depth in the rhizoboxes than SB165. Examination of wheat lines with contrasting rooting habits is recommended for further work. A significant negative relationship between floret development stage and organ cooling capacity was recorded with the temperature depression for both SB020 and SB165.

The results demonstrate that in the UK, it is possible to assess both CTD and STD in the field. The unpredictability of the UK climate poses serious limitations on organ TD becoming a permanent tool that wheat breeders would consider using. In particular, the results illustrate that assessing the effect of abiotic stress at a particular stage in development, in an open field-scale trial, is associated with serious risk of failure. Collaborative wheat breeding for abiotic stress resistance may be viable in alternative sites within the ME11 wheat mega environment that could result in the transfer of germplasm to the UK.

CHAPTER 4

STARCH AND WATER SOLUBLE CARBOHYDRATES

4.1. CHAPTER SUMMARY

Due to climate change, an increase in the potential frequency and severity of high temperature stress events during early season growth has been reported. Stress during the sensitive stage of anthesis is particularly detrimental to wheat (*Triticum aestivum* L.) yields. A conflicting body of data relating to the relationship between increased spike temperature depression (STD) at anthesis and higher grain yields (GY) has emerged in Chapter 2 and 3. The contribution that reserve carbohydrates make to yield production under conditions of stress is not known and warrants examination. Assimilate remobilization is an adaptation mechanism identified as showing potential in mitigating the effects that abiotic stresses have on plant yields. Tissue samples of the flag leaf, peduncle and glumes from all treatment combinations of Experiment 2 and 4 were sampled at mid-anthesis. In Experiment 2, three lines of Seri-Babax were grown in controlled environment growth cabinet under a combination of two temperature and irrigation treatments at anthesis. In Experiment 4, two lines of Seri-Babax were grown in rhizoboxes and were also exposed to a combination of two temperature and irrigation treatments at anthesis. Starch and water soluble carbohydrate (WSC) content of the frozen plant tissue was assessed subsequently at the Lancaster Environment Centre (LU, UK). In both Experiment 2 and 4, starch content was significantly greater in the glumes compared to the peduncle and flag leaf, suggesting that the glumes acted as a significant, and previously underestimated carbohydrate reserve pool for starch. The absence of significant differences in the organ starch content, when categorized by genotype, indicated that starch content and accumulation in wheat were unaffected by periodic abiotic stress at anthesis. Starch content of the flag leaf and peduncle was found to be significantly higher in Experiment 2 than in Experiment 4, which may have been due to differences in experimental design. No genotypic variation in the WSC content was identified in Experiment 2 and 4. In Experiment 2, the glucose and sucrose contents were highest in the peduncle, followed by the glume and the flag leaf. Fructose content however was significantly higher in the glume

compared to the other two organs. In both experiments, sucrose content remained low and unchanged by the presence of elevated temperature and water-deficit stress. Only in Experiment 4 was the glucose content significantly affected by the elevated temperature and water-deficit stress treatments. In both experiments, organ starch/WSC content did not positively correlate to GY in either temperature treatments applied. Similarly, starch content of the flag leaf, peduncle and glumes was not associated with increased GY under contrasting irrigation treatments. Further examination of the effects that abiotic stress at anthesis have on the photoassimilate distribution and GY need to take place in field-grown wheat.

4.2. CHAPTER INTRODUCTION

4.2.1. Justification of Study

Heat and drought stress are the two primary abiotic stress factors constraining cereal yields (Araus *et al.,* 2002), with the effect that heat stress can have on grain yields becoming an increasing concern (Porter and Gawith, 1999; Battisti and Naylor, 2009; Semenov and Shewry, 2011). In several key wheat growing regions around the world today, periods of high-temperature stress are increasingly common during a crops life cycle. Often, these peaks in temperature coincide with sensitive growth phases such as anthesis, which can significantly affect grain yield (Wheeler *et al.* 1996a; Porter and Semenov, 2005; Farooq *et al.,* 2011; Semenov and Shewry, 2011). The increased frequency and intensity of these heat stress events during the growing season has been predicted by a number of models (Barnet *et al.,* 2006; Beniston *et al.,* 2007; Semenov and Shewry, 2011). It has therefore been proposed that breeding for heat-tolerance may be an option in addressing this problem (Reynolds *et al.,* 2000). One promising approach to doing this is by selecting for increased spike photosynthesis, which has the potential to significantly contribute to increased grain-filling (Tambussi *et al.,* 2005; Tambussi *et al.,* 2007; Parry *et al.,* 2011). The ability of the spike to delay chlorosis under conditions of stress, relative to the canopy, has been reported (Abbad *et al*., 2004). The optimization of photosynthesis on a whole-plant level is important when attempting to create a phenotype capable of maximizing yield under stress conditions. With early season heat and drought stress becoming an increasingly likely occurrence (Stratonovitch

and Semenov, 2015), it is critical that an understanding of the relative distribution of photoassimilates at anthesis in wheat, as well as the susceptibility of these assimilates to abiotic stress, is gained. In this study, the starch and water soluble carbohydrates, the products of photosynthesis, were quantified in the flag leaf, peduncle and glume of Seri-Babax wheat under optimal growth conditions and after exposure to heat and drought stress at anthesis.

4.2.2. Starch and Water Soluble Carbohydrates in Wheat

Plants use two distinct strategies for carbohydrate storage in vegetative tissue. The first is in the form of insoluble starch grains, and the second is in the form of sucrose or sucrose derivatives (fructans), collectively classed as water soluble carbohydrates (WSC). Starch is stored in chloroplasts and sucrose is stored in the vacuoles of the mesophyll cells. Starch stored in the chloroplasts is primarily utilized for the synthesis of fructose-6-P, which in turn is used in the regeneration of ribulose-1,5-bisphosphate (RuBP), the substrate of ribulose-1,5-bisphosphate carboxylase/oxgenase (Rubisco). Along with vacuole-based sucrose, starch can also be converted into sucrose through a triose-mediated pathway, which is used to replenish the cytoplasmic sucrose. Once combined with amino acids and soluble minerals, this sucrose can then be loaded into the phloem for transport to non-photosynthetic tissue or tissue acting as a sink. Once unloaded from the phloem at the target site, sucrose can either be utilized for growth and respiration or if there is excess sucrose available, it is stored in a soluble form in vacuoles, as a starch molecule in amyloplasts or as a polymer in the form of proteins.

During the vegetative and up to early reproductive growth phases of cereal plants, carbon is stored temporarily in the stem and leaf tissue whilst awaiting remobilization for transport to sink organs during the period from grain filling to senescence. In the stem, WSC form the largest group of transient carbon assimilate. Pre-anthesis reserves of carbohydrate have been found to contribute up to 27% of the carbon in carbohydrates and 47% of the carbon in the protein of grain (Gebbing and Schnyder, 1999).

Stem storage of carbohydrates in the peduncle acts as a transient stopover location for assimilates moving from source to sink organs. During dark periods, carbohydrates stored in source tissue can be remobilized and transported to sinks throughout the plant, thereby maintaining a continuous supply of assimilates during a 24h period, despite assimilation only taking place during daylight hours. Wardlaw (1990) and Evans (1993) found that between 10 and 30% of the final grain weight could be attributed to carbohydrates temporarily stored in the stems of maize, rice, wheat and sunflower. Under conditions of terminal drought stress, WSCs have been shown to bolster both grain yield and biomass production, two traits closely linked to increased water uptake and water use efficiency (Asseng and Turner, 2007). The ability to remobilize WSC from the stem and leaves has been found to effectively mitigate the negative effects of post-anthesis drought stress on grain filling (Blum, 1998; Araus *et al.,* 2002; Reynolds *et al.,* 2007, Rebetzke *et al.,* 2008). Suboptimal growth conditions during grain-filling can therefore be partially offset by the ability to remobilize carbohydrates and move them into key sink organs, such as grains. Blum (1998) reported that the remobilization capacity of stem-stored carbohydrates ranged from 6-100% depending on the genotype, environmental limitations and experimental protocol. The contribution to grain yield of pre-anthesis reserves in wheat has been reported to be up to 57% (Gallagher *et al.,* 1976).

The assimilate reserve pools also perform a protective role within crops (Hendry and Wallace, 1993; Vergauwen, 2000; Hincha *et al*., 2003; Valluru, 2008) as well as a role in osmoregulation within the plant (Hendry, 1987). The role of fructans in a protective capacity has primarily focused on low temperature and drought tolerance (Hendry and Wallace, 1993), in a large part due to fructans high water solubility. The stabilizing role of sugars in membrane protection against elevated temperature stress has been briefly examined in the past (Santarius, 1973). The occurrence of water-deficit and elevated temperature stress is not only limited to grain filling and may occur earlier in the season as well, during anthesis for example (Stratonovitch and Semenov, 2015). Here, water-deficit may last for several days, which can significantly inhibit photosynthesis. In corn (*Zea mays*) an insufficient supply of assimilates to the reproductive structures in plants can cause abortion of the floral organs (Westgate and Boyer, 1986). Zinselmeier *et al.* (1999) found that the remobilization of starch reserves to the reproductive tissue of corn, supports

reproductive development if photosynthesis is inhibited. McLaughlin and Boyer (2004) detected high levels of both starch and glucose in the reproductive structures of corn. They concluded that high concentrations of these assimilate reserves were utilized by the plant when drought stress occurred, which assisted in preventing floral abortion. The availability of glucose is important in preventing disruption to the metabolism of the reproductive structures in corn. Glucose is an intermediary product of both starch breakdown and the hydrolysis of sucrose via invertase in crops.

Grain filling does not stop during dark periods. Starch accumulation in plant tissue is associated with the provision of carbohydrates to the plant during dark periods (Smith and Stitt, 2007). The diurnal fluctuations in photosynthetic rate and dark respiration in wheat have been well reported (Singh *et al.,* 1993; Deng *et al.,* 2000). During dark periods, or when midday temperature stress reaches a peak and stomatal closure occurs, photosynthesis ceases in wheat (Deng *et al.,* 2000). During these periods, respiration continues to occur and the conversion of photoassimilates is required at all times. In *Poa* species, starch accumulation has been observed during light periods followed by a decline in starch content after the subsequent dark period (Borland and Farrar, 1987). In wheat, Jenner and Rathjen (1972) observed an accumulation of sucrose during the morning, peaking at midday, followed by a decline through the afternoon and the dark period.

The use of WSC and the remobilization capacity as a trait-based tool in breeding programmes has been suggested as a promising approach to identifying genotypes with an increased yield potential and improved grain-filling traits. Several authors have reported quantitative trait loci for the remobilization of WSC in wheat (Salem *et al.,* 2007; Yang *et al.,* 2007; Rebetzke *et al.,* 2008). The genetic variability that exists within the species makes this a likely candidate trait for future crop improvement programs. Selective breeding in wheat for higher stem WSC content has shown potential for increasing yields under drought (Foukes *et al.,* 2002). Although some work has taken place that examines the effect of heat and drought stress on the plant around anthesis (Talukder *et al.,* 2013), more needs to take place in order to expand the current, limited knowledge base on the subject.

Levitt (1972) divided drought resistance strategies into two distinctly different categories: dehydration avoidance and dehydration tolerance. Physiologically speaking, traits that maintain plant hydration are classified as dehydration avoidance strategies. These may include traits such as deep rooting, early flowering or epicuticular wax deposition. Traits that maintain plant functionality despite a loss of water due to adverse water-deficit stress surrounding the plant, even if this is only a partial functionality, are considered dehydration tolerance imparting traits. These include the remobilization of WSC or the production of heat shock proteins and chaperones.

4.2.3. Photoassimilate Partitioning in Wheat Organs

The partitioning, also referred to as allocation, of the carbon assimilated as a result of photosynthesis in a plant, is the balance and movement from where these assimilates are produced (sources) to where the assimilates end up (sinks). A crop such as wheat has several distinct source and sink organs, although all plant tissue is at some stage a sink for photosynthate. All growing tissue can be considered a sink, whereas a source is any plant structure that provides the photoassimilates required for both above and below ground biomass accumulation. Certain organs may act as both a source and sink simultaneously. An example of this is the spike, where the developing grains are sinks for photosynthates, but the tissue surrounding it acts as a source by carrying out the production of assimilates through photosynthesis. Previous studies have identified the spike as being a major contributor to grain filling and an important source of photosynthetic assimilates, not only under drought and heat stress, but also under optimal growing conditions (Araus *et al.,* 1993; Tambussi *et al.,* 2005; Tambussi *et al.,* 2007; Sanchez-Bragado *et al.,* 2014). The contribution of the flag leaf to the production of photoassimilates has been extensively documented. High temperatures have been reported to inhibit assimilate export from leaves (Jiao and Grodzinski, 1996).

The peduncle plays a number of critical roles in wheat development. It is central in the translocation of assimilates from the canopy to the developing grains (Wardlaw, 1990), but it also plays important roles in reducing the risk of leaf-borne pathogenic diseases affecting the spike (Gebbing, 2003) and in maintaining a positive plant

water balance during heat or drought stress by acting as a temporary storage site (Wardlaw, 2002). Depending on the wheat cultivar, Wang *et al.* (2001) suggested that CO_2 assimilation by peduncle may contribute between 9 and 12% of the grain dry mass, highlighting the important photosynthetic capacity of the peduncle.

In the past, little attention has been given to the role of spike photosynthesis in yield development, largely due to the methodological constraints imposed by the complex geometry of the spike compared to other, less complex organs such as the flag leaf. The two primary sites of photosynthetic activity in the spike are the glumes and awns (Gebbing and Schnyder, 2001; Tambussi *et al.,* 2007). In addition to being the key sites of CO_2 fixation in the spike, the glumes may also perform the vital task of re-fixation of CO_2 from respiration occurring in the forming grains (Gebbing and Schnyder, 2001). The presence of awns on wheat cultivars grown in hot, arid environments has been well documented (Grundbacher, 1963). Under these typically adverse conditions, awned lines were found to exhibit greater yield stability than unawned lines (Derera and Stoy, 1973). Furthermore, glumes have been identified as having a superior tolerance to abiotic stress than other plant organs. Under conditions of drought stress, glumes have been observed to maintain a higher relative water content (RWC) than flag leaves (Wardlaw, 2002) as well as exhibiting greater water use efficiency (Bort *et al.,* 1996). In addition, delayed senesce of the glumes compared to the flag leaf during grain filling has been noted, suggesting the relative importance of maintaining glume function to the plant during the latter stages of grain maturation (Bort *et al.,* 1996; Lopes *et al.,* 2006). Blum (1986) concluded that the carbon exchange rate is similar for the flag leaf and glumes but several times greater for the awns. The presence of awns on a spike has been suggested as a suitable selection criterion for wheat grown in hot and arid environments (Blum, 1986), although Rebetzke *et al.* (2016) found that the biomass allocation associated with awn development may have a negative effect on spikelet number and floret fertility under low-stress conditions.

4.2.4. Organ Temperature Depression and Photoassimilates

The ability to synthesize and re-mobilize starch and WSC has the potential to infer temporary stress tolerance to crops. An essential stage in advancing breeding of this

trait will be the ability to identify cereal genotypes that are able to produce and store these assimilates early in the growing cycle and, if required, successfully maintain the photosynthetic production process throughout periods of abiotic stress. Canopy temperature depression (CTD) has been found to be a suitable indicator of both heat stress avoidance (Amani *et al.,* 1996; Reynolds *et al.,* 1998) and drought tolerance (Blum *et al.,* 1989; Rashid *et al.,* 1999). High CTD has been linked to yield increases of wheat (Fischer *et al.* 1998) and consequently high CTD has been used as a selection trait for breeding stress avoidance (Amani *et al.,* 1996; Blum, 1996; Reynolds *et al.,* 1996; Fischer *et al.,* 1998; Rashid *et al.,* 1999; Ayeneh *et al.,* 2002). Spike temperature depression (STD) has been quantified and its potential usage as a screening tool is discussed in detail in Chapter 2 and Chapter 3.

Until now, no efforts have been made to explore the interaction between temperature depression (TD) in the spike and flag leaf, and the photosynthetic assimilate composition in these corresponding organs. This is primarily due to the lack of research focusing on the organ level TD of wheat in a controlled environment (CE). With the knowledge that TD of plant organs is a promising trait to monitor stress avoidance of a crop, it is crucially important to understand how abiotic stress at anthesis influences photoassimilate production and distribution within a plant. The ability of a plant to store photoassimilates at critical development stages, such as at anthesis, as well as the ability to maintain these reserves through short periods of stress, may accord resistance and be a likely candidate for future crop improvement.

4.2.5. Roots and Photoassimilate Partitioning

One of the limitations that must be considered when interpreting results from a pot-based CE experiment, such as the ones outlined in Chapters 2 and 3, is the severe restrictions placed on the roots. Approaching the examination of thermal dynamics at anthesis, photosynthate partitioning and yield stability in wheat with a holistic view is therefore essential.

Lopes and Reynolds (2010) found that a reduction in stem based WSC was associated with increased root weight at depth in field-grown Seri-Babax lines. The

authors concluded that the accumulation of stem-based WSC and a deeper root profile are contrasting drought adaptation strategies. The model of yield under drought developed by Passioura (1977) has led to the widely accepted conclusion that water uptake, principally through investment in deeper root profiles, is the primary driver of yield under water limiting conditions. This premise is however dependent on the assumption that deep water is available within the soil profile. As the ability to remobilize WSC during grain filling will increase the harvest index, WSC have been recognized as a potentially valuable breeding goal. Rebetzke *et al.* (2008) concluded that higher levels of WSC, under drought conditions, are associated with yield when deep water in the soil profile is unavailable. Additionally, Lopes and Reynolds (2010) stated that WSC are accumulated at, or before, anthesis, a period coinciding with the establishment of the mature root profile in wheat. The investment of photoassimilates into WSC would therefore reduce the availability of photoassimilates for root development and *vice versa*. Consequently, the accumulation of stem WSC and a deep root profile may be considered as contrasting, not complimentary, approaches to drought adaptation.

As the stem is the largest potential sink of WSC in wheat, the dynamics of stem and leaf tissue storage have been extensively examined (Kühbauch & Thome, 1989; Bancal & Triboi, 1993). The examination of non-leaf photosynthetic organs' contribution of grain yield has recently drawn much attention (Parry *et al*., 2011). The significant contribution that the spike, consisting of the photosynthetically active glumes and awns, has on net photosynthesis has been demonstrated (Tambussi *et al*., 2007). Changes in the sucrose content between dark and light periods, as well as the unique ability of the glumes to re-fix respiratory CO_2 for photosynthesis, has been reported (Gebbing and Schnyder, 2001). Gebbing *et al*. (1998) reported that glumes contribute only 5% of net WSC mobilization from the above ground biomass, compared to 24% from the leaf sheaths and 67% from the stem. Although some work has examined the dynamics of assimilate partitioning in multiple wheat organs (Sanchez-Bragado *et al*., 2014), information relating to the effect of abiotic stress at anthesis on the composition of starch and WSC in the flag leaf, peduncle and glumes is lacking.

4.2.6. Chapter Objectives

The objectives of this chapter are therefore to:

a) Examine how the composition, at mid-anthesis, of starch and WSC in the flag leaf, peduncle and glumes is affected by abiotic stress from tissue samples collected during Experiments 2 and 4;
b) Identify the relationship between organ starch/WSC content and grain yield, under contrasting conditions of abiotic stress at anthesis.

4.3. MATERIALS AND METHODOLOGY

4.3.1. Summary of Growing Conditions

A detailed description outlining the crop husbandry practices and environmental conditions in which the plant material used for this chapter was grown can be found in Chapter 2.3 and Chapter 3.3. The growing conditions for Experiment 2 and Experiment 4 are briefly summarized below.

4.3.1.1. Experiment 2

Three lines (SB020, SB155 and SB165) of the Mexican spring wheat cultivar Seri-Babax (SB) were sown into 180mm plastic pots and grown in Saxcil growth cabinets at Plant Environment Laboratory (UoR, UK). The pots were filled with a vermiculite, sand, gravel and compost mix (2:1:2:0.5 ratio). The plants were thoroughly watered during the period up to anthesis and grown at 25°C/16°C day/night cycle with a 16h photoperiod. At the start of anthesis, four treatments were applied to the plants: ambient glasshouse temperature (25°C/16°C day/night cycle) with full irrigation (AT+WW), ambient glasshouse temperature with reduced irrigation (AT+WS), elevated temperature (35°C/26°C day/night cycle) with full irrigation (HT+WW) and elevated temperature with reduced irrigation (HT+WS). At mid-anthesis, tissue samples from the flag, peduncle and glumes were harvested, frozen in liquid nitrogen and stored in a -80°C freezer. Upon completion of anthesis, the plants were returned to a growing regime consisting of full irrigation and day/night temperatures at 25°C/20°C. A comprehensive description of Experiment 2 can be found in Chapter 2.3.

4.3.1.2. Experiment 4

Two lines SB (SB020 and SB165) were grown in rhizoboxes in a naturally lit glasshouse at the Plant Environment Laboratory (UoR, UK). The rhizoboxes were constructed out of 5mm thick polyvinyl chloride and measured 1.0m tall, 0.3m wide and 0.15m deep, with the front wall made from 5mm thick clear-as-glass acrylic. The plants were grown in a soil classified as sandy agricultural topsoil with the

addition of slow-release fertilizer granules. The rhizoboxes were individually wrapped in an adjustable reflective sleeve made of a high temperature resistant insulation material and supported on steel stands at a 30° angle, with 0.1m between the boxes. The plants were grown in the glasshouse at ambient day/night temperatures with a 14h photoperiod and thorough irrigation up to 10 days before anthesis. At the start of anthesis, four treatments were applied to the plants: ambient glasshouse temperature with full irrigation (AT+WW), ambient glasshouse temperature with reduced irrigation (AT+WS), elevated temperature (35°C between 11.00-14.00h) with full irrigation (HT+WW) and elevated temperature with reduced irrigation (HT+WS). At mid-anthesis, tissue samples from the flag leaf, peduncle and glumes were harvested, frozen in liquid nitrogen and stored in a -80°C freezer. Upon completion of anthesis, the plants were returned to a growing regime with ambient temperatures and full irrigation. A comprehensive description of Experiment 4 can be found in Chapter 3.3.

4.3.2. Starch Determination Protocol

The following protocol used was based on a modified Megazyme starch assay protocol. The protocol was modified to allow for sample preparation using 2ml microcentrifuge compatible tubes that are readily available in most laboratories. The methodology used required the preparation of three solutions (sodium acetate buffer, potassium hydroxide solution and GOPOD reagent solution) in order to determine starch content. Preparation took place according to the manufacturers instructions. Details relating to how the three solutions were prepared can be found in the Appendix (Protocols for Megazyme Solution Preparation).

Tissue samples of the glume, flag leaf and peduncle were harvested at mid-anthesis (GS65) within three hours of the onset of daylight. The samples were flash frozen in 15ml Falcon tubes before being stored in a -80˚C freezer until analysis. In order to standardize sample collection, the guidelines detailed in Chapter 2.3.6 were used when determining how a single replicate of each plant organ was collected.

Once in a laboratory environment, the tissue samples were rapidly transferred into labelled and pre-weighed 2ml microcentrifuge tubes and returned to the -80˚C

freezer until all samples were in 2ml microcentrifuge tubes, ready for the next stage. The samples were freeze-dried according to the manufacturers instructions for a total of 46h at -20˚C and 1.0bar pressure. Once complete, a single 6mm ceria stabilized zirconium oxide grinding satellite (OPS Diagnostics, Lebanon, NJ, USA) was added to each tube before the sample was finely powdered using a bench top grinding mill.

A series of 2ml screw-cap microcentrifuge tubes were labelled and their weights recorded. 20mg of the freeze-dried, powdered sample was added to each corresponding 2ml screw-cap tube described in the previous stage. The weight of the plant tissue in each tube varied between 18mg and 22mg, with the actual weight accurately recorded. Processed in batches of 24, 1ml of aqueous ethanol (80% v/v) was dispensed to each microcentrifuge tube before the tubes were incubated in a heating block at 80˚C for 20 minutes with intermittent vortexing and left to cool. Once cooled, the tubes were centrifuged at 14,000rpm for 5 minutes. Ensuring that none of the pellet at the base of the tube was disturbed, the supernatant of each tube was transferred into a correspondingly pre-labelled and weighed 15ml disposable plastic centrifuge tube. A further 1ml of aqueous ethanol (80% v/v) was added to each tube containing the pellet before being thoroughly vortexed. The tubes were centrifuged as above and the supernatant was carefully transferred and combined with the previous supernatant. A further 1ml of aqueous ethanol (80% v/v) was added to each tube containing the pellet before being vortexed and incubated in a heating block at 80˚C for 10 minutes, again with intermittent vortexing. The tubes were centrifuged and the supernatant transferred, as described above. A final 1ml of aqueous ethanol (80% v/v) was added to each tube containing the pellet before the tubes were vortexed and centrifuged, as described previously, and the supernatant transferred into the 15ml disposable plastic centrifuge tube, each of which now contained just under 4ml of supernatant. The micro centrifuge tubes containing the pellet were placed in a centrifugal evaporator and dried, then kept at 4˚C. Each of the tubes containing the supernatant was weighed and the weights recorded. 0.5ml of the supernatant in each tube was transferred to a pre-labelled 2ml microcentrifuge tube. These were placed in a centrifugal evaporator, with the lids off and the heating unit on, until all of the solvent had evaporated. Once evaporated, 0.5ml of distilled water was added to each tube before being vortexed vigorously in order to rehydrate

the soluble sugar residue. The microcentrifuge tubes containing the rehydrated soluble sugars were stored at -20˚C until further processing.

A volume of 400µl of 0.2M KOH was dispensed into each tube in order to rehydrate the starch pellet before being vortexed and incubated in a 90˚C water bath for 15 minutes. Once cool, 1.28ml of 0.15M sodium acetate buffer was added to each tube and vortexed. Immediately, 20µl of thermostable alpha-amylase and 20µl amyloglucosidase was added to each tube, vortexed and placed in a 50˚C heating block for 30 minutes with intermittent vortexing. Once cool, the tubes were again vortexed and centrifuged at 14,000rpm for 15 minutes. 100µl of the supernatant was then transferred to pre-labelled 1.5ml microfuge tubes. 600µl of the GOPOD reagent solution was added to each tube, shaken briefly and then placed in a heating block at 50˚C for a further 30 minutes. Once cool, a 200µl aliquot of the sample was pipetted in triple replicate onto a 96 well microtitre plate. Along with the starch aliquots, a series of blanks and D-glucose controls were included on each microtitre plate used. The D-glucose controls were prepared using diluted volumes of D-glucose standard solution (1.0mg/ml in 0.2% (w/v) benzoic acid) and water to make controls, which were 0.00, 1.11, 2.22, 3.33, 4.44 and 5.55mM. As with the sample preparation described above, 600µl of the GOPOD reagent solution was added to each of the D-glucose controls, before vortexing, incubation in a 50˚C heating block for 20 minutes and left to cool. The absorbance was read and recorded at 510nm in a microplate reader within 60 minutes of colour formation.

4.3.3. High Sensitivity Starch Determination Protocol

The initial starch absorbance reading of certain samples was found to be below the lowest D-glucose reading on the calibration curve (1µl). Consequently, two stages of the protocol were modified to allow detection of starch using a high sensitivity assay (HSA).

The first stage of developing this HSA was by making a GOPOD reagent solution that was 4 times as concentrated as the GOPOD reagent solution described in section 4.3.2 (above). 50ml of the GOPOD reagent buffer was added to a 0.5L glass bottle tightly covered in aluminium foil, containing 250ml of distilled water. 20ml

of this concentrated GOPOD reagent buffer was then added to the GOPOD reagent enzymes supplied by Megazyme. The contents of this bottle were then quantitatively transferred into the 0.5L glass bottle containing the concentrated GOPOD reagent buffer solution. The bottle containing the concentrated GOPOD reagent solution was kept in a fridge at 4˚C.

The second modification required to transfer the standard protocol into the HSA, focused on the ratio between the sample solution and the concentrated GOPOD reagent solution. The total volume was maintained (700µl), but the sample solution volume increased to 550µl and the amount of GOPOD reagent solution decreased to 150µl.

Finally, in order to detect the starch using this new HSA, a new series of D-glucose standards had to be created that contained the same quantity of D-glucose in the same volume used for the samples (550µl). Thus 0, 0.5, 1, 2, 4 and 8µl of D-glucose standard solution (1.0mg/ml in 0.2% (w/v) benzoic acid) were added to 550, 549.5, 549, 548, 546 and 542µl of distilled water, respectively.

4.3.4. Water Soluble Carbohydrate Determination Protocol

The following protocol describes the methodology used for determining soluble sugar content of samples through enzymic analysis and can be traced back to Jones *et al.* (1977). The process involves the stepwise addition of hexokinase (HK), phosphoglucose isomerase (PGI) and invertase (INV) to a buffered solution containing the sample, along with adenosine triphosphate (ATP), nicotinamide adenine dinucleotide (NAD^+) and glucose-6-phosphate dehydrogenase (G6PD). The change in absorbance at 340nm as a result of each enzymic addition, allows for the determination of the glucose, sucrose and fructose content of each sample. Similarly to the starch determination protocol described in 4.3.2 (above), this protocol is accurate and only requires the use of equipment readily available in most laboratories.

The methodology used required the preparation of a buffer in order to determine soluble sugar content. The buffer was prepared at the start of the procedure and its preparation is outlined below:

Imidazole/$MgCl_2$ Buffer Preparation

A 100ml imidazole/$MgCl_2$ assay buffer (100mM, pH 7.5) was made by adding 10ml of imidazole (1M) and 1ml of $MgCl_2.6H_2O$ (1M) to 80ml of water. The pH was adjusted to 7.5 using HCl (1M) and the volume of the imidazole/$MgCl_2$ assay buffer was made up to 100ml.

On each day of sample analysis, a fresh batch of the assay buffer was prepared using the Imidazole/$MgCl_2$ buffer described above. The components required to produce a single 200µl aliquot of this assay buffer (the amount pipetted into each microplate well) is outlined in Table 4.1.

Table 4.1 – The components of a single 200µl aliquot of the assay buffer used.

Assay buffer component	Volume required for 1x 200µl assay (µl)
Imidazole/$MgCl_2$ buffer	195
ATP	2
NAD^+	2
G6PD	1

The PGI used did not require any dilution of the 700U/ml stock, so that only as much PGI required for the day was transferred from its container to a labelled 2ml tube. As soon as they were prepared, the assay buffer and all the enzymes used were kept at 4˚C. The HK and the INV however, required additional preparation from their stock state before they could be used. The procedure detailing the preparation of these enzymes is detailed below.

HK Preparation

The HK used, consisted of a 2-fold dilution of the 1500U/ml HK with the imidazole/$MgCl_2$ buffer.

INV Preparation

A fresh solution of INV was prepared at the start of each day of sample analysis. The INV used (6000U/ml), was made by combining 20mg of the 500U/mg INV crystalline stock to 1ml of the imidazole/$MgCl_2$ buffer.

On the day of analysis, the samples to be assayed were removed from the -20˚C freezer and left to thaw for approximately 1 hour. Once fully thawed, the samples were vortexed and then clarified in a centrifuge for 5 minutes at 14,000rpm. For each sample, a 20µl aliquot of the clear supernatant was pipetted in triple replicate onto a 96 well microtitre plate. Along with the 20µl sample aliquots, a series of 20µl D-glucose and sucrose controls were included on each microtitre plate used. The D-glucose controls were prepared using diluted volumes of D-glucose standard solution (1.0mg/ml in 0.2% (w/v) benzoic acid) and water to make controls that were 0.00, 1.11, 2.22, 3.33, 4.44 and 5.55mM. Similarly, a series of 20µl sucrose controls were included on each microtitre plate used. The sucrose controls were prepared by the addition of 0, 30, 60, 120, 240 and 480µl of 5.0mM sucrose solution to 600, 570, 540, 480, 360 and 120µl of distilled water, respectively. The sucrose controls used represented 0, 0.25, 0.5, 1, 2 and 4mM sucrose, respectively. 200µl of the assay buffer solution was added to each well on the microplate and, once stabilized, the initial absorbance at 340nm was recorded in a microplate reader. Subsequently, 1.5µl of the HK solution was pipetted into each well, mixed well and the absorbance was monitored until stable (approximately 20 minutes). The endpoint absorbance was read and recorded at 340nm in a microplate reader 25 minutes after the addition of the HK solution. Following this step, 1.5µl of PGI was added to each well before being mixed thoroughly and the absorbance monitored until stable. Similarly to the HK, the endpoint absorbance for the PGI was recorded after approximately 25 minutes. 1.5µl of the INV solution was then added to each

well, mixed and again, the absorbance was monitored until stable. The final endpoint absorbance was recorded as well.

4.3.5. Calculations

4.3.5.1. Starch Calculations

In order to convert the absorbance readings from the microplate reader into starch values, the calculations from the Megazyme Total Starch Assay Kit had to be adapted to suit the modified protocol used. First, a calibration curve had to be created in order to estimate the D-glucose content of the samples. The D-glucose standards (in µl) used had to be converted into nmol by multiplying by a factor of 5.5. The absorbance readings were then plotted against the D-glucose standards.

Once plotted, the equation obtained from the calibration curve allowed for the calculation of glucose in each sample using the following formula:

$$\text{D-Glucose (nmol)} = \frac{\Delta A - c}{m}$$

where ΔA is the absorbance at 510nm read against the reagent blank, c is the y-intercept obtained from the calibration curve, and m is the slope of the calibration curve equation.

The volumes and mass of dry matter used had to be accounted for in order to normalize the readings. The following formula was therefore applied to calculate the starch content of the sample:

$$\text{Starch (mg/g DW)} = D-glucose\ (nmol) \times \frac{GOPOD\ V}{Sample\ V} \times FW \times TW \times MWG \times \frac{162}{180} \times \frac{1}{1000}$$

where GOPOD V is the volume of GOPOD reagent used (µl), Sample V is the volume of sample solution used (µl), FV is the final volume, TW is the tissue weight used, $\frac{1}{1000}$ is the conversion from µg to mg, MWG is the molecular weight of

glucose, $\frac{162}{180}$ is the adjustment from free D-glucose to anhydro D-glucose (as found in starch).

4.3.5.2. Water Soluble Carbohydrate Calculations

Figure 4.1 (see Appendix) summarizes the coupled assays utilized for the determination of WSC in a sample. The individual reactions taking place during the determination of sample soluble sugar content, are detailed in Fig. 4.2 below:

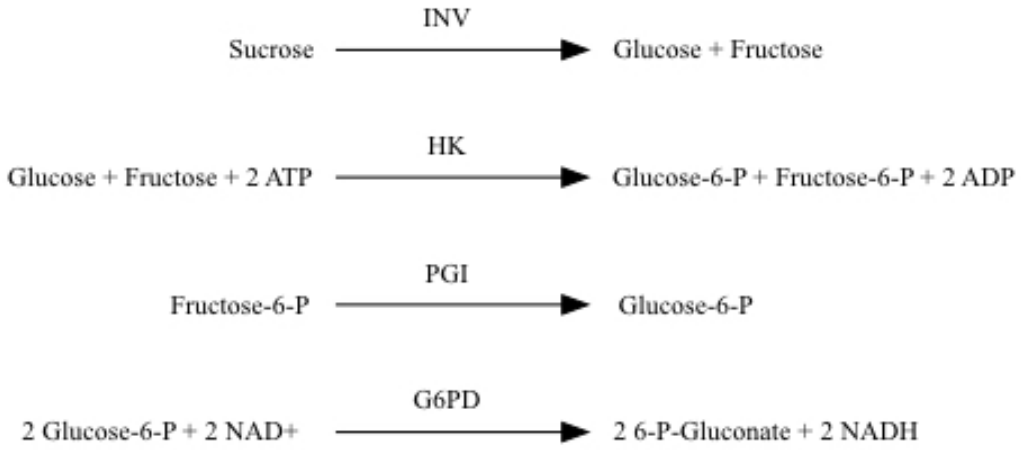

Fig. 4.2 – The individual enzyme-driven reactions occurring during the determination of soluble sugar content. Adapted from Jones *et al.* (1977).

A calibration curve had to be created in order to estimate the D-glucose content of the samples. The absorbance readings were then plotted against the D-glucose standards. Once plotted, the equation obtained from the calibration curve allowed for the calculation of glucose in each sample using the following formula:

$$\text{D-Glucose (nmol)} = \frac{\Delta A - c}{m}$$

where ΔA is the absorbance at 510nm read against the reagent blank, c is the y-intercept obtained from the calibration curve, and, m is the slope of the equation obtained in the calibration curve equation.

The glucose content was calculated by subtracting the amount of glucose measured immediately before the addition of HK from the amount measured after the addition of HK. The fructose content was calculated by subtracting the amount of glucose measured immediately before the addition of PGI from the amount measured after the addition of PGI. The sucrose content was calculated by subtracting the amount of glucose measured immediately before the addition of INV from the amount measured after the addition of INV.

Finally, the volumes and mass of dry matter used had to be accounted for in order to normalise the readings. The following formula was therefore applied to calculate the WSC content of the sample:

$$\text{WSC (mg/g DW)} = GCont/FCont\ /SCont \times \frac{Eth\ V}{Sample\ V} \times TW \times MW \times \frac{1}{1000}$$

where EthV is the total volume of 80% ethanol used for extraction (µl), SampleV is the volume of the sample extract used in the coupled assay (µl), TW is the original mass of the plant DW tissue used, MW is the molecular weight of either glucose, sucrose or fructose, depending on which is being calculated and $\frac{1}{1000}$ is the conversion from µg to mg,

4.3.6. Statistical Analysis

REML, ANOVA and correlation analysis were performed using Genstat Version 16 (VSN International, Hemel Hemstead, UK). GraphPad Prism (GraphPad Software, San Diego, CA, USA) was used as a supplementary graphing tool. For the Experiment 2, separate pots within growth cabinets were considered independent replicates. For Experiment 4, separate boxes within individual growth tents were considered independent replicates. Effects were considered to be significant at $P<0.05$.

4.4. RESULTS

4.4.1. Starch Content

4.4.1.1. Experiment 2

When categorized by genotype, significant differences in the total starch content of the tissue was identified (*P*=0.017). SB020 was the genotype that displayed the highest starch concentration (7.41mg/g DW) followed by SB165 (5.70mg/g DW) and finally SB155 (3.41mg/g DW). When the starch concentration of the organs examined was compared, significant differences were identified (*P*=0.025). The highest concentration of starch was found in the glumes (7.54mg/g DW), followed by the flag leaf (5.19mg/g DW) and finally the peduncle (3.78mg/g DW). However, no significant differences were identified between organs of separate genotypes (*P*=0.271). No significant difference in starch concentration was identified due to the HT treatments applied (*P*=0.113). HT was not found to significantly affect starch concentration amongst genotypes (*P*=0.867) and similarly, HT failed to significantly impact starch concentrations between the organs examined (*P*=0.738). Similarly, when starch concentration was categorized by irrigation treatment, no significant differences were identified (*P*=0.371). Irrigation treatments failed to result in any significant differences being uncovered for either the plant organs (*P*=0.963) or the genotypes (*P*=0.197) examined.

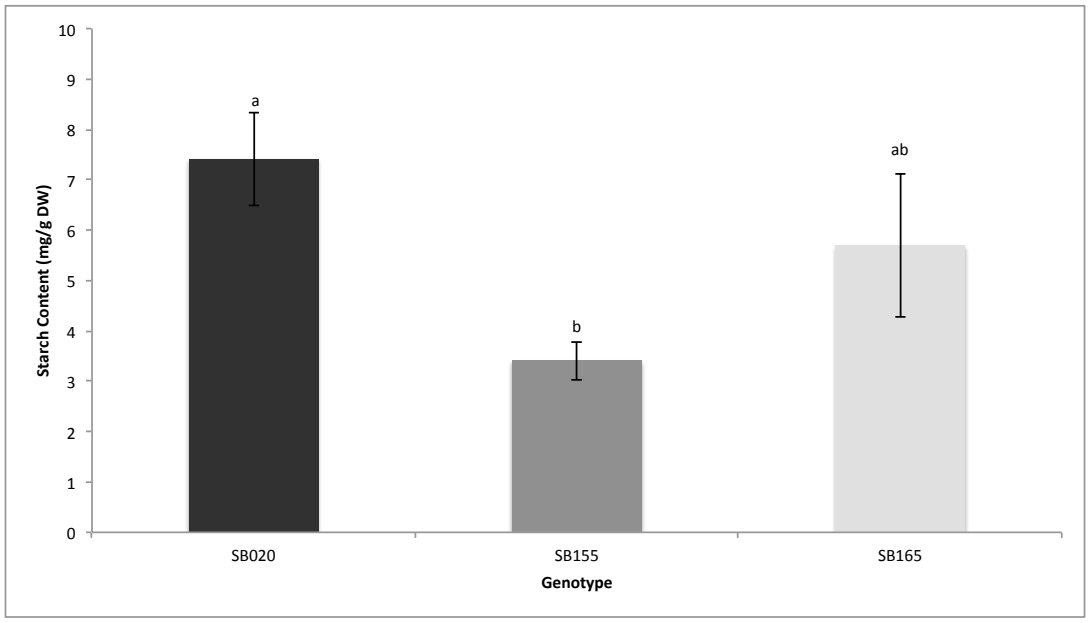

Fig. 4.3 – The pooled organ starch content of each genotype across all treatment combination, recorded at mid-anthesis in Experiment 2. Error bars represent standard error. Letters indicate significant difference at *P*<0.05.

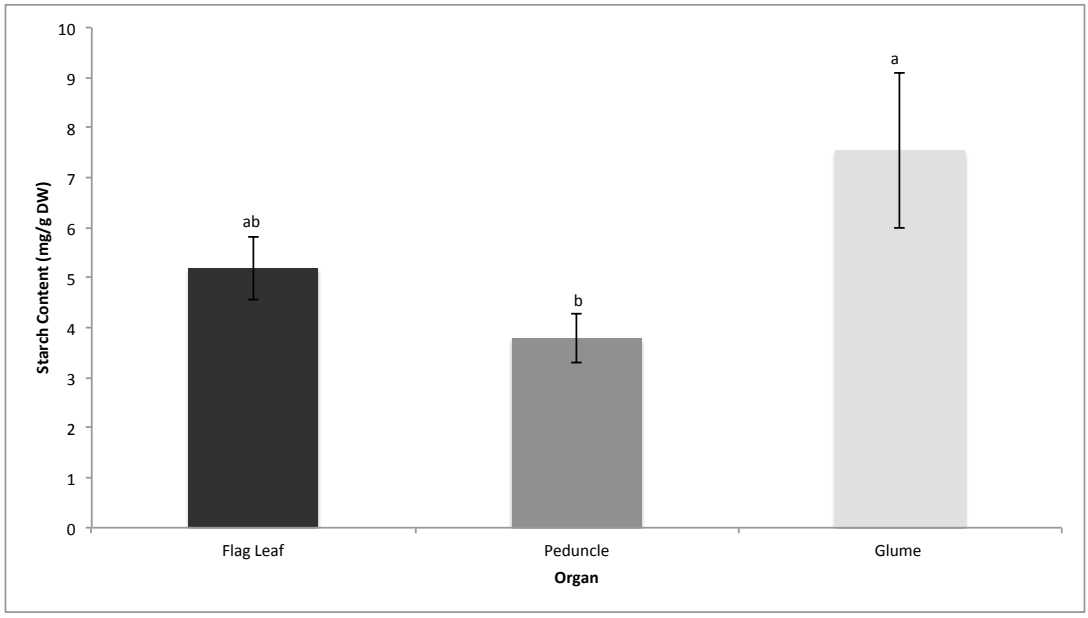

Fig. 4.4 – The mean genotypic flag leaf, peduncle and glume starch content across all treatment combinations recorded at mid-anthesis in Experiment 2. Error bars represent standard error. Letters indicate significant difference at *P*<0.05.

4.4.1.2. Experiment 4

When categorized by plant organ, significant differences were identified between the flag leaf, peduncle and glumes ($P<0.001$). The starch content of the glumes of both SB020 and SB165 was significantly higher than in the flag leaf and peduncle of either genotype (7.86 and 9.25mg/g DW, respectively). However, no significant differences in the starch concentration of organs were identified between genotypes ($P=0.681$). Genotypes displayed no significant differences in the combined starch contents of the flag leaf, peduncle and glumes when categorized by heat and irrigation treatments ($P=0.828$). Under optimal growing conditions (AT+WW), there were no significant differences in starch content between the two genotypes ($P=0.471$). There were no significant differences in combined starch content as a result of the heat treatments applied ($P=0.667$). Furthermore, there was no significant effect identified as a result of HT treatment on starch content between both genotypes and the organs investigated ($P=0.921$ and $P=0.994$, respectively). Although slightly higher in the WW treatment compared to the WS treatment, no significant differences in starch content between irrigation treatments were identified ($P=0.082$). Similarly, no effects were observed when the interaction between genotypes and irrigation treatments were examined ($P=0.349$). Irrigation treatments were not found to significantly affect the starch concentrations in plant organs either ($P=0.580$).

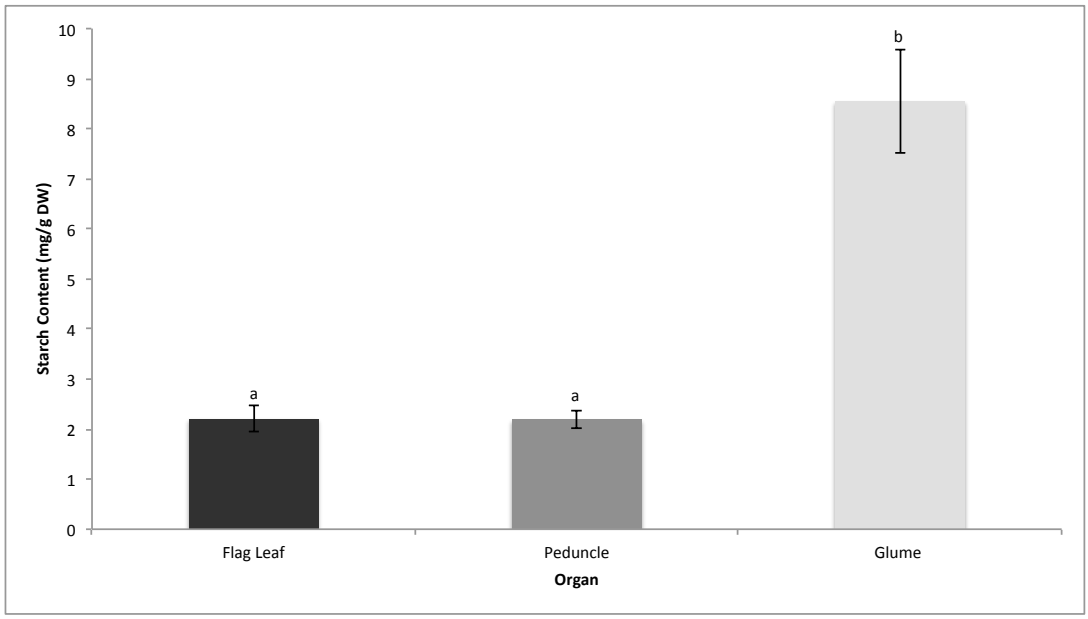

Fig. 4.5 – The mean genotypic starch content of the flag leaf, peduncle and glume across all treatment combinations, recorded at mid-anthesis in Experiment 4. Error bars represent standard error. Letters indicate significant difference at *P*<0.05.

4.4.1.3. Total Starch Content per Plant

When the starch content of the flag leaf, peduncle and glumes were pooled into total starch content on a per plant basis, significant differences were identified between genotypes (*P*=0.001) with SB020 containing significantly more total starch per plant (21.39mg/g DW) than SB155 (10.23mg/g DW) or SB165 (13.18mg/g DW). The two irrigation treatments applied resulted in significant differences to the total starch content (*P*=0.008) with WS resulting in a lower total starch content (12.40mg/g DW) than the WW treatment (17.47mg/g DW). The combined effect of the two temperature and irrigation treatments applied at anthesis resulted in significant differences in total starch content (*P*=0.015). Plants in the AT+WW treatment contained significantly more total starch (21.41mg/g DW) than the other three treatment combinations. In Experiment 4, total starch content per plant did not differ between genotypes (*P*=0.398) or a result of the temperature (*P*=0.612) and water-deficit (*P*=0.105) treatments imposed. There was no significant interaction identified between the treatments (*P*=0.869) or between the interaction of the genotypes and the treatments (*P*=0.789).

4.4.2. Water Soluble Carbohydrate Content

4.4.2.1. Experiment 2

No significant genotypic differences in glucose content were found between the genotypes examined (P=0.365) in Experiment 2. Similarly, the temperature and irrigation treatments did not significantly affect glucose content (P=0.261 and P=0.740, respectively). Significant differences were identified between the organs examined (P=0.005), with the flag leaf containing the lowest amount of glucose (1.20mg/g DW), followed by the peduncle (1.53mg/g DW) and finally the glumes (2.01mg/g DW). No significant differences in sucrose content were identified between the genotypes examined (P=0.084), the temperature and irrigation treatments applied (P=0.120 and P=0.077, respectively) or between the organs (P=0.207). No significant genotypic differences in fructose content were found between the genotypes examined (P=0.588). Similarly, the temperature and irrigation treatments did not significantly affect fructose content (P=0.564 and P=0.693, respectively). Significant differences were identified between the organs examined (P=0.002). The fructose content was significantly greater in the glumes (1.06mg/g DW) than in the flag leaves or peduncle (0.69 and 0.79mg/g DW, respectively). Fig. 4.6 illustrates the pooled genotypic and treatment glucose, sucrose and fructose content of the flag leaf, peduncle and glumes recorded in Experiment 2. Fig. 4.7 illustrates the pooled genotypic and treatment glucose, sucrose and fructose content recorded in the two temperature and irrigation treatments applied in Experiment 2.

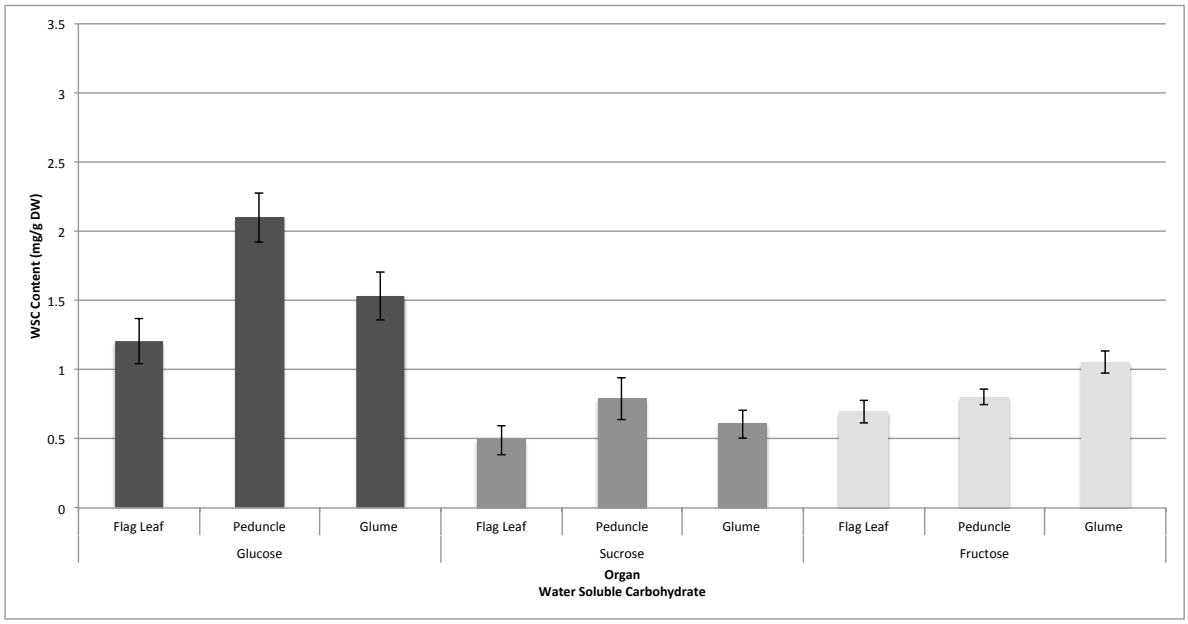

Fig. 4.6 – The pooled genotypic and treatment glucose, sucrose and fructose content of the flag leaf, peduncle and glumes recorded in Experiment 2. Error bars represent standard error.

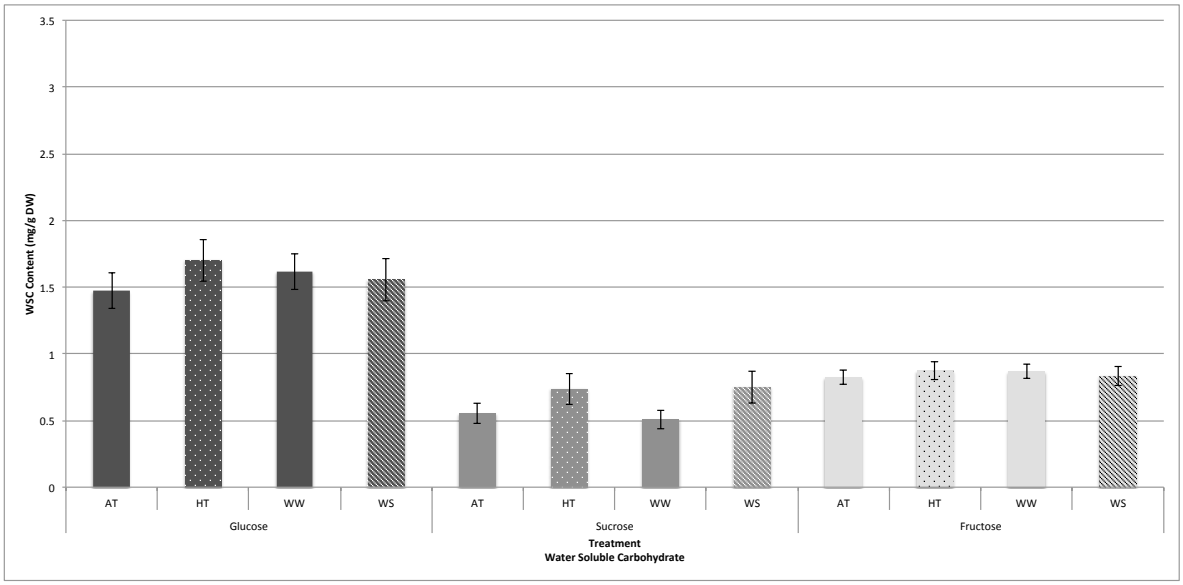

Fig. 4.7 – The pooled genotypic and treatment glucose, sucrose and fructose content of the flag leaf, peduncle and glumes in the two temperature and irrigation treatments applied in Experiment 2. Error bars represent standard error.

4.4.2.2. Experiment 4

In Experiment 4, significant differences in glucose content were identified between the two temperature treatments ($P<0.001$), as well as between the organs examined ($P<0.001$). The HT treatment resulted in significantly lower glucose content (1.37mg/g DW) than was measured in the AT treatment (2.21mg/g DW). The organ with the lowest glucose content was the flag leaf (0.70mg/g DW) with the peduncle containing slightly more (1.97mg/g DW). The glumes contained the highest concentration of glucose (2.70mg/g DW). Glucose content varied significantly between the irrigation treatments ($P=0.023$), with WS resulting in significantly less glucose (1.53mg/g DW) than in the WW treatment (2.06mg/g DW). No significant genotypic differences in glucose content were identified ($P=0.824$). No significant genotypic differences in sucrose content were identified ($P=0.918$). Similarly, no significant differences in sucrose content were identified between the two temperature ($P=0.668$) and irrigation treatments ($P=0.981$) applied. Sucrose content did not differ between the organs examined either ($P=0.094$). Fructose content did not differ significantly between the genotypes examined ($P=0.595$) or as a result of the two temperature treatments applied ($P=0.396$). Significant differences in fructose content were however found between the two irrigation treatments ($P=0.013$), with the fructose content being significantly lower in the WS treatment (0.69mg/g DW) compared to the WW treatment (0.90mg/g DW). Fructose content differed significantly between the three organs examined ($P=0.004$), with the content being the highest in the glumes (0.94mg/g DW) followed by the peduncle (0.85mg/g DW) and finally the flag leaf (0.60mg/g DW). Fig. 4.8 illustrates the pooled genotypic glucose, sucrose and fructose content of the flag leaf, peduncle and glumes recorded in Experiment 4. Fig. 4.9 illustrates the glucose, sucrose and fructose content recorded in the two temperature and irrigation treatments applied in Experiment 4.

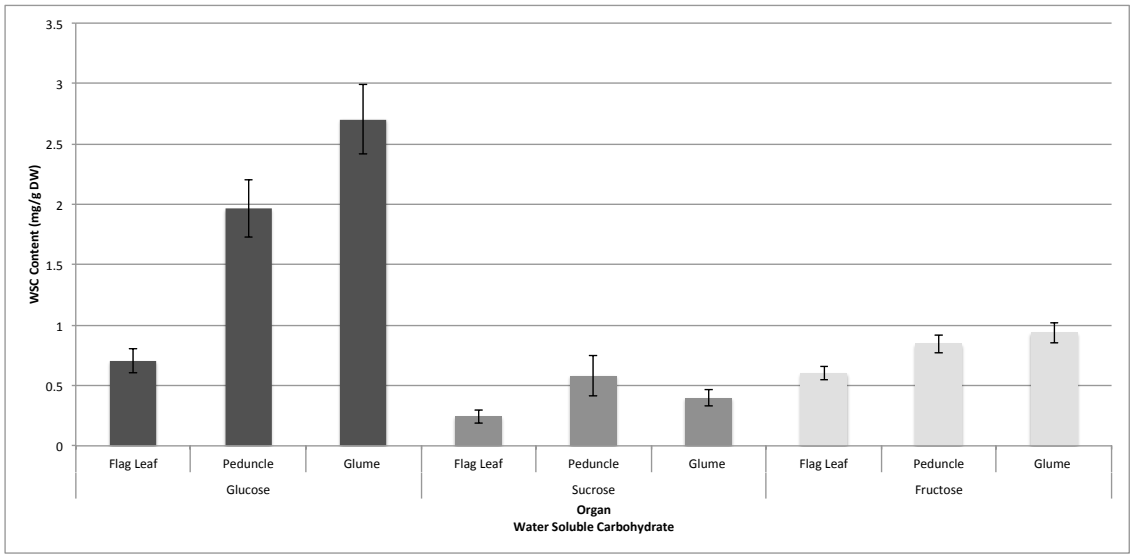

Fig. 4.8 – The pooled genotypic and treatment glucose, sucrose and fructose content of the flag leaf, peduncle and glumes recorded in Experiment 4. Error bars represent standard error.

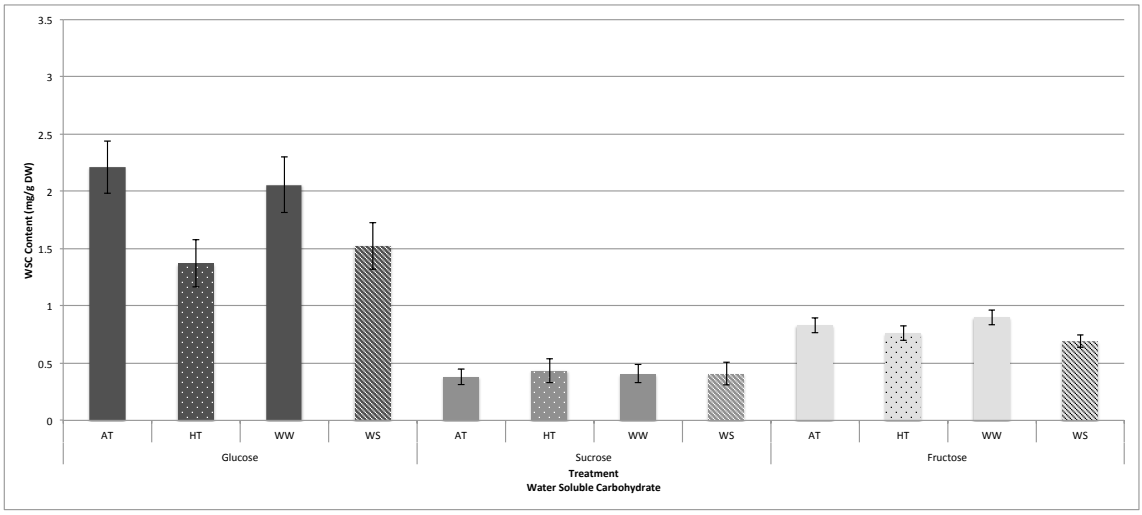

Fig. 4.9 – The pooled genotypic glucose, sucrose and fructose content of the flag leaf, peduncle and glumes in the two temperature and irrigation treatments applied in Experiment 4. Error bars represent standard error.

4.4.2.3. Total WSC Content per Plant

When the sucrose, glucose and fructose contents of the three organs were pooled into total WSC content on a per plant basis, no significant differences were identified in Experiment 2 between the genotypes (P=0.191), temperature or water-deficit treatments (P=0.164 and P=0.658, respectively) or as a result of the

interaction between the treatments (*P*=0.580) and the genotypes (*P*=0.979). Total WSC per plant was unaffected by the treatments imposed at mid-anthesis. In Experiment 4, the total WSC per plant did not vary between genotypes (*P*=0.842) but the temperature and irrigation treatments applied significantly affected total WSC content per plant (*P*=0.024 and *P*=0.48, respectively). The elevated temperature treatment (HT) significantly reduced total WSC (7.69mg/g DW) compared to the AT treatment (10.35mg/g DW). Similarly, the presence of water-deficit stress in the WS treatment (7.87mg/g DW), reduced the total WSC per plant significantly compared to the WW treatment (10.17mg/g DW).

When the ratio of WSC to starch content was examined for both experiments, no significant differences were identified between genotypes or the treatments applied.

4.4.2.4. Total Glucose, Sucrose and Fructose Content per Plant

In Experiment 2, no genotypic differences in glucose, sucrose or fructose were identified (*P*=0.421, *P*=0.597 and *P*=0.106, respectively). No significant difference existed between the total glucose, sucrose or fructose content per plant as a result of the two contrasting temperature (*P*=0.273, *P*=0.570 and *P*=0.135, respectively) or irrigation treatments applied at anthesis (*P*=0.783, *P*=0.697 and *P*=0.090, respectively). Similarly, the interaction between genotypes and treatments did not result in significant differences in total sugar content per plant. As in Experiment 2, no genotypic differences in glucose, sucrose or fructose were identified in Experiment 4 (*P*=0.933, *P*=0.903 and *P*=0.622, respectively). However in the rhizobox grown wheat, significant differences existed between the two temperature treatments (*P*=0.005), with total glucose per plant being significantly reduced in the HT treatment (4.11mg/g DW) than in the AT treatment (6.72mg/g DW). The two temperature and irrigation treatments had no significant effect on total sucrose per plant (*P*=0.616 and *P*=0.977, respectively). Total fructose per plant was unaffected by the temperature treatments applied (*P*=0.434) whilst the difference between the irrigation treatment resulted in significant differences (*P*=0.028). Restriction of irrigation in the WS treatment (2.08mg/g DW) resulted in significantly less fructose per plant than in the WW treatment (2.70mg/g DW).

4.4.3. Starch Content and Grain Yield

4.4.3.1 Total Starch Content

4.4.3.1.1 Experiment 2

A significant relationship between the total starch content (comprised of flag leaf, peduncle and glume starch content, see 4.4.1.3) and the grain yield was found for SB155 (P=0.021) whilst no relationship was identified for SB020 (P=0.215) and SB165 (P=0.561). Fig. 4.10 illustrates this significant relationship.

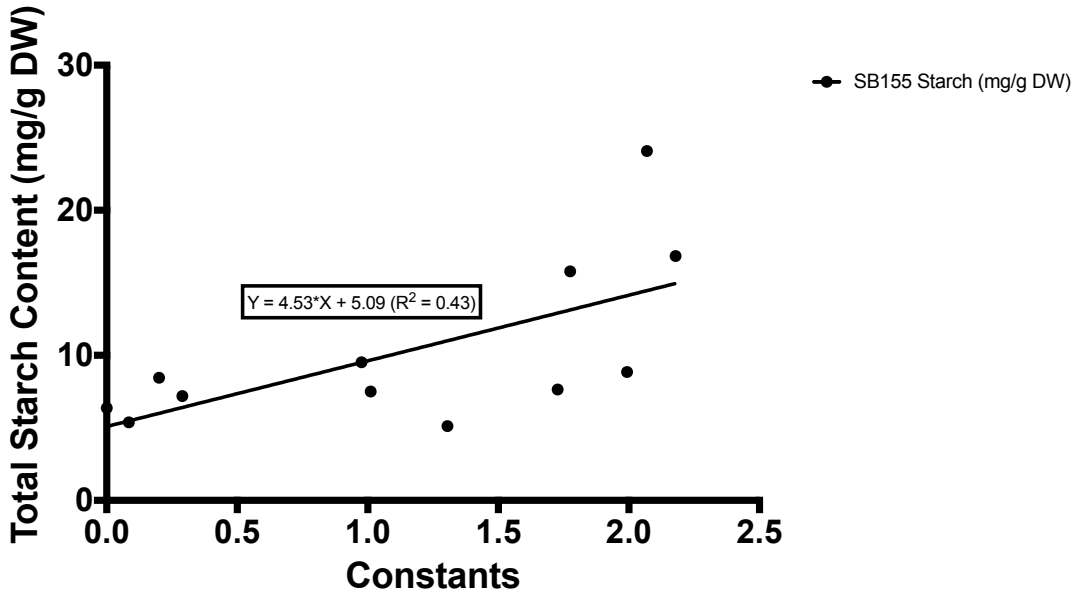

Fig. 4.10 – The significant relationship identified between total starch content of SB155 and grain yield, categorized by genotype, in Experiment 2.

When the relationship of whole plant starch content and grain yield was categorized by the temperature treatment, the effects of the AT treatment (P=0.105) and the HT treatment (P=0.437) were not significant. Similarly, the relationship between the starch content of the whole plant and grain yield was not significant in the WW (P=0.116) or the WS (P=0.585) treatments.

4.4.3.1.2 Experiment 4

When data was categorized by genotype, temperature or irrigation treatment type, no significant relationships between total starch content per plant and grain yield were found in Experiment 4.

4.4.3.2 Organ Starch Content

The relationship between the starch content of individual organs and grain yield was examined for both Experiment 2 and 4. This was performed in order to examine whether increased organ starch and (in section 4.4.4.2) WSC content are related to higher grain yields which, if true, may be of significance to wheat breeders attempting to confer increased heat or drought resistance to a population. The implication of this examination is however not that the starch content of an individual organ is directly related, or proportional, to the grain yield of the corresponding plant – the relationship is not mutually exclusive and a host of other factors present during the life cycle of the plant may affect the final grain yield.

4.4.3.2.1 Experiment 2

Table 4.2 summarizes the relationships between the organ starch content and grain yield in Experiment 2. When the genotypic starch content of each of the three organs, pooled by treatments, was examined, a significant relationship was identified for the peduncle of SB155 (P=0.007). Greater starch content in the peduncle was associated with greater grain yields. When categorized by temperature treatment, no significant relationship was identified between organ starch content and temperature treatment. However, when categorized by irrigation treatment it was found that under WW conditions, increased starch content of the peduncle was related to a higher grain yield (P=0.009).

Table 4.2 – A summary of *P*-values generated from the examination of the relationships between organ starch content and final grain yield in Experiment 2.

Organ	Treatment		P-value	Sig?
Flag Leaf	Genotype	SB020	0.245	ns
		SB155	0.918	ns
		SB165	0.843	ns
	Temperature	AT	0.657	ns
		HT	0.756	ns
	Irrigation	WW	0.223	ns
		WS	0.445	ns
Peduncle	Genotype	SB020	0.159	ns
		SB155	0.007	*
		SB165	0.085	ns
	Temperature	AT	0.176	ns
		HT	0.997	ns
	Irrigation	WW	0.009	*
		WS	0.159	ns
Glume	Genotype	SB020	0.898	ns
		SB155	0.121	ns
		SB165	0.621	ns
	Temperature	AT	0.608	ns
		HT	0.963	ns
	Irrigation	WW	0.916	ns
		WS	0.241	ns

4.4.3.2.2 Experiment 4

When the relationship between tissue starch content and grain yield was examined on a per organ basis, no significant relationships between the organ starch content and grain yield were found in Experiment 4. Table 4.3 summarizes these findings.

Table 4.3 – A summary of *P*-values generated from the examination of the relationships between organ starch content and final grain yield in Experiment 4.

Organ	Treatment		P-value	Sig?
Flag Leaf	Genotype	SB020	0.113	ns
		SB165	0.192	ns
	Temperature	AT	0.706	ns
		HT	0.813	ns
	Irrigation	WW	0.744	ns
		WS	0.110	ns
Peduncle	Genotype	SB020	0.211	ns
		SB165	0.163	ns
	Temperature	AT	0.856	ns
		HT	0.887	ns
	Irrigation	WW	0.190	ns
		WS	0.622	ns
Glume	Genotype	SB020	0.633	ns
		SB165	0.845	ns
	Temperature	AT	0.283	ns
		HT	0.276	ns
	Irrigation	WW	0.689	ns
		WS	0.150	ns

4.4.4. WSC Content and Grain Yield

4.4.4.1 Total WSC Content (Experiment 2 and 4)

When categorized by genotype, temperature or irrigation treatment, no significant relationships were identified between the total WSC content per plant and grain yield in either Experiment 2 or 4.

4.4.4.2 Organ WSC Content (Experiment 2 and 4)

As was reported in 4.4.3.2.2, when the relationship between tissue WSC content and grain yield was examined on a per organ basis, no significant relationships between the organ WSC content and grain yield were found in either Experiment 2 or 4.

4.5. DISCUSSION

The glumes play a unique role amongst the photosynthetically active plant material due to their proximity to the grains. Up to 30% of the photosynthates made available to the grains may originate directly from the photosynthesis occurring in the glumes (Grundbacher, 1963). Contrary to Scofield *et al.* (2009), who reported that the exposed peduncle lacked significant amounts of starch, the results suggest that starch is found in the peduncle, flag leaf and glumes. The starch content of the glumes was found to be significantly greater than that of the flag leaf or peduncle in both Experiments 2 and 4, indicating the potential significance of glume starch content as a carbohydrate reserve. With data relating to total glume and flag leaf dry weight per plant not being available, it was not possible to determine whether starch concentration per organ was greater in the glumes compared to the flag leaf and peduncle.

As WSC content has been reported to be significant in the peduncle (Bonnett and Incoll, 1993; Gebbing, 2003), it was assumed that the largest starch reserves would also be found here. However, in Experiment 2, the peduncle starch content was lower than that of the flag leaf and in Experiment 4, it was comparable. Despite the differences in experimental design between Experiment 2 and 4, a higher starch content than in the two other organs was consistently observed in the glume. This finding highlights the important role of the glumes in carbohydrate storage, which has previously been underestimated. Although starch had previously been reported to only be present in small quantities in the glumes, the findings of this work challenge this notion.

Although the same plant material was used in both experiments, the limitations placed on the pot-grown wheat of Experiment 2, as discussed in Chapter 2.5, may account for differences in observations made between Experiment 2 and 4. The increased ability to freely root, along with the absence of negative tissue temperature depression observations, may explain why starch content varied between plant organs in the two experiments. The two experiments differed in a range of other ways, including differences in the lighting design and in the airflow over the plant organs. The increased soil volume available for rooting in the

rhizoboxes prevented the direct heating of the root zone experienced in the pots used during Experiment 2. Additionally, differences in the fertilization and nutrient availability of the contrasting growth mediums used in the two experiments, which would affect plant bulk and consequently storage capacity, may explain the observed differences. The two contrasting temperature treatments applied failed to significantly affect genotypic and organ starch contents in Experiment 2, demonstrating that starch accumulation between genotypes and organs is unaffected by short periods of heat stress during early anthesis. Similarly, the starch content at mid-anthesis was unaffected by drought during early anthesis, although the effect of drought at anthesis may be significant in the latter stages of the crop cycle. If the duration and/or intensity of the heat and drought stress were to be extended, it is likely that significant reductions in starch content would be observed. The effect that abiotic stress at anthesis has on the starch content of the three organs examined during the subsequent period of grain filling is unknown. Sampling of plant tissue at multiple stages during development (e.g. at vegetative growth, anthesis, early grain filling, mid-grain filing and late grain filling) in all three organs, would elucidate the changes that occur in the photoassimilate balance within the plant throughout its lifecycle. Diurnally, the photoassimilate synthesis and demands vary. Consequently, continuous sampling throughout the day would have been valuable. This sampling approach however requires significantly more assays and is therefore associated with an increased expense.

Starch contents of both the flag leaf and peduncle in Experiment 2 were significantly higher than those recorded in Experiment 4. As the starch content of the glumes remained comparable between the two experiments, it is unclear why such a significant reduction in flag leaf and peduncle starch content was recorded. Similarly unexplained, was the observation that the starch contents of the flag leaf and peduncle in Experiment 4 were virtually the same despite significant differences being detected in Experiment 2.

Similarly to the starch content in Experiment 2, no genotypic variation was found in WSC content in either Experiment 2 or 4. Significant genetic variation in WSC content exists (Ruuska *et al.,* 2006) and it is evident that the limited number of lines utilized in both experiments were not contrasting in their WSC potential.

Fructan has consistently been reported as the most prevalent WSC in wheat (Wardlaw and Willenbrink, 1994; Ruuska *et al.,* 2006) but it was not measured during this experiment. It was therefore anticipated that either sucrose or fructose would be the most prevalent of the WSC to be detected. However, glucose was found in the largest concentrations in both experiments. As fructan was not measured, it is possible that it could have been the most prevalent. The WSC composition varies between genotypes however and the ratio between them may be affected by changes in environmental conditions. As WSC was assessed only at mid-anthesis, it was not possible to observe the continuous change in WSC content of individual plant organs throughout the plant's lifecycle.

In both Experiment 2 and 4, the sucrose content remained low and unchanged by the presence of HT and WS. It was only in Experiment 4, the rhizobox experiment, that the glucose content was significantly affected by the elevated temperature and water-deficit stress applied. The relatively short period of high-temperature stress and water-deficit applied to the plants prior to tissue sampling at mid-anthesis, resulted in reductions in the glucose and fructose contents compared to the cool and well-watered treatments of Experiment 4. An absence of any treatment differences in the total glucose, sucrose and fructose content per plant was reported in the pot-grown wheat in Experiment 2. In the rhizobox-grown wheat of Experiment 4 however, total glucose per plant was significantly greater in the AT treatment whilst restriction of water at anthesis significantly affected total fructose content per plant. On a per plant basis, total sucrose content remained unaffected by either the elevated temperature or water-deficit stress imposed in Experiment 4. Significant differences between treatments only became possible to detect once the roots were able to freely root in Experiment 4. The results illustrate the limitations of conducting physiological studies aimed at understanding the balance and distribution of WSC in pot experiments. However, the limitations posed by field-scale experiments, when examining the effect of future climate scenarios on crops in the UK, are manifold as illustrated by Experiment 3. The effect of short-term heat stress on WSC has only been examined recently (Talukder *et al.,* 2013). However, the work only examined the effect of a single day of heat stress, prior to anthesis and early grain-set on the WSC content of the peduncle. As heat stress is rarely found in isolation in natural environments, the value of this work would be greatly

improved with the inclusion of a drought element. Currently, the available literature remains unclear as to the effect that abiotic stress at anthesis has on the distribution and composition of WSC in a range of wheat organs.

Unlike the starch content in Experiment 2, the glucose and sucrose contents were highest in the peduncle, followed by the glume and the flag leaf. Fructose content however, was significantly higher in the glume compared to the other two organs. The data from Experiment 4 agrees with the findings made in Experiment 2 with regards to organ sucrose and fructose, but the glucose content was significantly greater in the glumes for rhizobox grown wheat. It is unclear why glume glucose concentration was significantly greater in rhizobox-grown wheat.

It is hypothesized that the ability to accumulate and break down starch close to the vital sink sites, the glumes surrounding the developing grains, may provide an evolutionary advantage to crops grown in arid and hot environments. Rather than producing a large amount of stem stored WSC, which requires storage and remobilization to the sink organs, a wheat cultivar adapted to dry and hot conditions may preferentially synthesize starch in the glumes which can then be utilized for protection of the floral organs and early grain development. Additionally, the breakdown of starch for grain filling may take place at greater rates during the cooler temperatures experienced at night. This work did not set out to examine this and therefore, positive confirmation of this theory requires detailed further experimentation. A limitation of the results is that they only reveal the starch and WSC balance between the plant organs at mid-anthesis. In order to comment on the contribution of individual organs to grain yield, details relating to the assimilate balance post-anthesis are required.

WSC however, do not always correlate with increased grain yield (Užík and Žofajová, 2006; Dreccer *et al.,* 2009). No significant correlations between WSC and grain yield were observed for either Experiment 2 or 4. The WSC remobilization capacity of the lines studied SB020, SB155 and SB165 was not known and the genotypes were not selected specifically for their contrasting remobilization capacity. Consequently, a lack of correlation between WSC content and grain yield was a possibility from the outset of the experiment. WSC was not recorded from a

field-grown wheat crop and therefore no comparisons between that and the CE grown wheat of Experiments 2 and 4 could be made.

The relationship between total starch content and grain yield was largely non-significant, except for SB155 when categorized by genotype in experiment 2. When the relationship between total plant starch/ WSC content and grain yield were examined in experiment 4, no significant relationships were uncovered. A limited number of significant relationships between organ starch content and grain yield were uncovered in experiment 2 whilst no such relationships were found in experiment 4. In both experiments, increased starch and WSC content were not associated with higher yields as a result of the HT and WS stress treatments. The significant observations made on an organ level all occurred in the peduncle, identifying this organ as the most likely candidate for further research efforts into the relationships between a wheat plants photo-assimilate balance and grain yield. Several authors have previously suggested that increased carbohydrate availability is an important physiological trait for maintaining yield under stress conditions (e.g. Liu and Huang, 2000). Increased starch content alone may however not be sufficient to infer stress tolerance to a crop, as the efficiency of soluble starch synthase has been demonstrated to vary between cultivars (Zahedi *et al*., 2003). As an enzyme driven process, the thermostability of the enzymes involved at different temperatures, must be considered as well (Singletary *et al*., 1994). In order to determine whether the starch contained in the glumes is both synthesised and stored there, or whether the glumes merely act as a storage site for starch synthesised elsewhere (e.g. the canopy), further experimentation is required.

Whilst limited, the significant relationships identified between grain yield and organ starch content at anthesis warrant further examination. A greater number of lines screened under field conditions may elucidate these relationships clearly. It is likely that the contrasting growing mediums used between the two experiments, as well as differences in the timing, duration and intensity of the stress may be affecting the observed relationships. However, from the data gathered there is no evidence to suggest that under conditions of elevated temperature and drought stress, higher levels of starch and/or WSC at anthesis correspond to higher grain yields.

4.6. CONCLUSION

Contrary to the assumption that the largest starch reserve would be found in the peduncle, an organ identified as a significant storage site of WSC, it was the glumes that contained the highest starch content per milligram of plant tissue. Despite the differences in experimental design between Experiment 2 and 4 (pot-grown versus rhizobox-grown wheat), the observation of high starch content in the glumes remained consistent. Elevated temperature and water-deficit stress significantly impacted the WSC composition, but only in Experiment 4. The absence of significant variation between the stress treatments applied in Experiment 2 suggests that the value of using this experimental design approach to assessing WSC content may be limited.

The high starch content observed in the glume is hypothesized to be an adaptation mechanism of wheat grown in hot and arid environments. The basis for this assumption is that in growing regions in which high temperature stress is frequently encountered at midday, photosynthesis declines rapidly in response. In order to maintain grain filling and protect the developing floral organs from damage, a stress tolerant wheat genotype may use this starch assimilate pool to maintain vital processes in the spike. The rapid formation of a mature grain is important for the survival of wheat grown in marginal environments, as high temperature and water-deficit stress during grain filling is a regular feature of these cereal producing landscapes. The reliance on WSC as a pivotal source of assimilates to the developing grain, may in fact be less productive than an investment by the plant in starch reserve pools in the glumes. In addition, the starch reserve pool in the glumes may be utilized during the cooler night-time temperatures when photosynthesis is not occurring. Whilst this hypothesis requires the examination of wheat genotypes from a wide range of genetic backgrounds and geographical origins, extensive field-scale evaluation of the trait is essential. Increased stem WSC content has been identified as being a promising physiological trait that can improve heat and drought tolerance in wheat (Cossani and Reynolds, 2012). No similar body of work has focused on the capacity of the starch content in plant wheat organs to increase abiotic stress tolerance. The results obtained from this work warrant further examination in field-grown wheat.

CHAPTER 5

GENERAL CONCLUSIONS AND FUTURE RESEARCH RECOMENDATIONS

5.1. GENERAL CONCLUSIONS

With mean global temperatures predicted to rise by between 1-6°C by the end of the century (IPCC, 2014), the impact that these temperature changes may have on previously predicable precipitation patterns is of concern to cereal producers globally. Of particular significance to cereal production in the UK is the increased likelihood of short and extreme periods of high temperature occurring early in the growing cycle of the crop. These periodic heat stresses have been demonstrated to negatively impact yields in a number of key crops, including wheat (Porter and Semenov, 2005; Prasad *et al.,* 2006b). In wheat, the negative effect of these short-lived heat stress events is particularly pronounced during the sensitive developmental stage of anthesis (Porter and Semenov, 2005).

A number of unique findings are reported in this thesis. It is the most comprehensive study of the thermal dynamics of the wheat spike at anthesis to date. It has explored the interaction between the spike, its environment and the effect of abiotic stress at anthesis on wheat yields. The thesis examined the complications associated with assessment of tissue temperature depression in a field-scale setting in the UK and it reported the value of using rhizoboxes as a means to assess root development rapidly whilst maintaining control over the plants environment. Importantly, this thesis has identified the glumes as a previously underestimated site at which starch is found in significant quantities. The implications of this are potentially valuable to commercial wheat breeders as well as the wider crop physiology community. The key findings made are summarized in greater detail below.

In Chapter 2, greater temperature depression (TD) of the flag leaf compared to the spike during anthesis is reported. This increased cooling of the flag leaf indicates a greater transpirational cooling capacity, indicative of potentially greater

photosynthetic activity compared to the spike. The reporting of negative TD values in Chapter 2 is in line within the findings made by others in the past who have reported that in controlled environment (CE) experiments, tissue temperatures in many plant species rise to above that of the air temperature due to an increase in the thermal radiation load placed on the plants (Casperson, 1957) as well as restrictions associated with air flow (McCree, 1984). Lange (1959) found that the tissue temperature in a range of Mauritanian desert plants varied significantly, with some species, such as *Citrullus colocyndactylifera*, exhibiting tissue temperature 15.3°C below ambient air temperature whilst others, such as *Phoenix dactylifera*, exhibited tissue temperature up to 12.9°C above ambient air temperature whilst maintaining function. This thesis confirms the occurrence of both above- and below-ambient tissue temperatures in wheat. The effect of growing plants in isolation of one another instead of at plant densities encountered in the field, can lead to differing yield results (Rossiter, 1959) and the effect of experimental design on the interpretation of the results must be considered.

The thesis also reports that the observation of significant relationships between organ TD and floret development stage (FDS) varied between Experiments 1 and 2 as well as between the genotypes and treatments imposed. A number of significant interactions were recorded in which organ TD increased from the early to the latter stages of floral development. A greater cooling capacity in the latter stages of floral development supports a theory that increased spike temperature depression (STD) in the latter stages of anthesis is due to changes in the rate of translocation of assimilates from the senescing canopy to the developing grains of the spike.

Variation in the relationship between organ TD and grain yield was found between the two experiments conducted, suggesting that the CE growth chambers used to assess organ TD affected the physiological response of the plant to abiotic stress. The inconsistent relationship identified between organ TD and grain yield discourages the use of this methodology to further assess the effect of organ TD in wheat on yield formation. A possible explanation of the difference in the organ TD at anthesis between the spike and the flag leaf is that a differing photosynthetic capacity may exist between the two organs. Alteration to this photosynthetic capacity may be in part modified by the presence of abiotic stress and therefore a

potential route to crop improvement may exist. During Experiment 1, SB020 maintained the greatest flag leaf temperature depression (FLTD) and STD on all three days of observation, whilst the organ TD of other genotypes, SB019 for example, remained low on Day 1 and continued to decline further up to Day 3. The variation observed in floral development between different genotypes exposed to contrasting temperature regimes at anthesis, suggests that significant variation in the timing of the onset of senescence may occur. The completion of reproduction initiates the process of mass phloem flow associated with overall plant senescence from the canopy to the developing grains (Davies and Gan, 2012).

In both the experiments discussed in Chapter 2, SB020 was consistently identified as a stress resistant variety, which is in line with the findings made by Pinto *et al*. (2010) from field-scale trials at CIMMYT in Mexico. Additionally, it was identified in Experiment 1 as containing a high concentration of chlorophyll at anthesis in both the flag leaf and glumes. SB020 exhibited the greatest differences in FLTD and STD between treatments at anthesis indicating that the transpirational cooling capacity of SB020 under stress conditions was greater than the other genotypes examined. A high transpirational cooling capacity has previously been associated with the vascular systems' capacity to maintain the evaporative demands placed on the plant (Pinto and Reynolds, 2015). The data from SB020 indicate that the optimal phenotype for stress resilience at anthesis is a wheat plant with a high organ chlorophyll content and a high transpirational cooling capacity. From the data gathered it is not possible to definitively identify the cause of hotter tissue temperature in the other genotypes. It is however postulated that increased cooling in SB020 compared to the other two genotypes, is an artefact of differing plant water balance, variation in photosynthetic capacity and rate of stomatal closure during a stress event.

When categorized across all treatments during Experiment 2, the relative water content (RWC) of the flag leaf in SB020 and SB165 were comparable. However under water-deficit stress conditions SB020 maintained a significantly higher RWC than SB055 or SB165. A genotypic assessment of the stomatal density on the surface of the glumes and flag leaf, as well as an assessment of the stomatal closure rates due to stress must be quantified in order to confirm these assumptions. The

relationship between FLTD/STD and grain yield in Experiment 2, which demonstrated that increased organ TD is associated with lower yields, contradicts the findings made in Experiment 1 and conversely suggests that under certain conditions maintaining open stomata during stress periods instead of closing them may be associated with yield losses. It is possible that an inability to efficiently allocate plant water content may be responsible for the observation of cooler tissue resulting in lower grain yields.

Despite complications associated with successfully creating and maintaining a significant gradient between the two temperature and irrigation treatments in the field-scale polytunnel experiment detailed in Chapter 3, this thesis can report that both canopy temperature depression (CTD) and STD were successfully measured in the field. No association between organ TD and the binary floret scoring system was identified. It is likely that an increase in the duration and severity of the stress at anthesis may have resulted in a relationship being elucidated, although the current data is unable to support this hypothesis. The absence of significant differences in rooting depth between the treatments did not allow for examination of the relationship between deep rooting and increased organ TD. Previous work has successfully linked deep rooting to increased CTD (Lopes and Reynolds, 2010). Examination of the effect of deep rooting on STD must be quantified in the future using wheat lines with contrasting rooting morphology habits (Clarke, 2014). In the field-grown wheat, the insufficient abiotic stress at anthesis failed to result in yield differences between treatments. Consequently, no significant relationship between organ TD and yield at anthesis was found.

In the rhizobox experiment detailed also in Chapter 3, significant differences in rooting phenology at anthesis were identified at the 70-80cm and the 90-100cm soil horizons between the two Seri-Babax genotypes used with SB020 maintaining a greater root weight in the lowest soil horizon than SB165. The differences in rooting at the base of the rhizoboxes between the two genotypes was however less than 0.005cm/cm^3. Further work is needed to confirm the effect of deep rooting on organ STD and grain. A significant decline in organ cooling was detected in the ambient temperature treatment as anthesis progressed from the early to the latter stages of floral development. Although no significant relationship to floral

development stage was found, STD in excess of FLTD was observed from the start to the end of the observation period in the elevated temperature treatment. No significant correlations between organ TD and grain yield were uncovered in the rhizobox experiment.

In Chapter 4, the starch and water soluble carbohydrate (WSC) content of the flag leaf, peduncle and glumes under contrasting abiotic stress conditions at anthesis was examined. Starch was found in significant quantities in all three organs, with the glumes containing the highest starch content. Consequently, the wheat glumes were identified as a previously underestimated carbohydrate storage site at anthesis. Starch content of the flag leaf and peduncle in Experiment 2 was greater than that recorded in the same organs of Experiment 4. Differences in the starch content recorded between the two experiments may have occurred as a consequence of the different experimental design utilized between the pot- and rhizobox-based experiments. Starch and WSC were affected by the treatments applied in the rhizoboxes but not in the pot-based experiment. No genotypic variation in starch and WSC content was identified in either Experiment 2 or 4. Glucose comprised the largest fraction of the WSC measured in both experiments, with the sucrose content remaining low and unchanged in the presence of abiotic stress.

As photosynthesis is extremely sensitive to high temperature stress (Allakhverdiev *et al.,* 2008), when stress was applied at anthesis in Experiment 4, a complete, or at least partial, reduction in the photosynthetic rate of the plant tissue is likely to have occurred (Feng *et al.,* 2014). High thermal stress results in alterations to the thylakoid membrane, which negatively impacts the activity of photosystem II (Yamamoto *et al.,* 2008). By regulating carbohydrate metabolism under conditions of stress, sugars play an important role in maintaining plant function. As a consequence of the reduced photosynthate production under stressed conditions, it is hypothesized that a large portion of the glucose was remobilized as a substrate for cellular respiration. Glucose also plays a role in maintaining cell homeostasis (Gupta and Kaur, 2005). The effect of elevated temperature stress on the rhizobox-grown plants in Experiment 4 was demonstrated by the significant reduction in glucose content in the elevated temperature treatment compared to the ambient treatment. Had the intensity and duration of the temperature stress been greater, it is

likely that a further reduction in the glucose content would have been recorded. This in turn would have impacted the final grain yield due to the formation of smaller grains. A complete understanding of the distribution and quantity of starch and WSC in all organs and at each stage of development would have completed the picture on the effect of elevated temperature and water-deficit stress at anthesis on the photoassimilate balance within wheat plants.

Except for a limited number of significant, positive correlations, tissue starch content at anthesis was not significantly correlated to higher yields. In Experiment 2, the total starch content of SB155 was positively correlated to grain yield but failed to correlate under conditions of elevated temperature and water-deficit stress. Under well-watered conditions, starch content was positively correlated to increased grain yield in the peduncle, whereas under water-stressed conditions no such correlation was observed. In experiment 2, the significant correlations were observed in the peduncle whereas no such relationships were identified for the other two organs. In the limited cases where significant relationships were observed, the scope of the study did not elucidate whether the relationships were due to the starch acting in a protective capacity for the floral organs against elevated temperature and drought stress at anthesis by inferring membrane stability and cell homeostasis, or whether greater starch content at anthesis resulted in more carbohydrates being available for remobilization during the subsequent period of grain filling. The relationship between starch content and grain yield was inconsistent between Experiment 2 and 4, suggesting that the experimental approach used may significantly impacted the assimilation, storage and remobilization dynamics in wheat.

The data identifies starch content as being a potentially valuable phenotyping tool for selecting for stress resistance at anthesis in wheat. SB020 was identified as containing in excess of twice the starch content of the other varieties examined. Questions remain as to whether higher starch content is solely responsible for the improved grain yields or whether differences in the remobilization capacity exist between the genotypes as well. Screening of tissue starch content as early as seedling stage may be viable, thereby increasing the number of genotypes screened as well as speeding up the entire phenotyping process.

Although data relating to root zone temperature was lacking from the growth chamber and rhizobox experiments, the author can confirm an unequivocal difference between the elevated surface temperature of the pots used in the growth chamber experiments and that of the rhizoboxes. It is recommended that in future work, temperature fluctuations of the root zone are recorded in every pot used. The rhizoboxes demonstrated their enhanced ability over the pots to allow researchers to apply different levels of stress effectively without warming to the root zone, a common problem encountered in pot-based experiments and one believed to be in part responsible for the differing physiological responses observed between Experiment 1 and 2. No correlation between WSC content and grain yield was found in either Experiment 2 or 4, findings in line with those reported by Užík and Žofajová (2006) as well as Dreccer *et al.* (2009) who reported an inconsistent relationship between the two.

The stress applied during this experiments took place exclusively at anthesis (GS61-69). The stages preceding anthesis, in particular booting (GS41-49), have however, exhibited significant susceptibility to abiotic stress in past experiments (Saini and Aspinall, 1982; Westgate *et al.,* 1986; Alghabari *et al.,* 2014). Stress resistant wheat varieties have been reported to initiate flowering whilst still contained within the boot (Alghabari, 2014 *pers comm*). The infrared imaging methodology detailed in Chapter 2.3 and 3.3 would not be able to measure the temperature of the spike within the boot. This would require the insertion of thermistors in order to assess temperature. Had the stress been imposed at booting, it is hypothesized that TD of the flag leaf would have been comparable to that observed at anthesis, whilst the TD of the boot would have been greater than that of the spike at anthesis, due to the lower positioning of the boot compared to the exposed spike. The peduncle has previously been identified as a photosynthetically active organ with a high concentration of stomata (Kong *et al.,* 2010) and is consequently capable of transpirational cooling.

In order for STD to become a viable screening tool for cereal breeders, assessment of the trait in a the wide range of genotypes has to take place under field conditions. The planned work that was due to take place at CIMMYT in Mexico (see 3.2.5) would have elucidated this relationship. However, the experimental limitations that

were encountered led to the decision to abandon STD assessment in the field at CIMMYT. Significant modification of the protocol is required before field-scale evaluation of STD can take place due to limitations in the accuracy of image capture as well as the rate at which image capture and analysis can take place.

The work presented in this thesis addresses a relativity young concept in plant physiology: TD of the spike. With very limited literature relating to the thermal dynamics of the spike being available, many questions remain as to the regulation of, and potential impact that this trait might have for wheat breeders. This thesis has addressed a number of important research objectives and its findings reconfirm the negative effect that abiotic stress at anthesis has on grain numbers and weight in wheat. However, there is insufficient evidence to currently link increased STD at anthesis to improved grain yields.

This thesis has provided a novel insight into the thermal and photoassimilate dynamics of the wheat spike at anthesis. Consequently, a number of limitations were encountered which have to be individually considered.

The central limitation of the study was the absence of a body of literature that had previously examined aspects of spike thermal dynamics in any detail, particularly at anthesis. A large body of literature exists on CTD but data on STD does not. However, these gaps in the literature serve as an opportunity for further exploratory research into the thermal dynamics of the spike. Similarly, literature on the distribution of photoassimilates in wheat at anthesis, under a range of environmental conditions, is lacking. Consequently, no previous methodology could be replicated to assess organ TD and the distribution of assimilates throughout the plant. The methodology used successfully addressed a range of research objectives but further modification is required in order to develop the methodology into one capable of being integrated into current high throughput phenotyping platforms.

The difficulties encountered in establishing sufficiently large treatment differences in the temperature and water-deficit stress treatments during Experiment 3 were unfortunate but arose as a consequence of conducting field-scale phenotyping studies of this kind in the UK. Had the severity and duration of the stress imposed at

anthesis been greater, a more conclusive analysis of STD in field-grown wheat may have been gained. The limitations encountered during the polytunnel experiment however, act as a valuable case study for future work of this kind in the UK. With the increased recognition of the need to breed abiotic stress tolerance in wheat, similar work on this kind is likely to take place in the future.

A further limitation of this thesis was the limited number of genotypes of wheat examined in the four experiments conducted. In the first growth cabinet experiment, the study attempted to assess six genotypes during anthesis. As the capturing of IR images and detailed floret scoring had to take place within a short three-hour window at noon, using six genotypes proved impractical. Because the growth cabinets were full to capacity, using all six genotypes would have required the doors to be opened for significantly longer periods to access the plants in the centre of the growth cabinet. Similarly in Experiment 3, the capture of all the thermal data required within the narrow time frame from the multiple polytunnels used was not possible with more than one genotype. In the rhizobox experiment, the potential number of genotypes used was limited to the funds available to construct rhizoboxes, as well as the facilities in which to grow the plants. The examination of more genotypes in each experiment would have resulted in the ability to examine a larger portion of the Seri-Babax population for their physiological responses to heat stress. A number of modifications to the methodology would have had to have taken place to accommodate an increase in the genotypes used, with automation of data collection requiring a significant investment.

5.2. FUTURE RESEARCH RECCOMENDATIONS

The scope of this thesis focused on the examination of organ TD and the distribution of photoassimilates in wheat organs, exclusively at anthesis. A limited number of genotypes were examined in this thesis and consequently, further research into the physiological responses to elevated temperature and drought stress at anthesis is required in wheat from a wider genetic background. Detailed below are a number of other possible future research objectives that may increase our understanding of abiotic stress tolerance in wheat and therefore warrant further investigation.

Effects of Period Heat Stress in Wheat

Much of the work relating to heat stress in wheat, including the work conducted in Experiment 1 and 2, applies heat stress in excess of 8 hours (Wardlaw *et al.,* 1989a; Wardlaw *et al.,* 1989b; Gibson and Paulsen, 1999; Shah and Paulsen, 2003; Prasad *et al.,* 2006a; Kaur and Behl, 2010). In a crop's natural environment however, high temperatures are rarely encountered on these timescales. Similar to the experimental approach used by Talukder *et al*. (2010), in which high temperature stress was imposed for only three hours, more work must take place to examine the effect of periodic high temperature stress on grain yield in wheat. Not only the duration of a heat stress event, but also the time of onset during the day warrants further examination. For example, what is the effect of high temperature stress in wheat between 07.00-11.00h, 11.00-14.00h and 14.00-17.00h during anthesis in wheat? Coast *et al*. (2015) reported that an earlier flowering phenology, along with peak flowering occurring earlier in the day and a reduction in the overall duration of anthesis, are critical escape mechanisms against elevated temperature stress in rice. Due to the threat posed by elevated night-time temperatures as a consequence of climate change, it is critical that developing a model for optimal flowering phenology under abiotic stress conditions occurs in wheat.

Altered Flowering Synchrony

High temperature stress alters the synchrony of development in both the anthers and stigma, resulting in potential disruption to fertilization occurring in wheat. Adjusting the synchrony of flowering so that sensitive wheat cultivars flower earlier during the day, presents an interesting opportunity to increase rates of successful seed set. Associated to this, is the potential to examine the effect of altering the rate at which anthesis progresses to determine the impact on grain set of a short- versus a long-duration flowering phenotype under conditions of abiotic stress.

Spike Temperature Depression of Field-Grown Wheat

Examination of STD in field-grown wheat, under stable conditions of elevated temperature and drought stress, is required to conclusively establish the relationship of STD at anthesis and grain yield. It would equally be valuable to explore the dynamics of STD along with CTD during grain filling.

Development of Automated IR Capture

Significant modification to the methodology detailed in Chapter 2.3 and Chapter 3.3 is required to examine a large number of genotypes. If STD as a selection tool were to become interesting to commercial breeders, a significant investment in developing a protocol for automated data collection and image analysis is necessary. In early generation selection programs breeders screen hundreds, in some cases thousands, of lines simultaneously. The capabilities of field-sensing technology are rapidly increasing and what was thought previously to be impossible is now being realized. For instance, sensing platforms have been developed which are capable of detecting individual broadleaf weeds in a cereal field and targeting them with herbicides. Along with advances in drone and imaging technology, it is not inconceivable that it will be possible to fully automate the process of spike detection, imaging and GPS location cataloguing in the near future. Detailed assessments of anthesis may be more complex to automate, although this is linked to the level of detail required by future researchers.

5.3. CONCLUSION

The spike is a complex organ that is intrinsically linked to yield production in wheat. With the knowledge that climate change is likely to result in an increase in the frequency of extreme weather events that have the potential to significantly disrupt grain formation and yield development, further work into the genetic capacity of the spike to mitigate these harmful events is warranted. Previously used methods of achieving yield increases, including expanding the land area under cultivation or increasing agricultural inputs to a crop, are recognized as no longer a sustainable solution to feeding an increasingly hungry world. An increase in the research efforts invested into the spike is important in order to adapt the organ in which the most vulnerable stage of development, anthesis, take place. A failure to appropriately adapt wheat, in particular the spike, to the changing climate we face, may have significant ramifications on the cereal production capabilities, not only in marginal producing regions around the globe, but also here in the UK. This thesis has detailed two contrasting approaches wheat may use when responding to abiotic stress at anthesis: increased organ TD and the accumulation of carbohydrate-based photoassimilates. The concept of STD is novel and the results arising from this thesis conclude that field-scale examination of this trait is required in order to develop it further into a screening tool that could potentially be integrated into a high throughput phenotyping platform in the future. Currently there is insufficient data to unequivocally support the notion that an increased cooling capacity of the spike at anthesis is associated with improved grain yields. Further research examining the genetic variability of starch distribution in wheat organs is necessary in order to develop this trait into one with potential value to the cereal breeding community. The development of novel screening tools is a complex task but without exploring the wide range of physiological processes that occur in wheat, we are not going to be able to develop the novel analytical breeding tools we need to face a changing climate.

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APPENDICES

Fig. 1.1 – The global average price of wheat (in USD) between 2006 and 2016. Data provided by CBOT. Source: www.tradingeconomics.com.

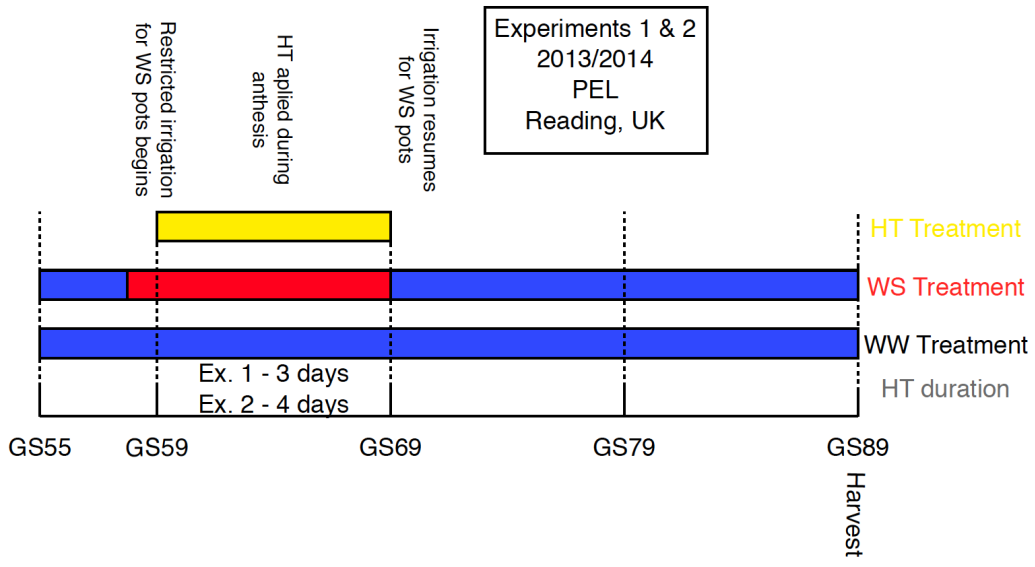

Figure 2.37 – A timeline illustrating when elevated temperature and water-deficit stress were applied to the plants in Experiment 1 and 2, in relation to Zadocks physiological growth stages.

Stage 1 **Stage 2**

Stage 3 **Stage 4**

Figures 2.38A, 2.38B, 2.38C, 2.38D – Photographic illustrations of the four stages determined during anther scoring. During stage '1', the anthers are very small, closely packed to each other in the glume and are green in color. In stage '2', the anthers have become slightly larger than they were during stage '1'. In addition, they have taken on a green/yellow color. During stage '3', the anthers are suspended on a filament and are bright yellow in color. They may be contained within the glume or hang out of the glume. In stage '4', the anthers are usually outside the glume. They have turned a distinctive white color, making them easily recognizable from the bright yellow observed during stage '3'. Reproduced from Steinmeyer *et al.* (2013) with permission from the authors.

Half-Fluffy (HF)

Fluffy (F)

Grain (G)

Figures 2.39A, 2.39B, 2.39C – Photographic illustrations of the three stages used for pistil scoring during the experiment. 'Half-Fluffy' is characterized by the pistil being tightly contained within the developing anthers. The 'Fluffy' stage has been reached when the white pistil is protruding outwards. 'Grain' differs from the previous stage in that the pistil hangs out of the floret and the filaments of the pistil look damaged. Often pollen is seen coating the pistil at this stage. These differences are clearly visible. Reproduced from Steinmeyer *et al.* (2013) with permission from the authors.

Table 2.6 – Summary of the change in average percentage of male florets observed during anthesis in the AT temperature treatment of Experiment 1, for each of the five genotypes used.

		AT			
SB Code	% Florets	FDS (1)	FDS (2)	FDS (3)	FDS (4)
	Day 1	100	0	0	0
	Day 2	71	17	8	4
SB019	Day 3	25	25	12	38
	Day 1	100	0	0	0
	Day 2	50	8	8	34
SB020	Day 3	38	8	8	46
	Day 1	96	4	0	0
	Day 2	63	10	10	17
SB155	Day 3	21	8	4	67
	Day 1	100	0	0	0
	Day 2	88	12	0	0
SB156	Day 3	50	13	4	33
	Day 1	100	0	0	0
	Day 2	38	38	14	10
SB165	Day 3	21	8	4	67

Table 2.7 – Summary of the change in average percentage of male florets observed during anthesis in the HT temperature treatment of Experiment 1, for each of the five genotypes used.

		HT			
SB Code	% Florets	FDS (1)	FDS (2)	FDS (3)	FDS (4)
	Day 1	92	8	0	0
	Day 2	33	21	13	33
SB019	Day 3	0	0	4	96
	Day 1	71	29	0	0
	Day 2	13	25	8	54
SB020	Day 3	0	0	0	100
	Day 1	71	13	13	4
	Day 2	0	8	8	84
SB155	Day 3	0	0	0	100
	Day 1	100	0	0	0
	Day 2	8	8	21	63
SB156	Day 3	0	0	0	100
	Day 1	88	12	0	0
	Day 2	33	38	8	21
SB165	Day 3	0	0	0	100

Table 2.8 – Summary of the change in average percentage of female florets observed during anthesis in the AT temperature treatment of Experiment 1, for each of the five genotypes used.

		AT		
SB Code	% Florets	FDS (HF)	FDS (F)	FDS (G)
	Day 1	67	33	0
	Day 2	17	75	8
SB019	Day 3	0	63	37
	Day 1	67	33	0
	Day 2	4	46	50
SB020	Day 3	0	54	46
	Day 1	54	46	0
	Day 2	13	75	12
SB155	Day 3	4	25	71
	Day 1	92	8	0
	Day 2	54	46	0
SB156	Day 3	13	54	33
	Day 1	75	25	0
	Day 2	21	67	12
SB165	Day 3	4	29	67

Table 2.9 – Summary of the change in average percentage of female florets observed during anthesis in the HT temperature treatment of Experiment 1, for each of the five genotypes used. Numbers in brackets represent standard error.

		HT		
SB Code	% Florets	FDS (HF)	FDS (F)	FDS (G)
	Day 1	54	46	0
	Day 2	8	63	29
SB019	Day 3	0	0	100
	Day 1	50	50	0
	Day 2	0	38	62
SB020	Day 3	0	0	100
	Day 1	71	29	0
	Day 2	0	32	68
SB155	Day 3	0	0	100
	Day 1	92	0	8
	Day 2	4	46	50
SB156	Day 3	0	0	100
	Day 1	62	38	0
	Day 2	0	88	12
SB165	Day 3	0	0	100

Table 2.10 – Summary of the change in average percentage of male florets observed during anthesis in the AT temperature treatment of Experiment 2, for each of the five genotypes used.

		AT			
SB Code	% Florets	FDS (1)	FDS (2)	FDS (3)	FDS (4)
	Day 1	83	11	6	0
	Day 2	56	14	30	0
	Day 3	50	3	39	8
SB020	Day 4	0	0	17	83
	Day 1	56	11	25	8
	Day 2	39	2	59	0
	Day 3	25	3	33	39
SB155	Day 4	0	0	3	97
	Day 1	42	6	44	8
	Day 2	64	6	28	2
	Day 3	31	0	47	22
SB165	Day 4	0	0	14	86

Table 2.11 – Summary of the change in average percentage of male florets observed during anthesis in the HT temperature treatment of Experiment 2, for each of the five genotypes used.

		HT			
SB Code	% Florets	FDS (1)	FDS (2)	FDS (3)	FDS (4)
	Day 1	39	22	39	0
	Day 2	11	17	72	0
	Day 3	11	17	72	0
SB020	Day 4	0	0	11	89
	Day 1	36	8	53	3
	Day 2	31	3	64	2
	Day 3	19	14	50	17
SB155	Day 4	0	0	19	81
	Day 1	58	8	11	23
	Day 2	11	8	81	0
	Day 3	11	6	81	2
SB165	Day 4	0	0	19	81

Table 2.12 – Summary of the change in average percentage of female florets observed during anthesis in the AT temperature treatment of Experiment 2, for each of the five genotypes used.

		AT		
SB Code	% Florets	FDS (HF)	FDS (F)	FDS (G)
	Day 1	50	50	0
	Day 2	31	67	2
	Day 3	31	31	38
SB020	Day 4	0	2	98
	Day 1	42	22	36
	Day 2	14	55	31
	Day 3	11	22	67
SB155	Day 4	0	8	92
	Day 1	31	36	33
	Day 2	31	47	22
	Day 3	14	42	44
SB165	Day 4	0	0	100

Table 2.13 – Summary of the change in average percentage of female florets observed during anthesis in the HT temperature treatment of Experiment 2, for each of the five genotypes used.

		HT		
SB Code	% Florets	FDS (HF)	FDS (F)	FDS (G)
	Day 1	14	61	25
	Day 2	22	56	22
	Day 3	0	58	42
SB020	Day 4	0	6	94
	Day 1	36	31	33
	Day 2	19	42	39
	Day 3	17	28	55
SB155	Day 4	0	0	100
	Day 1	25	45	30
	Day 2	11	50	39
	Day 3	11	28	61
SB165	Day 4	0	0	100

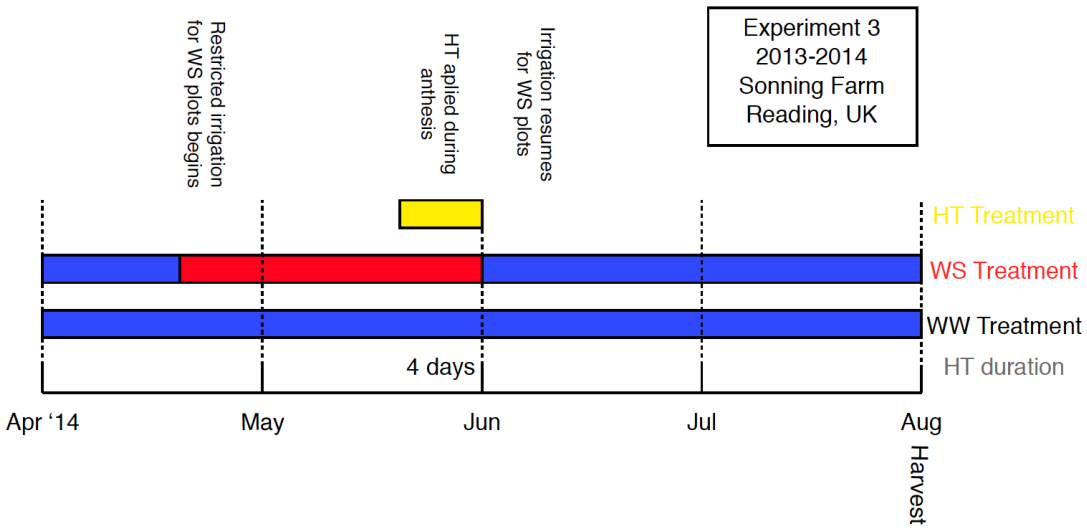

Figure 3.26 – A timeline illustrating, over the period of April-August 2014, when elevated temperature (HT) and water-deficit stress (WS) were applied to the plants in Experiment 3.

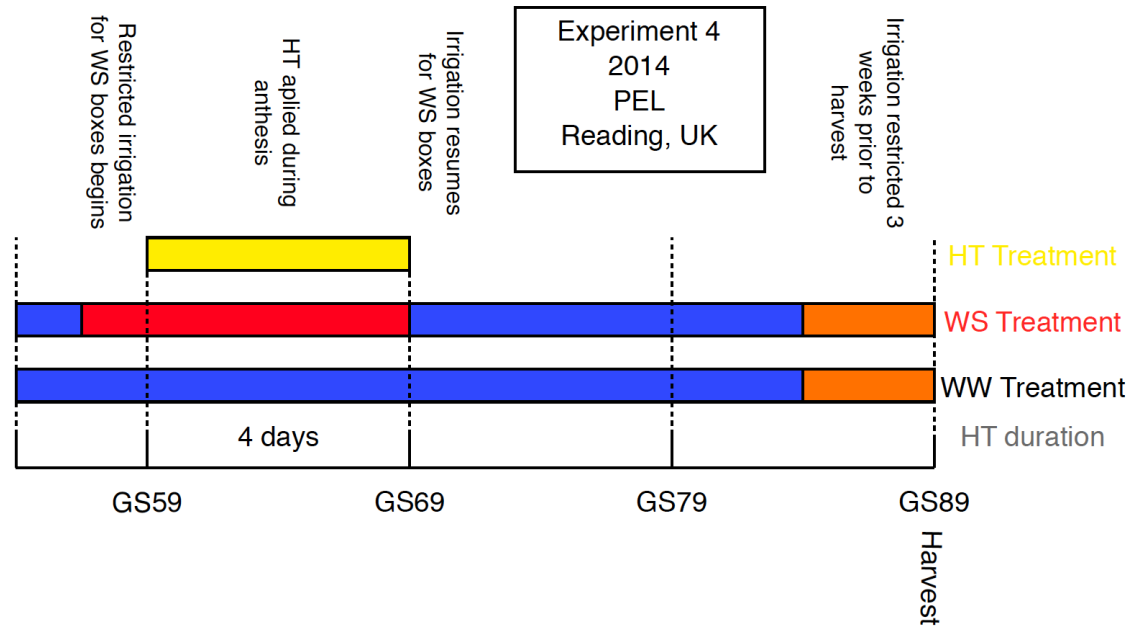

Figure 3.27 – A timeline illustrating when elevated temperature and water-deficit stress were applied to the plants in Experiment 4, in relation to Zadocks physiological growth stages.

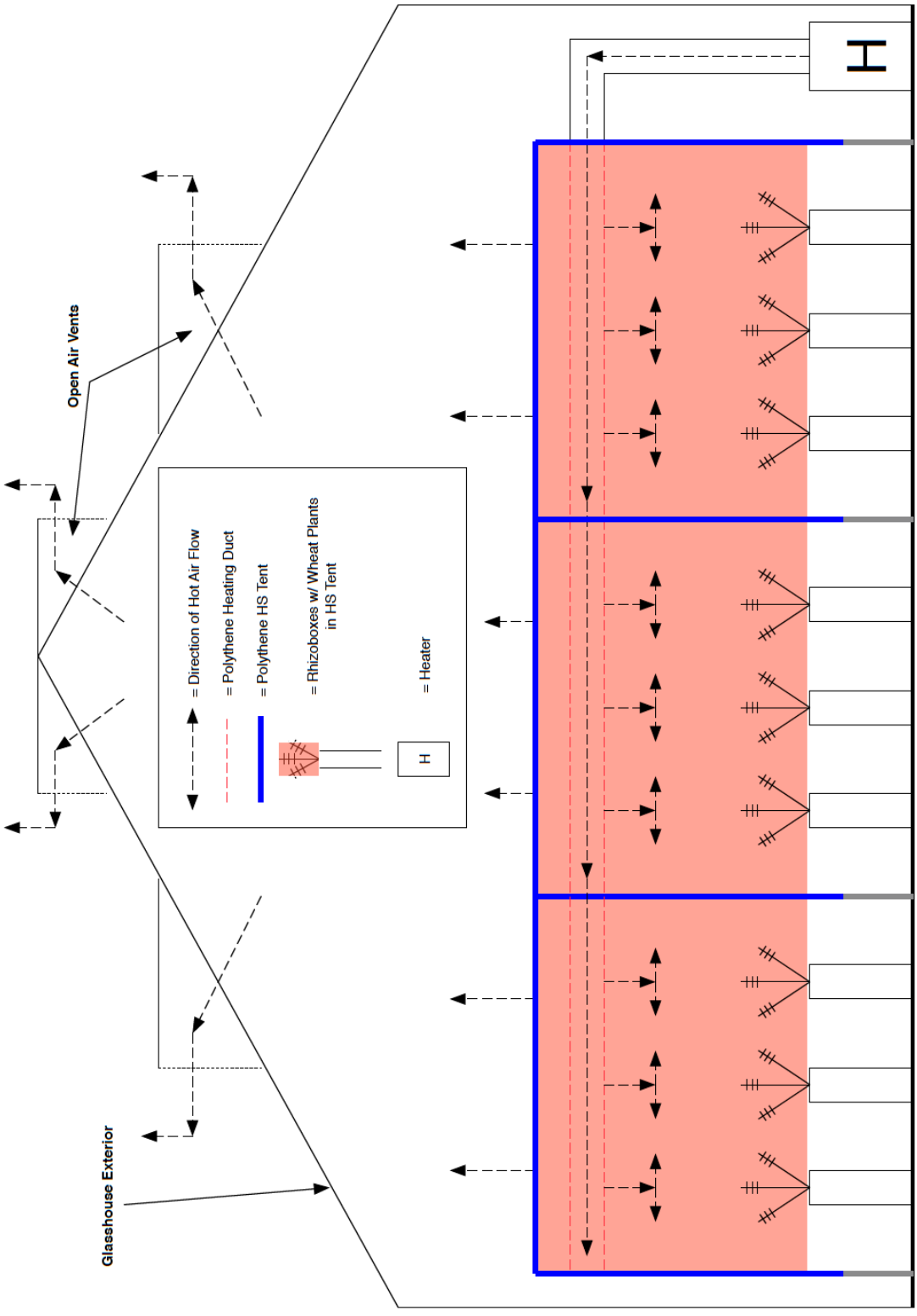

Figure 3.1 – The floor plan of the glasshouse used during Experiment 4.

Figure 3.2 – The glasshouse at the Plant Environment Laboratory (UoR, UK) used to conduct Experiment 4.

Figure 3.3 – The heat stress tent within the glasshouse pictured in Fig. 3.2.

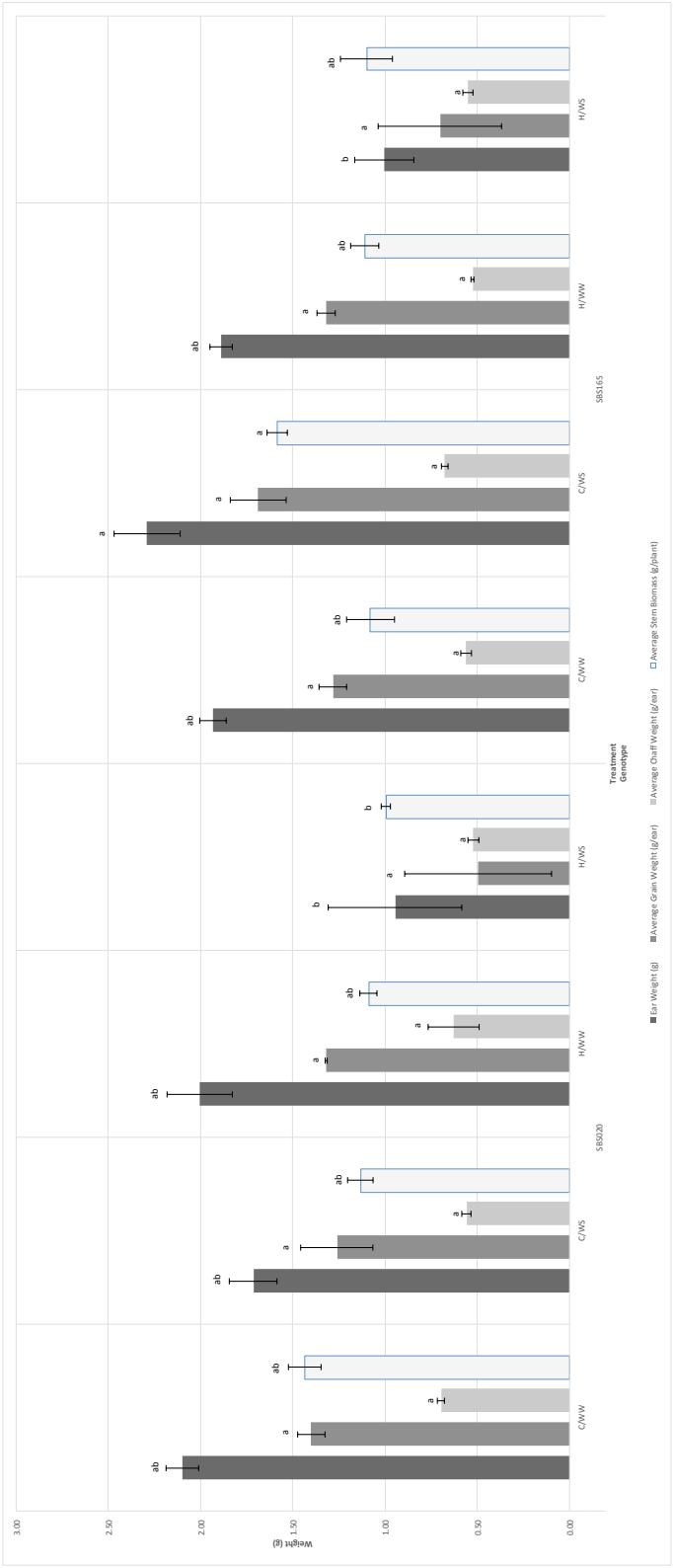

Figure 3.17 – Ear weight, grain weight, chaff weight and stem biomass collected from Experiment 4, categorized by genotype and treatment combination. Bars represent standard error. Different letters above bars indicate significant differences (*P*<0.05).

Table 3.5 – *P*-values generated from correlation analysis examining average CTD/STD during anthesis and grain yield, under a range of treatments in Experiment 3. Effects were considered significant at *P*<0.05.

Organ TD	Treatment/Treatment Combinations	P-value	Significant?
CTD	AT	0.404	ns
	HT	0.211	ns
	WW	0.082	ns
	WS	0.277	ns
	AT+WW	0.362	ns
	AT+WS	0.235	ns
	HT+WW	0.068	ns
	HT+WS	0.293	ns
STD	AT	0.735	ns
	HT	0.155	ns
	WW	0.161	ns
	WS	0.958	ns
	AT+WW	0.589	ns
	AT+WS	0.459	ns
	HT+WW	0.197	ns
	HT+WS	0.451	ns

Table 3.6 – *P*-values generated from correlation analysis examining average CTD/STD on Day 2 and grain yield, under a range of treatments in Experiment 3. Effects were considered significant at *P*<0.05.

Organ TD	Treatment/Treatment Combinations	P-value	Significant?
CTD	AT+WW	0.363	ns
	AT+WS	0.282	ns
	HT+WW	0.413	ns
	HT+WS	0.051	ns
STD	AT+WW	0.335	ns
	AT+WS	0.256	ns
	HT+WW	0.339	ns
	HT+WS	0.262	ns

Table 3.8 – Average temperature of in the AT treatment and in each of the HT tents, recorded between 11.00h and 14.00h on all five days of observation during Experiment 4. Values in brackets represent standard error.

Sensor Location	Day	Temperture (°C)
AT	1	21.32 (0.28)
	2	25.36 (0.61)
	3	26.36 (0.58)
	4	25.98 (0.31)
	5	32.06 (0.30)
HT (Tent 1)	1	34.67 (0.68)
	2	25.94 (0.48)
	3	36.56 (0.40)
	4	35.95 (0.46)
	5	36.51 (0.31)
HT (Tent 2)	1	35.33 (0.38)
	2	36.22 (0.86)
	3	37.82 (1.22)
	4	41.94 (0.37)
	5	40.56 (0.70)
HT (Tent 3)	1	33.10 (0.74)
	2	33.78 (0.71)
	3	35.37 (1.05)
	4	38.44 (0.29)
	5	38.15 (0.59)

Table 3.9 – Average, minimum and maximum nighttime (20.00h-06.00h) air temperatures recorded in the glasshouse during Experiment 4. Values in brackets represent standard error.

Day	Average Temp. (°C)	Minimum Temp. (°C)	Maximum Temp. (°C)
1	18.3 (0.11)	18.2	20.6
2	19.5 (0.18)	19.1	23.4
3	18.7 (0.23)	18.3	24.1
4	19.4 (0.14)	19.1	25.3
5	19.7 (0.33)	17.2	23.1

Table 3.10 – Summary of the change in average percentage of male florets observed during anthesis in the AT temperature treatment of Experiment 4, for both (a) SB020 and (b) SB165. Irrigation treatments were pooled.

Day	Average FDS (1)	Average FDS (2)	Average FDS (3)	Average FDS (4)
1	100.00%	0.00%	0.00%	0.00%
2	100.00%	0.00%	0.00%	0.00%
3	72.00%	17.00%	11.00%	0.00%
4	22.00%	28.00%	50.00%	0.00%
5	0.00%	11.00%	6.00%	83.00%

Day	Average FDS (1)	Average FDS (2)	Average FDS (3)	Average FDS (4)
1	100.00%	0.00%	0.00%	0.00%
2	78.00%	0.00%	22.00%	0.00%
3	61.00%	0.00%	28.00%	11.00%
4	33.00%	17.00%	17.00%	33.00%
5	0.00%	22.00%	11.00%	67.00%

Table 3.11 – Summary of the change in average percentage of male florets observed during anthesis in the HT temperature treatment of Experiment 4, for both (a) SB020 and (b) SB165. Irrigation treatments were pooled.

Day	Average FDS (1)	Average FDS (2)	Average FDS (3)	Average FDS (4)
1	100.00%	0.00%	0.00%	0.00%
2	94.00%	11.00%	0.00%	0.00%
3	67.00%	11.00%	22.00%	0.00%
4	11.00%	22.00%	50.00%	16.00%
5	0.00%	0.00%	0.00%	100.00%

Day	Average FDS (1)	Average FDS (2)	Average FDS (3)	Average FDS (4)
1	100.00%	0.00%	0.00%	0.00%
2	83.00%	6.00%	11.00%	0.00%
3	67.00%	17.00%	17.00%	0.00%
4	0.00%	17.00%	72.00%	11.00%
5	0.00%	0.00%	0.00%	100.00%

Table 3.12 – Summary of the change in average percentage of female florets observed during anthesis in the AT temperature treatment of Experiment 4, for both (a) SB020 and (b) SB165. Irrigation treatments were pooled.

Day	Average FDS (HF)	Average FDS (F)	Average FDS (G)
1	83.00%	17.00%	0.00%
2	89.00%	11.00%	0.00%
3	17.00%	83.00%	0.00%
4	6.00%	94.00%	0.00%
5	0.00%	28.00%	72.00%

Day	Average FDS (HF)	Average FDS (F)	Average FDS (G)
1	50.00%	50.00%	0.00%
2	44.00%	56.00%	0.00%
3	17.00%	83.00%	0.00%
4	0.00%	94.00%	6.00%
5	0.00%	33.00%	67.00%

Table 3.13 – Summary of the change in average percentage of female florets observed during anthesis in the HT temperature treatment of Experiment 4, for both (a) SB020 and (b) SB165. Irrigation treatments were pooled.

Day	Average FDS (HF)	Average FDS (F)	Average FDS (G)
1	89.00%	11.00%	0.00%
2	33.00%	67.00%	0.00%
3	6.00%	94.00%	0.00%
4	0.00%	83.00%	17.00%
5	0.00%	0.00%	100.00%

Day	Average FDS (HF)	Average FDS (F)	Average FDS (G)
1	89.00%	11.00%	0.00%
2	33.00%	67.00%	0.00%
3	0.00%	100.00%	0.00%
4	0.00%	83.00%	17.00%
5	0.00%	17.00%	83.00%

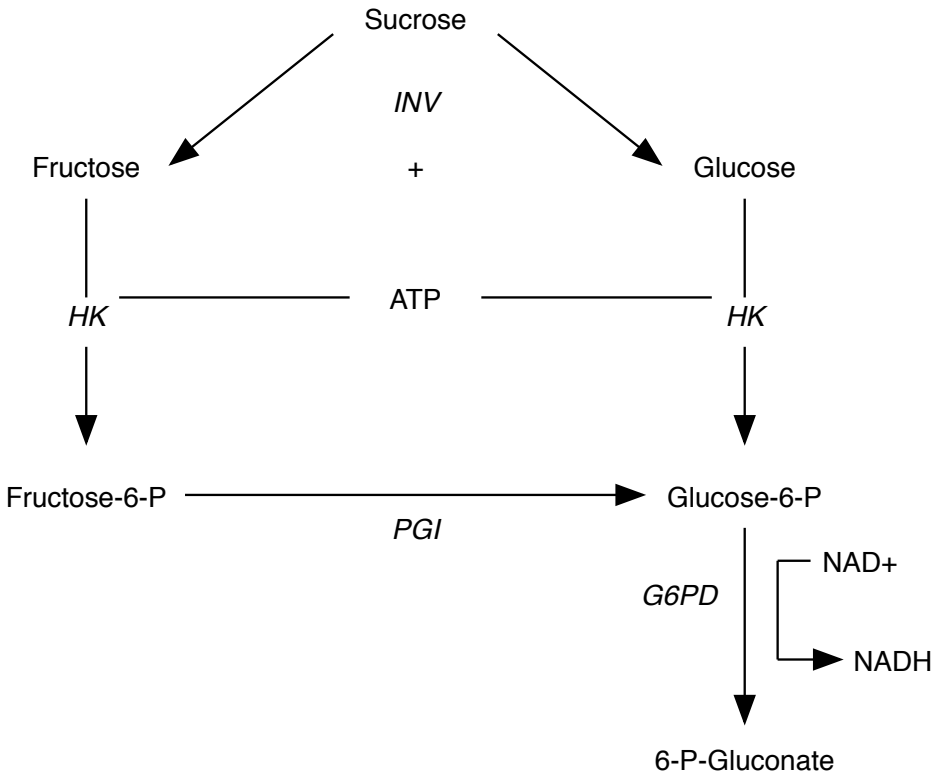

Figure 4.1 – The coupled assay pathways taking place during the determination of soluble sugar content.

Protocols for Megazyme Solution Preparation

Solution 1 – Sodium acetate buffer (0.15M)

A sodium acetate buffer (1.2M, pH3.8) was made up by adding 69.6ml of glacial acetic acid ($C_2H_4O_2$, 1.05mg/cm^3) to 800ml of distilled water. Once stirred, the volume was adjusted to 1L. The pH was adjusted to 3.8 using a 4M sodium hydroxide solution. In order to use this buffer for the subsequent stages, a 8x dilution of the buffer was prepared (0.15M, pH3.8). When sodium acetate buffer is referred to from this point onwards, it is the 8x dilution of the original buffer that is in question.

Solution 2 – Potassium hydroxide solution (0.2M)

A potassium hydroxide solution (2M) was made up by adding 112.2g of potassium hydroxide (KOH) to 900ml of distilled water. Once stirred, the volume was adjusted to 1L. In order to use this buffer for the subsequent stages, a 10x dilution of the buffer was prepared (0.2M). When All references to potassium hydroxide solution is referred to from this point onwards, it is the refer to the 10x dilution of the original potassium hydroxide solution that is in question.

Solution 3 – GOPOD Reagent Solution

This reagent is prepared using products supplied in the Megazyme Total Starch Assay Kit. 50ml of p-hydrobenzoic acid (pH7.4) and sodium azide (0.095% m/v), the GOPOD Reagent Buffer, was added to 900ml of distilled water in a 1L glass bottle and covered in aluminium foil, to protect the reagents against light. Once mixed, the total volume was adjusted to 1L. 20ml of the GOPOD reagent buffer solution described above was pipetted into the bottle containing the GOPOD Reagent Enzymes (a freeze dried powder of glucose oxidase, peroxidase and 4-aminoantipyrine). The contents of this bottle were then quantitatively transferred into the 1L glass bottle containing the GOPOD Reagent Buffer solution. Once mixed, the bottle was kept in a fridge at 4˚C.