University of Reading

Identifying beneficial traits for heat stress around reproductive phases of growth in wheat

Henry Mark Barber

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Declaration of Original Authorship

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

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Henry Mark Barber

Abstract

There is a need for heat tolerant traits in wheat to be identified in order to maintain and increase yields in future climates. The aim of this project was to assess genotypic variation in crop response to heat stress by comparing a southern European wheat genotype (Renesansa; *Rht-D1a, Rht8, Ppd-D1a*) with a UK genotype (Savannah; *Rht-D1b, Ppd-D1b, 1BL/1RS*) and their doubled haploid progeny. This would allow for the identification of traits and alleles that would benefit UK and European wheat production under climate change scenarios through the use of a combination of phenotyping, genotyping and crop modelling. Heat stress experiments were conducted in controlled environments to identify the most susceptible growth stages to heat stress within the population and to identify potentially tolerant traits. An appraisal of the crop model SIRIUS and how it simulates heat stress was undertaken. Finally, a field trial was conducted to identify which traits perform well in UK field conditions. Two periods of susceptibility in Savannah and Renesansa were identified as susceptible to heat stress, through reductions in grain number. The first period was identified around booting, with the second being identified one day before mid anthesis. The period around heading was found to be relatively tolerant. Compensation of reduced grain numbers through increases in grain size was limited and variable. *Rht8* was not found to influence heat stress tolerance. The photoperiod insensitivity allele *Ppd-D1a* was found to increase susceptibility to heat stress, while the semi dwarfing allele *Rht-D1b* was found to confer tolerance to it. *Rht8* was associated with reduced yield in UK field conditions. Simulations from SIRIUS suggest that yield loss due to heat stress could increase by three fold in central Europe by 2090, though it is not expected to be a major issue in the UK.

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Common Abbreviations

BN: billion

Cm: centimetre

D.F: Degrees of Freedom

DH: Doubled Haploid

GAI: Green Area Index

GS: Growth stage

H: Hour

Ha: Hectare

HI: Harvest index

Kg: Kilogram

NIL: Near Isogenic Line

NUE: Nitrogen Use Efficiency

PAR: Photosynthetically Active Radiation

Ppd: Photoperiod alleles

Rht: Reduced height gene

RUE: Radiation Use Efficiency

S.E.D: Standard Error of the Difference

Chapter 1: Introduction and Literature Review

1.1: Increased Yield in Crops

1.1.1: The need for higher yields

Wheat is one of the largest global food crops, with over 700 million tonnes being produced in 2013/14 (F.A.O., 2014). With world population expected to exceed 9.5bn by 2050 (UN, 2004), there is a need for a sustainable increase in food production (Godfray et al., 2010). Global meat demand is also expected to rise by 2030, especially from developing countries (F.A.O., 2003), meaning the demand for wheat as a source of animal feed is also likely to increase.

Another factor that will increase the need for higher yields is the availability of agricultural land. There is uncertainty with how much land will be available in the future for agricultural expansion, though a trend is emerging showing that less land is available per person to grow food (Alexandratos and Bruinsma, 2012). Therefore, it is likely that in order to feed a growing population, with less land available per person, crops yields will have to increase.

1.1.2: Previous methods of yield improvement

The two major methods in the past used to increase wheat yields have been to increase the proportion of biomass in the grain (increased HI) and increasing the resources available to the crop through increased nutritional inputs. Both were in part brought about by the green revolution. The green revolution, famously fronted for wheat by Norman Borlaug, was in response to an ever increasing population and increasing food demand (Lowell, 2008).

The reduction of crop height in the UK was brought about primarily through the introduction of reduced height genes (*Rht*). These brought two main benefits to UK wheat varieties; the reduction of lodging through shorter, stiffer straw and increased biomass in the ear, leading to increased grain yield (Gale and Youssefian, 1985). The Rht allele used in UK wheat genotypes originated from the Japanese Norin-10 genotypes and was first introduced in to UK agriculture as the variety Gaines (Gale and Youssefian, 1985). Semi dwarfism, displayed in Figure 1.1 (Flintham et al., 1997), is now well known to increase yields through reduced height and increased HI.

Figure 1.1: Effects of different Rht combinations on plant height. Photograph shows from left-right the tall control, Rht-B1b, *Rht-D1b*, RhtB1b+*Rht-D1b*, Rht-B1c and Rht-B1c+*Rht-D1b* lines of Maris Huntsman (Flintham et al., 1997).

The other method employed in the past to increase crop yields is increasing nutritional inputs, mainly nitrogen. A large amount of research has been carried out on the influence of nitrogen on wheat yield. Bayles et al (1978) found that increasing nitrogen applications to around 200kgha^{-1} maximised wheat yields. This research has led to a gradual increase in the amount of nitrogen applied to wheat, now averaging over 200kgha^{-1}, which is strongly correlated with increased yields (Hawkesford, 2014).

1.1.3: Possible future methods of yield improvements

Although previous increases in harvest index (HI) have increased yield in the past, it is unlikely that HI can be exploited further in wheat to increase yield. This is because plant heights of around 80cm have been found to give optimal yields (Flintham et al., 1997), as genotypes shorter than this have lower overall photosynthetically active radiation (PAR) interception due to smaller canopies (Austin, 1999). Harvest Index has not altered much in UK genotypes since the 1980’s, staying at around 50%, optimising biomass distribution with canopy size and light interception (Reynolds et al., 2009).

One option which could help to boost yields in the future is to improve efficiencies in the crop, namely radiation use efficiency and nitrogen use efficiency. Currently, as nitrogen inputs increase, nitrogen use efficiency (NUE) decreases (Loddo and Gooding, 2012), if this could be increased at high levels of nitrogen then this could help to increase yields. RUE is currently constrained, increasing this through methods such as increasing photosynthetic capacity could also increase yields (Reynolds et al., 2009). Crops will also have to be adapted to grow in future climates in order to maintain and increase yield potential (Semenov et al., 2014).

1.2: Climate Change Scenarios and Projections

Global mean temperatures are expected to rise by at least 1.5°C by 2100 compared to temperatures in the mid 1800’s (IPCC et al., 2013). At a regional level in Northern Europe, the rise in average global temperature is expected to cause an increase in the frequency of extreme weather events as well as a rise in average temperature (IPCC et al., 2013). These extreme weather events in the UK and Europe include increased risk of summer drought and heat waves (IPCC et al., 2013).

There are over 50 climate models that have been used by the CMIP5 (Coupled Model Intercomparison Project Phase 5) (Taylor et al., 2012). Combining these with differing emission scenarios causes a large divergence of future projections (Semenov and Stratonovitch, 2015), meaning a variety of different scenarios should be considered when looking at future crop production.

1.3: Effects of Climate Change on Food Production

Abiotic stress is one of the main threats to future global food security (Kadam et al., 2014) and will need to be addressed if we are to meet the challenge of feeding 9bn people by 2050 (Godfray et al., 2010). The next section highlights the two main abiotic stresses associated with yield loss (Barnabas et al., 2008) and gives an overview of previous work carried out on these stresses.

1.3.1: The effect of drought stress on crop production

Drought stress is one of the biggest risks to sustainable future crop production (Ahuja et al., 2010). The issue of drought stress is increasing largely due to the intensification of global agriculture increasing water demands (Mishra and Singh, 2010), as well as changing precipitation patterns due to climate change (Semenov and Stratonovitch, 2015).

Drought stress is known to have an effect on all commercially grown crops, including rice (Sheoran and Saini, 1996), sorghum (Mutava et al., 2011) and maize (Barnabas et al., 2008). Although abiotic stresses are often seen as an issue for future UK climates, there are many examples of heat and drought stress already causing serious damage to crops (Kadam et al., 2014).

For example, 30% yield losses due to drought were seen in the USA in 1988 and 1993 across a range of crops including soybean, maize and wheat (Rosenzweig and Parry, 1994; Kadam et al., 2014). Another example of drought stress has been seen in Thailand in 1998 and 2004 where rice yields were severely reduced, affecting up to 8 million people (Pandey et al., 2007; Kadam et al., 2014).

The mechanisms that cause drought stress to reduce crop yields are similar across most crops. In rice, it has been shown that drought stress around the pollen production phases of growth (microsporogenesis; Craufurd et al., 2013) can damage pollen and reduce grain numbers by up to 70% (Sheoran and Saini, 1996). A reduction in grain number and harvest index was also observed in Sorghum (Mutava et al., 2011) and maize (Barnabas et al., 2008).

1.3.2: The effect of drought on wheat production

The effect of drought stress on wheat can vary depending on the timing of the stress. Firstly, early season drought stress can affect germination, lower establishment and degrade early rooting systems (Almaghrabi, 2012). Similarly in other crops, drought stress around the reproductive phases of growth can lower fertility, reducing grain numbers and therefore yield (Barnabas et al., 2008).

A large amount of work has gone into detail looking at the effects of drought stress at these stages, including looking specifically at pollen formation (Saini and Aspinall, 1981; Saini et al., 1984), as well as more generally at the effects on grain yield (Alghabari et al., 2014; Pirttioja et al., 2015). Fertility and therefore grain numbers per ear are thought to be reduced due to the build-up of abscisic acid (Westgate et al., 1996).

Late season drought can reduce the grain filling period through premature leaf death, leading to earlier senescence and lower yields (Barnabas et al., 2008). Although drought stress is a major problem for wheat production on its own, drought x heat interactions are generally known to cause larger problems (Barnabas et al., 2008). Research by Alghabari et al (2014) suggests that a drought stress occurrence that coincides with heat stress can exacerbate the heat stress response. This is thought to be because heat stress can reduce grain set and combined with abscisic acid build up can increase the response compared to just one stress (Weldearegay et al., 2012).

There has been some progress made in wheat in regards to drought tolerance. The most notable example of this is the Australian variety Drysdale, which improves drought tolerance through increased water use efficiency, achieved by increasing carbon assimilation relative to water lost through transpiration under low water conditions (Richards et al., 2002; Condon et al., 2004).

As drought stress can negatively affect the crop during many different phases of growth, breeding strategies in the future need to consider ways to make wheat more tolerant, which could potentially occur in a number of different ways. For example, deeper rooting under water limited conditions could help to maximise uptake of available water (Araus et al., 2002). A number of different strategies need to be used, including QTL analysis to identify any potential beneficial traits under stressed conditions (Suzuky Pinto et al., 2010).

Although drought is widely agreed to be a major issue currently for global agriculture, especially crop production, there is some evidence to suggest that heat stress will become a larger threat to yields compared to droughts, in future climates. (Stratonovitch and Semenov, 2015) suggest that in future climates crops are likely to mature earlier due to higher ambient temperatures and this will mean that crops are likely to escape most drought periods that occur later in the summer. Stratonovitch and Semenov (2015) also state that heat stress will in fact be the bigger problem across Europe as this subtle shift in growing seasons coincides with likely periods of high temperature events, although drought tolerance is still a desirable trait if you wish to extend growing seasons to maximise yield.

1.3.3: The effect of heat stress on crop production

Heat stress poses a threat to many different crops globally and because of this a lot of work has gone in to studying the effects of it, most commonly around the reproductive phases of growth (Barnabas et al., 2008). There are already examples of where heat stress has had a negative effect on crop production. Notable examples include in 1972 when reduced yields in the Soviet Union due to heat tripled world grain prices (Battisti and Naylor, 2009; Kadam et al., 2014). More recently, in 2003, Western Europe was affected by high temperatures, severely damaging maize and fruit yields (Battisti and Naylor, 2009; Coumou and Rahmstorf, 2012; Kadam et al., 2014).

Early work by Satake and Yoshida (1978) suggests that rice is most susceptible around microsporogenesis and anthesis, however, this and subsequent reproductions (Satake and Yoshida, 1981; Craufurd et al., 2013) show a hand drawn response (Fig 1.2) with no apparent replication within the experiment, as well as a lack of clarity over the growth stages which were stressed at each timing.

More recent work on rice adds to the amount of information available, showing that microsporogenesis is indeed the most susceptible stage, with anthesis nearly being as susceptible to brief periods of high temperature (Prasad et al., 2006; Martinez-Eixarch and Ellis, 2015). The reproductive phases of growth being the most susceptible to heat stress is also a trend that has been seen in other crops including Sorghum (Prasad et al., 2008), faba bean (Bishop et al., 2016) and Maize (Barnabas et al., 2008). Elevated temperatures can also influence crops outside of the reproductive phases of growth. Increases in ambient temperature can move anthesis earlier in the season (Semenov et al., 2014), reducing time to senescence and lowering yield (Barnabas et al., 2008).

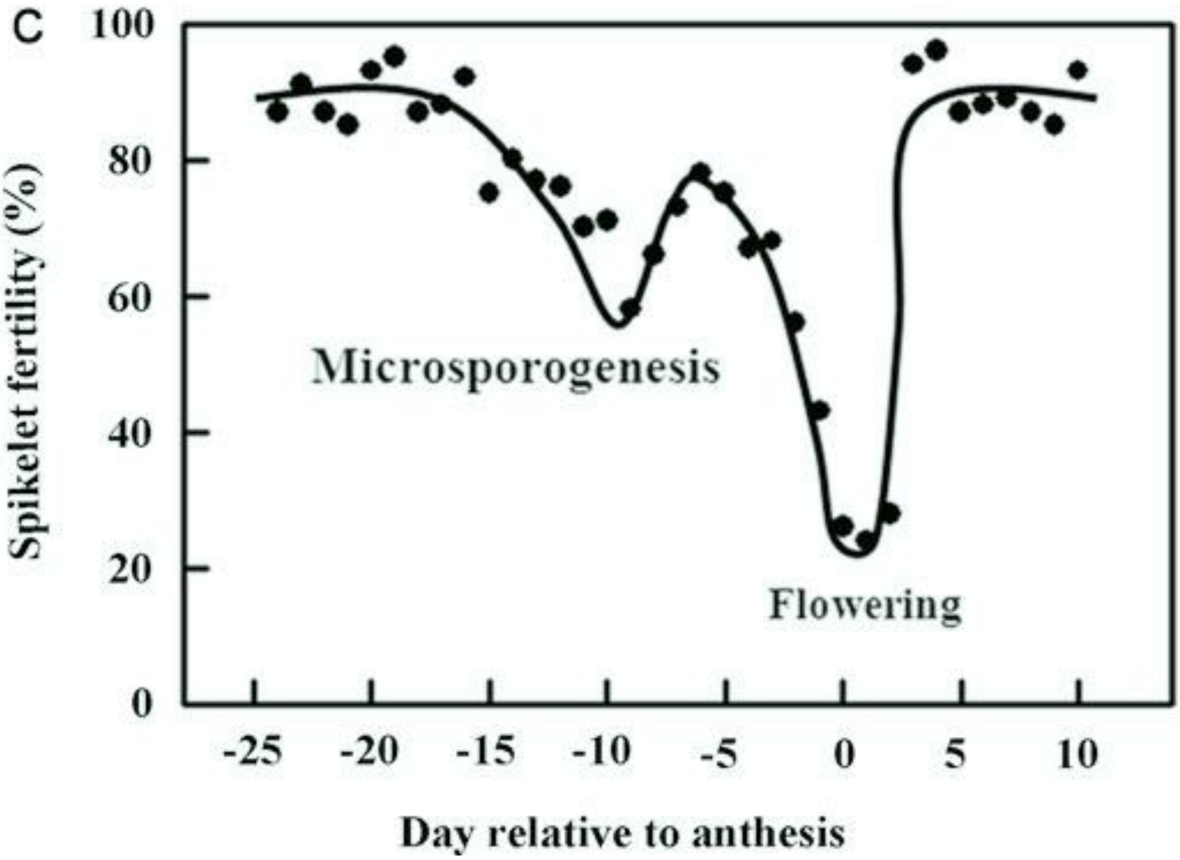

Figure 1.2: Sensitivity to high temperature at flowering in rice (Craufurd et al., 2013), redrawn from Satake and Yoshida (1981).

1.3.4: The effect of heat stress on Wheat

1.3.4.1. Heat stress events critical to wheat yield

Firstly, it is important to clarify what a “high temperature” stress is for the wheat plant, which is commonly grown in more temperate, northern climates. For stress events around the most sensitive growth stages, 31°C has been found to be able to significantly reduce yield in wheat (Wheeler et al., 1996; Porter and Gawith, 1999). However, this should not be considered to be a consistent value as it can be influenced by four main factors; duration of stress; timing of stress, ambient humidity and water availability.

A common theme amongst previous work on wheat is that the plants are subjected to heat stress over multiple days. For example, the work conducted by Ferris et al (1998) subjected wheat to 4 days of high temperature which reduced the critical temperature to below 30°C. Multi day stresses were also used in other trials including Alghabari et al (2014, 2016). Although a multi-day stress is useful in seeing the overall effect heat stress has on wheat yield, it becomes harder to analyse what specific growth stage is most susceptible to heat stress, especially as meiosis is usually completed within a day in wheat (Saini and Aspinall, 1982). Therefore, shorter periods of stress, which would allow a more accurate assessment of susceptible growth stages, are a more ideal approach. This was a method used by Langer and Olugbemi (1970), who used 3 hour transfers to high temperature environments and still saw an effect of high temperature. Saini and Aspinall (1982) also state that a reduction in grain number can be seen from just one day of wheat being under high temperature. Table 1.1 displays some more examples of heat stress experiments which also have interactions with other environmental factors, such as duration of stress, humidity and water availability.

Table 1.1: Examples of critical temperatures derived from heat stress experiments on wheat during the reproductive phases of growth, varying due to duration of stress, humidity and water availability. Critical temperature in this Table is defined as the temperature that wheat yields would be significantly decreased under the stated conditions.

Influencing Factor	Factor Description	Implied Critical Temperature (°C)	Example Reference
Duration of Stress	2 days	>30	(Prasad and Djanaguiraman, 2014)
	4 days	31	(Ferris et al., 1998)
	5 days	24	(Prasad and Djanaguiraman, 2014)
Humidity	Increase in humidity	Would lower critical temperature	(Tashiro and Wardlaw, 1990)
Water Availability	High	31	(Wheeler et al., 1996; Porter and Gawith, 1999)
	Low	29	(Alghabari et al., 2014)

As shown in Table 1.1, there are several factors in heat stress experiments, not related to genotype that can influence the response of wheat to temperature. Along with the duration of stress, ambient humidity and water availability can also influence the severity of the heat stress response.

Although some work has gone in to assessing the interactions between heat and humidity in rice (Abeysiriwardena et al., 2002; Weerakoon et al., 2008; van Oort et al., 2014), there are relatively low amounts of information regarding humidity x temperature effects on wheat. Studies have shown that higher humidity can increase the severity of yield loss due to heat stress (Dawson and Wardlaw, 1989; Tashiro and Wardlaw, 1990), also lowering the critical temperature that wheat is affected by heat stress. It is thought that this relationship is due to the effect transpiration rates have on the crops response to heat stress in an attempt to keep the crop cool. Actual floret temperature plays a very important role in determining the effect of high temperature stress (Suzuky Pinto et al., 2010; Steinmeyer et al., 2013).

1.3.4.2. General response to heat stress

The interaction between elevations in temperature and CO_2 in future climates is an interesting one. Elevated CO_2 levels are expected to help increase yields (Semenov, 2009), however elevated temperatures could offset any potential increases from this (Wheeler et al., 1996). Increases in average temperature are likely to make flowering times earlier in the year (Stratonovitch and Semenov, 2015) and also make maturity times earlier, reducing the amount of light intercepted over the season, lowering yields (Barnabas et al., 2008).

It has been reported that high temperature events late in the growing season can negatively impact wheat. Whilst grain number is usually determined by this stage, high temperature during later phases of development can impact the grain itself. High temperature has been seen to lower overall grain size, leading to lower yield (Stone and Nicolas, 1994; Liu, Asseng, Liu, et al., 2016) as well as having negative implications on grain quality (Jagadish et al., 2014).

Heat wave events in field are not likely to be one consistent temperature (Lukac et al., 2012), therefore the majority of heat stress experiments try to reflect that and have a range of temperatures within one stress experiment. There is a “stressful” temperature, which is deemed to be the temperature in the experiment high enough to have negative implications on the plant. The “base” or control temperature is that used in the experiment deemed not to be stressful. This is not always the “optimum” temperature, which is the temperature where development of the plant is at its most rapid. Finally, it is important to acknowledge “acclimation” periods. Depending on the specific methodology this can occur in two ways. Either the plant is transferred directly from a base temperature to a stressful one, e.g. from outside conditions in to a growth cabinet. Or an acclimation period can occur when a plant in a growth chamber experiences the temperature being raised from base to stressful. The severity of the acclimation period can affect the plant negatively and this effect needs to be accounted for when analysis heat stress experiments.

1.3.4.3. The response to heat stress around booting

One of the most susceptible phases of growth to heat stress is meiosis (Saini and Aspinall, 1982; Barnabas et al., 2008; Alghabari et al., 2014, 2016), which is generally considered to be coincident with booting, starting at GS39 (Zadoks et al., 1974b). The Zadoks Growth Scale in wheat (Zadoks et al., 1974a, 1974b) has been hugely influential in improving agronomic inputs, including nitrogen and fungicide applications, through improvement in timings of applications (Barber et al., 2015). The reproductive phases of growth start around the appearance of the flag leaf, at the onset of booting (Tottman, 1987). However, the specific start point of meiosis relative to external growth stage is variable, influenced by both genotype and environment (Zadoks et al., 1974b; Barber et al., 2015). This becomes an issue when assessing the effect of heat stress around meiosis.

Whilst meiosis occurs around booting, the reproductive phases of growth are defined as starting at the beginning of stem extension, coincident with the double ridge stage of the meristem (Barber et al., 2015). After the double ridge stage, the spike elongates and progresses up the stem as it develops. Spikelet production finishes upon the appearance of the terminal spikelet. A full description of the reproductive phase of growth is provided by Barber et al (2015; Appendix 2).

Meiosis can be categorised in to 4 distinct phases during each division, prophase, metaphase, anaphase and telophase. In wheat, meiosis is usually synchronous between both male and female organs, and for an individual tiller is usually completed within a day (Saini and Aspinall, 1982). Detailed descriptions and illustrations of meiosis in wheat for both male and female organs have been produced by Bennett (Bennett et al., 1971; Bennett, Finch, et al., 1973).

There is currently no method of externally identifying when meiosis is actually occurring in the plant (Barber et al., 2015). This can leave some uncertainty with heat stress trials as to whether meiosis was “hit” or whether there was any “escape from stress”. Therefore there is some level of assumption with heat stress trials that they have been successful in targeting meiosis. Some experiments have assumed meiosis to occur as stated at the emergence of the flag leaf ligule (Tottman, 1987; Subedi et al., 2000; Alghabari et al., 2014) and some stressing crops during the main phases of booting where swelling has occurred (Saini and Aspinall, 1982).

Escape of heat stress through meiosis occurring at different growth stages must be avoided when comparing different genotypes of wheat for tolerance to heat stress. There are two main ways of achieving this: The first, most common way is to stress plants around booting for multiple days to ensure that meiosis is successfully targeted in each of the genotypes. The length of stress used to cover this period varies, from 3 day stresses (Saini and Aspinall, 1982; Alghabari et al., 2014), to being stressed for the whole duration of booting (Dawson and Wardlaw, 1989).

Although extending the duration of stress, especially for the whole of booting, is likely to ensure meiosis will be affected, it comes with some negative aspects. Firstly, extended periods of high temperature are not a likely occurrence in future climates in major wheat growing areas (IPCC et al., 2013) and therefore this style of experiments lose some focus from what the reality in a commercially grown field of wheat may be. Secondly, this method still cannot always guarantee that meiosis was stressed in the plant. We cannot make this guarantee for a number of reasons. As mentioned previously, the exact timing of meiosis isn’t consistent with external growth stage. Also, the length of time it takes for a plant to go through the whole of booting is genotype and plant density dependent and can vary (Barber et al., 2015). Therefore it is entirely possible that a 3 day stress from the start of booting on 2 different genotypes could hit meiosis in one but miss it in the other, which would skew the comparison of the two genotypes.

The other method of ensuring that meiosis is stressed during experiments is to use much shorter, more discrete, durations of stress, but increasing the number of treatments used. This is a method that has been used in rice to study microsporogenesis, originally in Satake and Yoshida (1978) and the subsequent reproductions (Satake and Yoshida, 1981; Craufurd et al., 2013). In wheat, the method of multiple stresses across a range of different growth stages has appeared in Prasad and Djanaguiraman (2014), although each stress lasted for 5 days, removing the discreteness gained from this method.

Although this method is intensive in the amount of work required obtaining a full dataset, if done over all booting growth stages it can give a more realistic response to heat stress as well as covering all possible susceptible growth stages. This also allows a comparison of all of the different growth stages and how their response differs between genotypes (Barber et al., 2015).

Whist a volume of work, discussed above, has focussed on the effects of heat stress around meiosis leading to yield loss; it is also worth considering another effect of heat stress around the booting stages of development. It has been reported that abiotic stress around this timing can lead to floret abortion (Barnabas et al., 2008; Reynolds et al., 2009; Weldearegay et al., 2012). The number of fertile florets is a key determination of grain number (Reynolds et al., 2009) and as well as interacting with temperature it is also influenced largely by resource availability, such as nitrogen availability (Barnabas et al., 2008).

Unless detailed inspection of the plant is carried out around these times, it is difficult to determine definitively whether grain number reductions observed are due to floret abortion or direct damage to the meiotic process. What is clear however is that temperature has a strong interaction with grain development around booting, which warrants further investigation

1.3.4.4. The response to heat stress around anthesis

Anthesis is the final phase of reproductive development in wheat (Zadoks et al., 1974b) and is susceptible to a variety of biotic and abiotic stresses, which can significantly impact yield. Examples of these stresses include damage caused by *Fusarium culmorum* and *Fusarium graminearum* (AHDB, 2014) as well as damage due to cold stress (Subedi et al., 1998). However heat stress is the main abiotic stress around flowering that will cause grower issues in future climates (Semenov, 2009).

Unlike meiosis, which can usually be completed within a day (Saini and Aspinall, 1982), flowering across a whole plant can last over a number of days, due to variation in flowering timing across spikelets (Lukac et al., 2012).

Central spikelets usually flower first, after which flowering spreads to the top and bottom spikelets (Percival, 1921; De Vries, 1974), with the majority of flowering taking place in the morning (De Vries, 1974). As well as this, delayed development of tiller ears can increase the duration of flowering across a canopy (Devries, 1973), therefore flowering is considered to be occurring when 50% of the ears in a sample are in flower (Zadoks et al., 1974b; Lukac et al., 2012). Flowering duration is also thought to be genotype dependent, as demonstrated with the differences in flowering durations due to different *Ppd*-1 alleles (Jones et al., 2016).

Due to the importance of floral processes on the determination of final grain yield, through the determination of final grain number, damages due to heat stress must be understood. The review by Barnabas et al (2008) gives a thorough explanation to understand the processes behind these effects.

Earlier work by (Ferris et al., 1998) found a four day stress period immediately before mid anthesis (GS65) caused a significant reduction in grain set. These findings were also supported by a more recent work of Alghabari et al (2014), who also used multiple day stresses around flowering. Some attempt at identifying more discrete differences in effect of flowering GS on temperature response has been made, with 2 day stresses on the Asian cultivar, Chinese Spring, showing that the 2 days prior to mid anthesis as being particularly susceptible (Prasad and Djanaguiraman, 2014).

Various mechanisms have been proposed to explain why the period around anthesis is susceptible to heat stress. Similarly to heat stress around meiosis, yield reductions at this timing are due to reduced grain numbers through reduced grain fertility (Ferris et al., 1998; Barnabas et al., 2008).

Numerous reports show that this is caused due to heat shrivelling and damaging pollen, thus lowering fertility, summarised by Barnabas et al. 2008. Pollen dehiscence from anthers can also be reduced, lowering the amount of viable pollen which reaches the stigma, lowering fertility, as seen in rice (Prasad et al., 2006) and described in wheat (Barnabas et al., 2008). In crops which have a larger ability to outcross, such as faba bean, some compensation can occur through undamaged pollen from other plants, which can also be aided by pollinators (Bishop et al., 2016). This is less likely to happen in wheat because it is a primarily self-pollinating crop (Lukac et al., 2012), therefore any damage that happens to the pollen in wheat can be critical.

The theme of pollen damage through heat stress is a common one in previous literature, which leaves the question, why are male reproductive systems (anthers and pollen) more susceptible than female ones? One suggested reason is that the tapetum, a layer of cells in the anther wall, causes this increased sensitivity to heat stress (Dolferus et al., 2011). The tapetum is a highly metabolically active group of cells, which require a high number of mitochondria to function properly (Warmke and Lee, 1978). After meiosis, the tapetum cell layer dies and the resources from these cells go into assisting with healthy pollen development (Raghavan, 1988). Therefore any stress during this period which damages or prematurely kills tapetum cells (Gothandam et al., 2007) could damage pollen development and therefore grain set (Dolferus et al., 2011).

Out crossing across spikes is commonly observed to be well below 10% (Hucl, 1996), however there is less clarity over the amount of crossing across spikelets (Lukac et al., 2012).

However it is clear that within spikelet pollination is a crucial factor in the success of pollination and grain set. Therefore it seems clear why the crop has developed synchronous reproductive systems, with synchronicity between male and female systems during both meiosis (Bennett et al., 1973b; Saini et al., 1983) and anthesis (Lukac et al., 2012). It has been noted that heat stress can cause asynchrony to occur at meiosis (Bennett et al., 1973a) and anthesis (Lukac et al., 2012). This has the potential to lower yield through less successful pollination and is difficult to offset as increases in out crossing to compensate is limited and the stigma becomes less receptive to pollen the longer they are viable (Imrie, 1966).

As defined by Zadoks et al (1974b), peak flowering (GS65) is defined as over half of the ears assessed visibly flowering. In wheat, this growth stage is commonly observed when anthers are visibly extruded from their florets and flowering is easily identifiable. The only alternative to this is to open individual florets and examine their progression through flowering, as performed by Lukac et al (2012) and Jones et al (2016), although this can be a time consuming and labour intensive method. Previous heat stress studies show a trend that the days leading up to this stage tend to be the most susceptible (Ferris et al., 1998; Prasad and Djanaguiraman, 2014). It therefore seems odd that the peak susceptibility around anthesis does not line up with what is commonly observed and stated as the peak flowering period, the peak susceptibility instead coming slightly before this time. An explanation for this is that dehiscence from the anther largely occurs pre extrusion, whilst the anther is still in the floret (Joppa et al., 1968).

This could be due to the delayed nature of the floret opening in some genotypes (Sage and Isturiz, 1974) meaning the anther dehisces before it can extrude. This early dehiscence would therefore mean that the bulk of pollination occurs before peak external flowering growth stages, leading to the most susceptible external GS being 61 (early flowering) rather than 65 (mid flowering).

1.3.4.5. Current limitations

There has been a large volume of work attempting to quantify and explain the relationship between wheat and heat stress, however there are a number of areas within this topic which need addressing.

Whilst there have been a number of attempts to quantify the relationship between growth stage and heat stress effect, around booting (Saini and Aspinall, 1982; Alghabari et al., 2014) and flowering (Ferris et al., 1998; Prasad and Djanaguiraman, 2014), these have all involved multiple day stresses. More discrete stresses of shorter duration are required across multiple growth stages to add detail to specific growth stage responses, as internal reproductive mechanisms do not consistently coincide around the same growth stage (Barber et al., 2015). In particular, clarity is needed around the relationship of meiosis and booting, as well as the earlier phases of flowering.

The majority of papers mentioned previously have mainly focussed around one of the susceptible timings and as such there is a lack of clarity as to which is considered the most susceptible to heat stress. The only examples seen which have stressed booting and anthesis within the same experiment include the Satake and Yoshida (1978) experiment in rice and the Alghabari et al (2014) experiment in wheat.

An attempt was made to stress wheat across the whole reproductive period of growth by Prasad and Djanaguiraman (2014). However, fertility reductions were not seen in this study around meiosis. Furthermore, there was a lack of claritiy with regard to the precise growth stages stressed, as the Feekes scale was used to assess growth stage (Feekes, 1941). This is less clear than the Zadoks scale (Zadoks et al., 1974b) and the stresses were for 5 days, which covers a large range of growth stages, increasing uncertainty in the observations (Prasad and Djanaguiraman, 2014).

Future experiments need to stress across both phases of growth, especially when comparing genotypes, to add more certainty over the susceptibility of each stage to make comparisons easier. In order to achieve this, the multiple short duration stress approach carried out in rice (Satake and Yoshida, 1978), needs to be replicated for wheat. This “double dip” response also needs to be statistically proofed to add to the evidence of the response, which is something that Satake and Yoshida (1978) and its reproductions (Satake and Yoshida, 1981; Craufurd et al., 2013) lack.

Some have hypothesised that increased flowering durations can limit yield losses from heat stress events (Lukac et al., 2012; Jones et al., 2016). The idea being that increasing flowering duration lowers the number of spikes or spikelets that come in to contact with a heat stress event, increasing the proportion of healthy spikelets and maintaining yield (Lukac et al., 2012). This is a form of escape from stress rather than tolerance to it. This is also a theory that is difficult to test in controlled environments and conducting a field heat stress experiment can be difficult, especially in the UK.

Jones et al (2016) hypothesises that increasing insensitivity to day length (photoperiodism) through the *Ppd-D1a* allele (which also causes an increase in flowering duration) could be used as a beneficial trait in future climates. This idea needs testing and more detail on the *Ppd-D1a* allele and its relationship with heat stress is also needed.

As well as testing very specific traits like above, the search for heat stress tolerance must be continued. Methods must be developed for screening high numbers of genotypes for their heat stress responses, the data from which can be used to identify any heat tolerant traits through genetic analysis, such as QTL analysis.

Identifying heat tolerant genotypes is usually too difficult in the field due as avoidance mechanisms are often confused with tolerance (Dolferus et al., 2011). There are currently few known heat stress tolerant traits or genotypes, especially in Europe. It is easier to identify susceptible genotypes, such as Chinese Spring (Prasad and Djanaguiraman, 2014), than it is tolerant ones. This is because susceptible genotypes can be easily identified due to yield loss under stressed conditions, whereas it is not always clear whether a genotype which appears to retain yield under stress is tolerant or whether the susceptible period in that genotype escaped the stress. Any claims made for heat tolerant genotypes are usually mistaken for an avoidance mechanism and these claims arguably are not based on a deep volume of evidence and the genotypes aren't well adapted for current or future European climates.

1.4: Current Alleles Relevant to Adaptation to Abiotic Stress

There is in fact some potential to exploit potential future climates with regards to wheat yield. If wheat is adapted correctly a yield increase, rather than decrease, could be seen (Challinor et al., 2014). There are three main methods to counteracting abiotic stresses in crops, they are; escaping the stress through shifting susceptible periods of growth earlier in the season, lowering the risk of encountering extreme weather; making the crop tolerant to the stress by maintaining high yield potential even when the crop encounters extreme weather during a vulnerable period of growth and protecting the crop from stress through increased water availability to aid evaporative cooling. This section explains the genetic basis behind each of the different mechanisms and identifies alleles for further research that are relevant for future European climates.

1.4.1: Escape: Avoid periods of stress through timing

There are two potential methods of escaping heat stress around the reproductive phases of growth. The most common method is shifting meiosis and flowering to an earlier time in the growing season to avoid late season drought and heat stress. The other method is increasing the duration of flowering, in order to spread the risk and lower the number of spikelets that would be affected by abiotic stress at any one time.

Thermal time to anthesis is a very important phenotypic measure for yield potential, as the pre reproductive (vegetative) phases of growth are very important for biomass accumulation and ultimately grain yield (Reynolds et al., 2009). Increasing the thermal time to anthesis has been strongly linked with increased biomass accumulation and ultimately grain yield (Barber et al., 2015).

Therefore if we are to manipulate this important development stage and make it earlier in the season, it is important to understand the mechanisms which control its timing. There are three main mechanisms which influence the time of anthesis; these are earliness per se (Eps), vernalisation (*Vrn*) and photoperiodism (*Ppd*) (Snape et al., 2001; Langer et al., 2014).

Of the three main traits which influence flowering time, the vernalisation requirement of wheat is perhaps the best understood. In the UK there are two main types of wheat (*Triticum aestivum*) grown; winter wheat and spring wheat (AHDB, 2015b). The difference between the two is the vernalisation requirement; winter wheat requires vernalisation whereas spring wheat does not (Snape et al., 2001).

Vernalisation is the requirement of a period of low temperature by the plant before floral initiation can occur (Law et al., 1976). This allows the crop to be sown in autumn whilst ensuring that the risk of frost damage on floral processes can be reduced (Snape et al., 2001). Vernalisation requirement is usually considered an evolutionary adaptation of escaping winter abiotic stresses, primarily cold stress through frosts; its presence is likely to delay the date of anthesis (Snape et al., 1985). The importance of a vernalisation requirement is becoming increasingly important to avoid cold stress due to earlier sowing times of European winter wheat's (Snape et al., 2001).

There are a number of different loci on the wheat genome which contribute to vernalisation response with the most important alleles originally named *Vrn1, Vrn2* and *Vrn3* (Distelfeld et al., 2009).

Regulation of vernalisation in temperate cereals is predominantly controlled by *Vrn1* alleles (Yan et al., 2003), which have been mapped on chromosomes 5A (*Vrn-A1*) (Dubcovsky et al., 1998), 5B (*Vrn-B1*) (Iwaki et al., 2002) and 5D (*Vrn-D1*) (Law et al., 1976). Alleles of *Vrn* genes have varying effects on vernalisation requirements (Slafer, 1996), although *Vrn-A1* is considered to have the largest influence on vernalisation requirement (Pugsley, 1971; Dubcovsky et al., 1998). Vernalisation responses can also interact with photoperiod responses as when vernalisation requirements are not met, this can slow down pre floral development in the reproductive stages (Gonzalez et al., 2002). Although wheat's are usually confined to two categories, winter and spring, few can also be classified as facultative, meaning they can be sown both in autumn and spring (Snape et al., 2001).

This can occur due to the predominance of the *Vrn-A1* loci in reducing the vernalisation requirement compared to other loci (Snape et al., 1976), therefore, adjusting the allele at *Vrn-A1* can make wheat become facultative (Snape et al., 2001). There are a number of facultative wheat's currently commercially available in the UK (AHDB, 2016), which are spring wheat's suitable for late autumn sowing, such as Mulika. The best known example is Paragon, with was first recommended in 1999 and has become a regular genotype used in experimentation, most recently by Kowalski et al (2016).

Unlike vernalisation responses, which have been extensively studied (Snape et al., 2001), much less is known about 'earliness per se' (*Eps)* genes that also affect flowering time, independent of both photoperiod and vernalisation (Snape et al., 2001).

Variation in flowering time that occurs when both photoperiod and vernalisation are controlled for is usually attributed to *Eps* (Appendino and Slafer, 2003). Effects of *Eps* are usually attributed to more subtle variations in heading and anthesis dates (Griffiths et al., 2009). Owing to the fact that less is understood about *Eps*, or intrinsic earliness, less adaptation has occurred because of it, it is yet to be fully exploited when searching for ideal flowering times (Appendino and Slafer, 2003). More recent studies have begun to identify and study effects of certain *Eps* alleles. *Eps-*A^{m}*1* was seen to decrease time to heading resulting in fewer spikelets per ear in *T. monococcum*, which also translated in to spikelet number effects on group 1 chromosomes in regular hexaploid wheat (Lewis et al., 2008).

Although some understanding of *Eps* is coming through, it has yet to be exploited in commercial wheat for the escape of stress in the way that vernalisation or photoperiod sensitivity has.

Perhaps the most relevant trait in regards to the escape of late season abiotic stresses is photoperiod sensitivity. Sensitivity to photoperiod dictates the length of time taken to reach reproductive phases of growth from sowing (Grogan et al., 2016). Winter wheat is typically sensitive to photoperiod, classified as a ‘long day’ plant (Griffiths et al., 2009), meaning that it requires a minimum amount of day light to proceed to the reproductive phases of growth. However, wheat can also be grown as photoperiod insensitive, being able to flower under short day conditions (Griffiths et al., 2009).

As with vernalisation, sensitivity to photoperiod can also vary due to allelic differences. The allele that confers the strongest insensitivity to day length is *Ppd-D1a* (Jones et al., 2016). *Ppd-D1* is located on chromosome 2D and is fairly closely linked to the semi dwarfing allele *Rht8* (Gasperini et al., 2012).

Ppd-D1a is the most common source of photoperiod insensitivity in both wheat grown in southern Europe (Jones et al., 2016) and Asia (Kiss et al., 2014). There are 5 known *Ppd-D1* alleles, with *Ppd-D1a* the most commonly used (Beales et al., 2007).

Other *Ppd-1* alleles on group 2 chromosomes in wheat have been identified, including *Ppd-A1a* on 2A and *Ppd-B1a* on chromosome 2B (Jones et al., 2016). Although these alleles confer insensitivity to photoperiod, they do so to different degrees through different methods.

The mechanisms in which the different alleles confer insensitivity to day length have been well documented previously (Beales et al., 2007; Wilhelm et al., 2009; Diaz et al., 2012; Kiss et al., 2014; Jones et al., 2016).

Phenotypic effects of photoperiod insensitivity are also well documented. The primary effect of insensitivity to photoperiod is on the date of anthesis. *Ppd* alleles enable the plant to initiate floral processes earlier than photoperiod sensitive plants, meaning that anthesis date is brought forward (Snape et al., 2001). This effect is well established across numerous genotypes in different environments (Worland, 1996; Snape et al., 2001; Foulkes et al., 2004; Distelfeld et al., 2009; Griffiths et al., 2009; Diaz et al., 2012; Gomez et al., 2014; Kiss et al., 2014; Langer et al., 2014; Barber et al., 2015; Grogan et al., 2016; Jones et al., 2016), although the number of days that anthesis is brought forward can vary. The most recent work by Jones et al (2016) found that the stronger *Ppd* insensitive alleles also increase the duration of flowering, which they suggest could be a mechanism to further avoid stress, which has been suggested previously (Lukac et al., 2012), although this needs further testing as a method of resilience to heat stress.

Reducing the thermal time to anthesis through insensitivity to photoperiod is commonly used as an escape mechanism from late season abiotic stress (Gomez et al., 2014).

This trait is particularly prevalent in Southern European wheat’s (Worland et al., 1994), where adaptability to local climates has been considered vital to enhance yields for a number of years (Worland, 1996). *Ppd-D1* was introduced to central and southern European cultivars from the Japanese wheat Akamonugi (Worland, 1996; Snape et al., 2001) and appears in many modern cultivars, such as Renesansa (Addisu et al., 2009).

In climates which experience late season drought and heat stress, such as Australia and Mexico, *Ppd* insensitive alleles (earlier flowering) have been shown to improve yield compared to sensitive alleles (Maphosa et al., 2014), although the benefit of these alleles becomes less clear in central European countries when the climates suffer from a large amount of variability (Worland et al., 1998a).

However, in more temperate climates where late season stress is less of a factor, *Ppd* insensitive alleles are consistently found to decrease grain yield due to a number of negative phenotypic effects (Addisu et al., 2010). Due to the shorter thermal time to anthesis, *Ppd* insensitivity is also associated with lower light interception and ultimately lower grain yield (Addisu et al., 2010). These findings are generally consistent across studies, with Foulkes et al (2004) also finding reduced GAI and above ground biomass due to photoperiod insensitivity.

It is therefore clear that escaping late season abiotic stress comes at a cost in respect of final yield; through the use of less desirable phenotypic traits and that more work is needed to study the effect of photoperiod insensitivity for future climates, both in Northern and Southern Europe. Firstly, all work on the *Ppd* alleles focuses around the escape of stress, little is known about the tolerance of these alleles to stresses such as heat and drought.

Secondly, whilst we know which alleles are most suited for different climates, it will be important for future breeding strategies whether sensitivity or insensitivity to photoperiod will have the biggest benefit in an area in future climates. In Southern Europe, the shorter thermal time to anthesis found in *Ppd-D1a* may in fact become detrimental to yield as warmer climates further accelerate time to senescence (Semenov et al., 2014), exacerbating the effect of these alleles, lowering grain yield.

In Northern Europe, it has been suggested that *Ppd* insensitive alleles may actually become beneficial due to an increase in late season abiotic stress (Foulkes et al., 2004). This is where the combining phenotypic experiments with crop models could be better integrated, using crop models to test current genotypes in future climates as well as to test different adaptive alleles in different climates.

1.4.2: Increasing tolerance to stress

One of the great agronomic breakthroughs of the 20th century was reducing the height of wheat plants, increasing the harvest index and grain yield through increased portioning of biomass to the grain (Chapman et al., 2007). This was a direct result of the "green revolution" and the introduction of semi dwarfing *Rht* alleles to commercially grown genotypes (Peng et al., 1999).

Yield increases from the introduction of semi dwarfing alleles generally were also due to an increase in grain number (Flintham et al., 1997), as well as reductions in lodging, particularly beneficial in temperate climates (Addisu et al., 2010). However, over expression of this trait can often have negative impacts on yield (Flintham et al., 1997), grain quality (Casebow et al., 2016) and reduce competiveness against weeds compared to tall genotypes (Addisu et al., 2010).

This suggests there is an optimal height for yield, usually considered to be around 80cm (Addisu et al., 2010; Gooding et al., 2012a). *Rht* alleles often interact with agronomic and quality factors such as nitrogen use efficiency, farming system and quality factors such as protein content and Hagberg falling number. Semi dwarfing alleles have been found to improve NUE in conventional systems; however they can have a negative influence in organic systems (Gooding et al., 2012a).

There is some evidence that *Rht* alleles reduce Hagberg falling number (Gooding et al., 2012b), although this effect is influenced by the particular *Rht* allele and farming system.

It is clear then, that different semi dwarfing alleles have been used in different climates to optimise their benefit. There are two main categories of semi dwarfing alleles, gibberellin insensitive and gibberellin sensitive. Both are currently considered better suited to be used in different climates.

The common source of gibberellin insensitive semi-dwarfing comes from *Rht-B1b* and *Rht-D1b,* originally *Rht1* and *Rht2* (Gale and Youssefian, 1985; Hedden, 2003). These alleles are located on chromosomes 4B and 4D, respectively (Ellis et al., 2002).

These alleles reduce plant height through reducing sensitivity to the plant hormone, gibberellin (Hedden, 2003). Gibberellic acid (GA) is one of the primary plant hormones that promote growth (Leopold, 1964; Hooley, 1994), meaning that suppressing the response to these hormones will reduce stem extension (Peng et al., 1999), therefore reducing height of the plant.

Rht-B1b and *Rht-D1b* are the common sources of semi dwarfing in Northern Europe (Worland et al., 1994), originating from the Japanese variety Norin-10 (Hedden, 2003).

The primary source of GA sensitive semi dwarfing comes from the allele *Rht8* on chromosome 2D, which is closely linked to *Ppd-D1a* (Gasperini et al., 2012). *Rht8* is usually associated with reductions in height of around 10% compared to 20% reductions from GA insensitive alleles (Worland et al., 1998b). Rather than reducing sensitivity to GA, *Rht8* reduces height through Brassinosteroid suppression whist remaining GA sensitive (Korzun et al., 1998; Worland et al., 1998b).

Rht8 is the only GA sensitive allele to be used commercially due to an increased coleoptile length compared to other *Rht* alleles (Rebetzke et al., 2007; Wojciechowski et al., 2009). This makes it favourable in drier climates to improve crop establishment and yield, but lowers yield in temperate climates due to lower a lower grain number per ear (Kowalski et al., 2016).

Whilst *Rht8* has been selected in drier climates for its benefits with crop establishment in drier conditions, more needs to be known about the relative tolerance of these alleles to abiotic stresses, particularly drought and heat stress. These alleles are of interest for heat stress tolerance due to preliminary work (Law and Worland, 1985).

Evidence suggested the introduction of dwarfing alleles *Rht1*, *Rht2* and *Rht3* (*Rht-B1b*, *Rht-D1b* and *Rht-B1c*, respectively) were less fertile when heat stress was applied around booting compared to their tall NILs (*Rht-B1a* and *Rht-D1a*), this was especially the case for *Rht-B1c* (Law et al., 1981; Law and Worland, 1985).

It has also been reported that GA insensitive *Rht* alleles also increase sensitivity to drought stress compared to GA sensitive alleles, however the heat stress relationship isn't clear (Alghabari et al., 2014). Although this provides some evidence that the *Rht* alleles differ for tolerance, more work needs to be carried out on this to provide a clearer picture on whether GA sensitive alleles are more tolerant to stress compared to GA insensitive ones. This would also add further evidence for or against the argument of Flintham et al (1997) who suggest that *Rht8* could become more beneficial in future northern European climates. It is important that the relative tolerance and benefits of these alleles be assessed in order to find the most beneficial semi dwarfing alleles for future climates in both Northern and Southern Europe.

1.4.3: Protecting the crop from stress

As well as avoiding stress and making the crop more tolerant to stress, it may also be possible to protect the crop from stress by optimising the use of field resources available to crops. Evaporative cooling can play an important role in reducing the impact of heat stress (van Oort et al., 2014), improving water usage and maximising water uptake can therefore become crucial in offsetting the effect of heat and drought stress (Barnabas et al., 2008).

Increasing water uptake to aid with evaporative cooling in European climates should primarily focus on increasing rooting at depth (Araus et al., 2002). Some progress has already been made on improving the water uptake and usage in wheat. The Australian cultivar Drysdale is perhaps one of the best known examples, with improved drought tolerance due to improved water use efficiency (Fleury et al., 2010; Maphosa et al., 2014).

Whilst improving water use efficiency (WUE) in wheat can be used to counter any potential drought stress, protection from heat stress is likely to need an increase in water uptake to aid evaporative cooling, especially water up taken from deeper in the soil (Araus et al., 2002). One possible source of this is through the introgression of traits from wild relatives. Shamrock, a UK cultivar, has an introgression from wild emmer (Simmonds et al., 2008) and is thought to have higher root mass at depth compared to other UK cultivars (AHDB, 2015a). As well as increasing root biomass at depth, altering the root architecture to focus more rooting at depth could also be used to increase water uptake to protect against both drought and heat stress (Richard et al., 2015). One possible method is to alter the angle of roots to ensure that rooting systems are more compact at shallow depths, to increase deeper rooting (Manschadi et al., 2006).

1.4.4: Relevance to plant breeding

It is important to ensure that beneficial traits that are identified in research are provided to farmers. The most effective way to achieve this is through the provision of these traits to plant breeders who can then implement them in to their breeding programs. The *1B/1R* translocation is a clear example in the UK of beneficial traits identified in research being provided to farmers through plant breeding (Schlegel and Korzun, 1997).

Quantitative genetics, particularly QTL analysis, is a relatively new technique which has been developed to identify particular groups of genes that influence a particular trait (Collard et al., 2005). QTL analysis can be conducted on most observable phenotypes and it provides an opportunity for researchers to identify key groups of genes which can improve current commercial genotypes. This may be particularly important when trying to “stack” disease resistance genes for example, as it can provide plant breeding with more accurate information as to how to implement them.

Quantitative genetics also provides an opportunity to more easily identify and introduce positive traits for tolerance to abiotic stresses. Identifying particularly stress tolerant QTL, such as those identified by (Draeger and Moore, 2017), would allow for breeders to more easily introduce these traits. Current methodology for abiotic stress examination limits the size of populations that can be used as this experimentation is generally quite slow and time consuming. Therefore, identifying a higher throughput method for heat stress screening would allow for larger populations to be tested and therefore more heat tolerant QTL to be identified.

1.5: Modelling the Effects of Climate Change on Wheat

1.5.1: Examples of Crop Models

There have been numerous attempts to simulate the growth of crops in varying conditions through the use of crop models (Jamieson et al., 1998a; Semenov et al., 2014; Liu et al., 2016a). Crop models are a tool which can be used to assess certain phenotypic traits (Craufurd et al., 2013) which can also be used to assess the impact of climate change, including the effect of high temperature (Craufurd et al., 2013; Semenov et al., 2014).

Whilst a large number of crop models solely focus on the effect of mean temperature (Challinor et al., 2014), a number do focus on modelling the effect of abiotic stress, including drought (Lobell et al., 2015) and heat (Liu et al., 2016a). Some of the most well-known examples of these include CERES-Wheat (Timsina and Humphreys, 2006), DSSAT-NWheat (Asseng et al., 2011), WheatGrow (Yan et al., 2000) and APSIM-Wheat (Keating et al., 2003).

There are a number of examples where these crop models have attempted to simulate the effects of heat stress. CERES-Wheat primarily focuses on the effect of water and nitrogen on wheat growth (Singh et al., 2008), though it has been used on a number of occasions to simulate the effect of heat in various locations.

Examples of model usage include the simulation of two US cultivars in the US Great Plains (Mearns et al., 1996), drought stress in New Zealand (Jamieson et al., 1998b) and wheat yields in Mexico (Lobell et al., 2005). APSIM-Wheat has also been used to simulate heat stress across various parts of Australia (Reyenga et al., 1999; Asseng et al., 2011), with its heat stress response being continually updated (Lobell et al., 2015).

1.5.1.1: The SIRIUS crop model

SIRIUS is a wheat simulation model that has been used on a number of occasions to accurately simulate the phenology and yield of wheat at different locations under differing conditions, (Jamieson et al., 1998b; Semenov, 2009; Stratonovitch and Semenov, 2015) and is perhaps the most commonly used crop model to simulate European wheat yields in future climates.

The crop model is calibrated to 15 locations across Europe (Semenov and Stratonovitch, 2015) and is also calibrated to a number of different genotypes which have been used in previous studies including; Chinese Spring (Stratonovitch and Semenov, 2015); Rongotea and Batten (Jamieson et al., 1998b)(Jamieson et al., 1998ab); Avalon and Mercia (Semenov, 2009). A schematic outline of how SIRIUS generates grain yield data is provided in figure 1.3. SIRIUS simulates biomass accumulation throughout the season and then uses basic partitioning rules to simulate final grain number and yield. This potential grain yield is then offset by any losses due to heat or cold stress, through the use of heat stress reduction factors (Stratonovitch and Semenov., 2015).

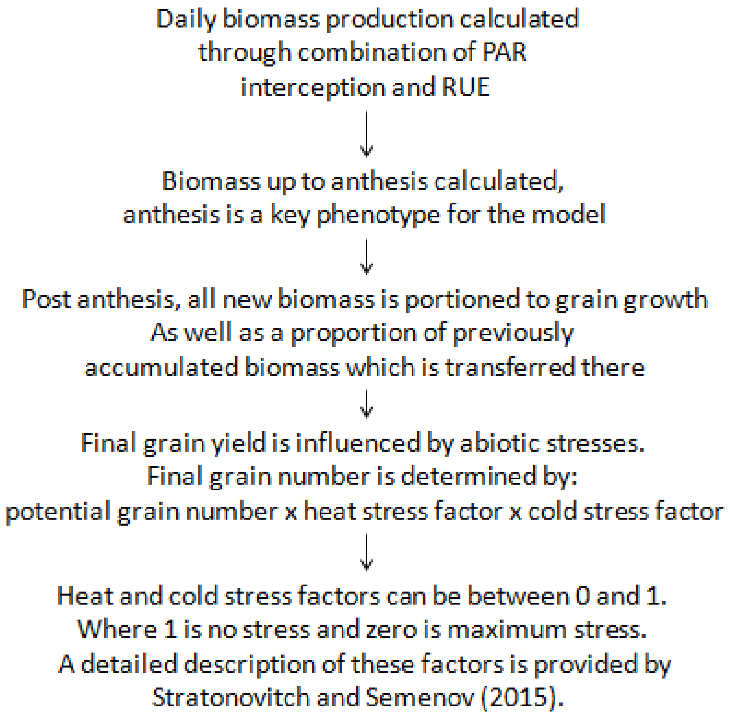

Figure 1.3: A schematic diagram showing the basic concept of how the SIRIUS crop model simulates grain yield. Factors that influence biomass accumulation, such as RUE and PAR interception are influenced in the model by the availability of inputs. A full description of the model is provided by (Jamieson, Semenov, et al., 1998).

SIRIUS has consistently simulated the reproductive phases of growth to be particularly susceptible to stress (Semenov et al., 2014) and have used this as a means of projecting future yields across Europe through the combination of the 18 GCMs in the CMIP5 ensemble (Semenov and Stratonovitch, 2015) and the LARS-WG stochastic weather generator (Semenov et al., 1998).

Through the use of different locations, genotypes and an ensemble of GCMs, SIRIUS has repeatedly identified that heat stress is likely to become a major hindrance on yield by the end of the century (Stratonovitch and Semenov, 2015). This is especially true for Southern Europe, therefore breeding for tolerance to heat stress must be considered a priority and no European cultivars can currently be considered tolerant to heat stress (Semenov, 2009; Semenov et al., 2014; Stratonovitch and Semenov, 2015).

1.5.2: Reducing Uncertainty through Ensembles

A major concern with crop modelling is the level of uncertainty involved with the projections due to the complexity of the systems that are being modelled. Direct comparisons of models show a large discrepancy when modelling heat stress response. A clear example of this comes from Liu et al (2016), comparing how 4 different crop models are able to replicate a heat stress data set (Prasad and Djanaguiraman, 2014). There were large differences in how the models simulated different yield parameters that were not consistent across the stress timings or for genotypes (Liu et al., 2016a). As there are large discrepancies between crop models when modelling the same thing, one way to overcome this is through the use of ensembles.

Arguably the most well-known crop model ensemble is from the AGMIP project, which involves some of the crop models described in section 1.5.1, including CERES-Wheat, APSIM-Wheat and SIRIUS (Asseng et al., 2013)

Although, when provided with enough calibration data, crop models can individually replicate grain yield, variation between models is observed primarily because of how individual models simulate the impact of climate change and climate change projections (Asseng et al., 2013). Using the median score of an ensemble of models has been found to be more accurate in projecting yield in stressed environments compared to single models (Asseng et al., 2015), suggesting ensembles do have use for modelling the effect of stress, even with high levels of variation. This process has also been used to assess comparative effects of CO_2 increases against rising temperatures (Makowski et al., 2015) as well as temperature water interactions across European locations (Pirttioja et al., 2015). Although ensembles are being used to account for variability within and between models, there is still work that needs to be done to improve crop models, especially responses to extreme weather.

1.5.3: Improvements and Future Work

As previously noted, Liu et al (2016) shows the large variation across crop models in replicating the effect of heat stress, likely due to the complexity of how wheat responds to heat and interacts with other influencing factors (Barnabas et al., 2008). Some strides are being made to improve the response of models to abiotic stresses, such as recent improvements in drought response based on an Australian dataset in the APSIM-Wheat model (Lobell et al., 2015).

The most recent heat stress data set to be incorporated in to the SIRIUS crop model was the same data set used by Liu et al (2016) (Prasad and Djanaguiraman, 2014; Stratonovitch and Semenov, 2015).

Although this provided some improvement to the response of SIRIUS, there is a clear need to calibrate the heat stress response to European cultivars grown primarily in European conditions (Semenov et al., 2014). The previous use of a data set involving an Asian Spring Wheat grown fully in a controlled environment (Prasad and Djanaguiraman, 2014) means there is further scope to improve the models response.

Nearly all previous studies using crop models focus on simulating current commercial genotypes of wheat in future climates (Asseng et al., 2013, 2015; Pirttioja et al., 2015; Stratonovitch and Semenov, 2015). Using SIRIUS, there have been attempts to design ideotypes for future climates, optimising phenotypic traits such as maturation date, leaf area and flowering time (Lawless et al., 2005). There could be future scope to use the crop model to compare different alleles of a gene in future climates, to actually help build these ideotypes. For example, using phenotypic data collected for different *Ppd* alleles (Addisu et al., 2010) to compare photoperiod sensitivity in different future climates would add evidence to previous speculation about future benefits (Worland et al., 1994; Foulkes et al., 2004).

1.6: Project Outline

1.6.1: Aims

This project aims to assess genotypic variation in crop response to heat stress by comparing a southern European wheat genotype with a UK genotype and their doubled haploid progeny, to identify traits and alleles that would benefit UK wheat production under climate change scenarios using a combination of genotyping, phenotyping and crop modelling.

1.6.2: Objectives

1. To clarify the discrete GS timings which are susceptible to heat stress
2. To characterize the response of different wheat genotypes, alleles and QTL to heat stress at particularly susceptible growth stages
3. To assess development, growth and yield characteristics of the parents and progeny of a Southern European wheat and UK wheat and their DH progeny
4. To use data gathered in 1 and 2 to parametize the crop model SIRIUS (and others where possible)
5. To use the SIRIUS crop model to predict yield and yield stability of different genotypes, alleles and QTL in climate change scenarios for the UK (and elsewhere when possible)

1.6.3: Key Research Questions

Based on the aim and objectives of this project, the following key research questions to be answered by the project can be drawn up:

- Does the growth stage of wheat’s highest susceptibility to heat stress change with genotype?
- Does the response to heat stress (effect of heat stress on grain number and grain weight) vary between cultivars?
- Does the crop model SIRIUS accurately simulate the effect of heat stress on wheat?
- To what extent can crop models take into account genotypic variation in GS-specific heat stress susceptibility?
- Is there sufficient variation amongst this one population to suggest that yield increases and improved yield stability is possible under climate change scenarios?

1.6.4: Overall Hypotheses

H_1: There are varietal differences in timing and severity of susceptibility to heat stress.

H_2: Southern European alleles of relevant adaptive alleles increase tolerance to heat stress.

H_3: Southern European alleles have a negative impact in non-stressed, Northern European conditions.

H_4: There will be differences in yield predictions and yield loss due to stress between different genotypes from the crop model SIRIUS when predicting yields in future climates across Europe

Chapter 2: Characterising the Heat Stress Response of Contrasting Wheat Cultivars

Content from this Chapter (data and text) appear in:

Barber HM, Lukac M, Simmonds J, Semenov MA and Gooding MJ (2017). Temporally and Genetically Discrete Periods of Wheat Sensitivity to High Temperature. *Front.PlantSci.* 8:51. doi: 10.3389/fpls.2017.00051 **(Appendix 1)**

Barber, H. M., Carney, J., Alghabari, F., & Gooding, M. J. (2015). Decimal growth stages for precision wheat production in changing environments? *Annals of Applied Biology*, *166*(3), 355–371. (**Appendix 2)**

2.1: Introduction

Numerous reports and assessments frequently conclude that Europe is likely to experience an increase in the frequency of extreme weather events, notably summer heat waves and droughts (IPCC, 2014). These stress events are likely to impact crop production across Europe (Semenov et al., 2014). Wheat is one of the largest global food crops, with over 700 million tonnes being produced in 2013 (F.A.O., 2014). Wheat is susceptible to stress events, particularly heat and drought (Barnabas et al., 2008). Therefore, gathering more information about how these stresses affect the crop is important as is the identification of genetic sources of tolerance.

2.1.1: The relationship of heat stress and growth stage

Wheat is sensitive to temperatures above 30°C around booting and flowering, an effect which is exacerbated with drought (Alghabari et al., 2014).

Previous reports on heat stress in wheat usually concern only one of the susceptible timings i.e. meiosis (Saini and Aspinall, 1982; Saini et al., 1984) or anthesis (Tashiro and Wardlaw, 1990; Ferris et al., 1998; Lukac et al., 2012; Pradhan et al., 2012; Steinmeyer et al., 2013; Liu et al., 2016b).

Fewer studies have attempted to quantify the response to stress at both of these timings: Alghabari et al. (2014) suggest meiosis is the most vulnerable stage, but Prasad & Djanaguiraman (2014) report that it is anthesis that is particularly susceptible. There have been few studies examining both periods, resulting in divergent opinions about which period, if any, is more susceptible than the other and whether this comparison is influenced by genotype and environment.

The majority of heat stress experiments focusing on reproductive phases of wheat tend to involve long durations of stress, to ensure that the sensitive phases of growth are stressed. At the booting stage, this is largely due to the short duration that meiosis occurs for in wheat, as it is usually completed within a day (Saini and Aspinall, 1982). Discrete detail is lacking when studying the interaction of heat stress and specific external growth stages. There is debate as to whether meiosis consistently occurs at the same external growth stage (Barber et al., 2015), as genotype and environment can have different influences on the relative timing of external (e.g. booting) and internal (e.g. meiosis) developmental phases (Zadoks et al., 1974a, 1974b).

In summary, there is no guarantee that the most common multi-day stress approach can consistently target e.g. meiosis. It is possible that multiple, short duration, stress episodes would ensure that meiosis is stressed whilst also adding information about discrete growth stage x heat stress interactions.

Previous work has often assumed that meiosis and anthesis represent two separate, discrete periods of susceptibility but there is currently little evidence to support this. Single experiments on rice and wheat suggest that there may be a period between meiosis and anthesis that is relatively tolerant to heat stress (Satake and Yoshida, 1978; Craufurd et al., 2013), but it is unclear as to the specific growth stages when this tolerance occurs.

2.1.2: Exploring the interaction between heat stress and genotype

Whilst there has been a large amount of research carried out on the effect of heat stress on wheat, relatively few studied addressed genotype x heat stress interactions. Genotypic interactions with heat stress timing also require clarification. Although some recent work has compared the heat stress response at anthesis across multiple genotypes (Liu et al. 2016), little work has quantified how genotype influences susceptibility across both stages, even though consecutive exposure of both stages to stress seems likely to occur in field conditions (Wardlaw et al., 1989). There is some evidence that there is variation in heat stress tolerance among wild wheat types (Pradhan et al., 2012) and this trait needs to be explored further.

There is a particular need to assess the tolerance of southern European cultivars of wheat, where heat stress is predicted to have the largest effects on yield (Stratonovitch and Semenov, 2015). Southern European cultivars have previously been selected for earliness (Snape et al., 2001; Langer et al., 2014) as an escape mechanism from stress, largely due to the addition of photoperiod insensitivity alleles (Jones et al., 2016).

Southern European wheat traits therefore need to be compared to northern European types to identify traits potentially beneficial for future climates (Foulkes et al., 2004).

2.1.3: Experiment outline

This experiment investigates whether the response to heat stress differs between genotypes and growth stages across the reproductive period of growth. This Chapter describes the use of replicated 1-day transfers of pot-grown wheat to be subjected to heat stress. The aim is to identify and characterise discrete periods of heat susceptibility during external growth stages extending from the second node detectable growth stage (GS 32; Zadoks et al. 1974) to the grain milky-ripe stage (GS 77) and hence encompassing meiosis and anthesis (Barber et al., 2015). This study will compare the Southern European wheat Renesansa (*Ppd-D1a, Rht-D1a*, and *Rht8*) to the UK-adapted wheat Savannah (*Ppd-D1b, Rht-D1b*, *1BL/1RS*). The successive use of short duration stresses ensures that the whole possible susceptible period is stressed and accounts for any genotype-dependant variation in the coincidence between GS and meiosis. Previous methods reduce the interpretative certainty from screens of genotypes against stresses applied according to GS, for example an apparently tolerant genotype may have ‘escaped’ the stress if meiosis occurred at a different GS to other more ‘susceptible’ genotypes.

2.2: Method

2.2.1: Plant material and growing conditions

The two cultivars used in this experiment were Savannah and Renesansa. Savannah had a high yield potential in North West Europe with low bread making quality and was recommended in the UK in 1998. Renesansa, a winter wheat listed in Serbia in 1995, has high yield potential and high bread making quality in southern Europe.

Plants used in these experiments were grown in pots (180 mm diameter) at the Plant Environment Laboratory at the University of Reading, UK (51 27’ N latitude, 00 56’ W longitude). Each pot contained 2.8 kg of growing media comprising 4:2:4:1 of vermiculite: sand: gravel: compost mixed with Osmocote slow release granules (2kgm-3) containing a ratio of 15: 11: 13:2 of N: P2O5: K2O: MgO. Seven seeds were sown per pot, thinned to four plants per pot at the two leaf stage. The pots were maintained outside under a protective net cage in four randomised blocks with guard pots of wheat placed around the perimeter of experimental blocks. Fungicide was applied as and when required. Pots were watered up to twice daily by an automatic drip irrigation system to maintain soil at close to field capacity.

2.2.2: Experimental design and heat stress conditions

The experiment, sown on the 16th December 2013, comprised a complete factorial of: the two cultivars, Savannah and Renesansa; day of transfer to Saxil growth cabinets (31 separate timings between May 2nd and June 13th 2014); and two temperature regimes (20/15°C and 35/30°C) within growth cabinets.

Possible confounding effects associated with temperature included water loss, due to lack of irrigation within the cabinets for the 24h treatment period. The mean weight of pots on entry was 3.40kg, whilst mean weights of pots on withdrawal were 3.19 kg and 2.98 kg (SED=0.016) for the 20/15°C and 35/30°C treatments respectively. More detailed studies on the water relations within this growing medium and system suggests that this degree of water loss would equate to 78% and 56% field capacity (FC; oven dry = 0% FC; (Gooding et al., 2003)) respectively, and that a FC of less than 70% maintained for 14 days during grain filling was required to reduce grain yield. A further confounded environmental variate was mean relative humidity (73% for 20/15°C and 47% for 35/30°C (SED=4.4)) whilst in the cabinets.

Transfers to Saxil growth cabinets began between 10:20h and 11:20h (BST) and continued for 24h (16h day, night time between 22:00h and 06:00h) before being returned outside to the pot's original randomised block position. Two temperature regimes were used in all experiments, day/night temperatures of 20°C/15°C for the control treatment and 35°C/30°C for the heat stress treatment. Pots were irrigated to field capacity before transfer, but were not irrigated whilst in the cabinets. Eight growth cabinets were used to maintain the block replication set up in outside cages. On the day of transfer main stems in each pot were tagged and assessed for growth stage (Zadoks et al., 1974b). Pots were weighed immediately before and after transfer to monitor water loss.

2.2.3: Post harvest and statistical analysis

Main stems and tillers were harvested separately after physiological maturity (GS 89) and dried (48h at 80°C). Ears and spikelets per ear were counted, after which grain was threshed from ears, and then re-dried, weighed and counted by a Kirby Lester K18 Tablet counter.

The primary statistical approach was a split plot ANOVA model of Block / Cabinet / Pot (GenStat 14th edn. VSN International Ltd). Polynomial regressions were fitted across day of transfer to growth cabinet using orthogonal polynomial contrasts in an analysis of variance i.e. treatment structure was pol (Day; n) * Temperature * Genotype, where n was the maximum level of polynomial to be fitted. Where quartic effects or deviations from them were significant, fits were compared with the double Gaussian model [equation 1] on an r^2adj basis. The maximal double Gaussian model permits the estimation of two 'bell-shaped' curves:

$$Relative\ Effect\ (\%) = 100 + b(2\pi {s_1}^2)^{-0.5}\ e^{-(t-m)^2/2{s_1}^2} +\ c(2\pi {s_2}^2)^{-0.5}\ e^{-(t-n)^2/2{s_2}^2)}$$

[Equation 1]

Where: Relative Effect is the result at 35°C (day temperature) expressed as a percentage of that achieved at 20°C; b and c are the size of the two peaks; m and n are when, in time t, they are centred; and s1 and s2 are the Gaussian shape factors (standard deviation) for the two peaks. The double Gaussian approach has previously been used to detect other phenologically-dependent responses in wheat time series data sets (Lu et al., 2014).

The FITNONLINEAR routine in GENSTAT 14 was used to compare regressions and allow a parsimonious approach to the inclusion of various parameters in the model fits. Additionally, the routine allowed simultaneous fits to different response variates (weighted for the inverses of their variances). Here it was used to investigate potential compensation in mean grain weights at the time when grain numbers were reduced by heat stress.

2.3: Results

Pot transfers began on the 2nd May, upon the start of booting in Renesansa (Fig. 2.1). Renesansa reached anthesis approximately 10 days before Savannah.

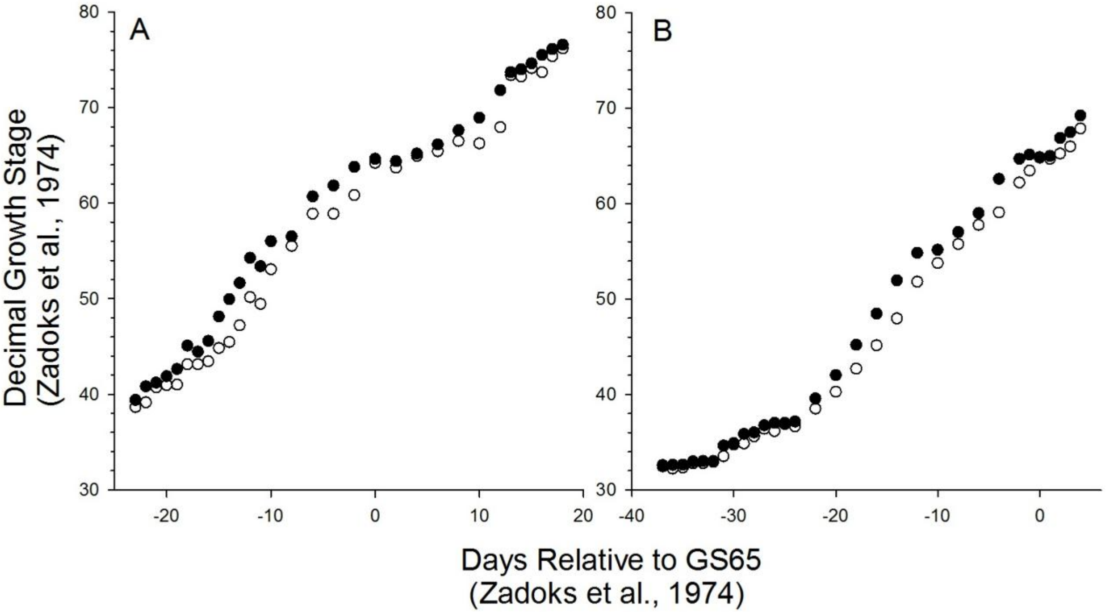

Figure 2.1: Effect of cultivar [(A) Renesansa; (B) Savannah] and day of transfer to controlled environment cabinets at 20/15 35/30◦C day/night temperature (16 h day) on the median growth stage when transferred (Clear circles), and when removed from the cabinet (Filled circles) 24 h later. Values are averaged across the two temperature treatments to show overall development of each genotype.

There were no significant (P>0.05) main or interacting effects of timing of transfer or temperature on ear number per pot or stem biomass per pot (mean for Renesansa and Savannah = 20.30g and 20.39g respectively; S.E.D. = 1.815; 345 d.f.).

Significant genotypic differences for ear number per pot were observed (p<0.001; mean for Renesansa and Savannah = 9.2 and 9.4 respectively; S.E.D= 0.057), but not for stem weight. Total biomass per pot was significantly affected by genotype (p<.001; mean for Renesansa and Savannah = 42.32g and 49.03g; S.E.D. = 0.322; 354 d.f.) and temperature x day x line (p = 0.002; S.E.D. = 2.537; 354 d.f.).

Differences were also seen in harvest index (HI) between genotypes (p<.001; mean for Renesansa and Savannah = 0.40 and 0.47; S.E.D. = 0.003; 347 d.f.) and for temperature x genotype x day (p = 0.006; S.E.D. = 0.024). Differences in total biomass and HI were likely caused by differences in ear weight due to the lack of differences seen in stem weight.

Initial analyses involved comparing differences in spikelet weight to identify any potential effects in the ear (Appendix 3). There were significant interactions between temperature and day of transfer (quadratic, p<0.001) and between genotype and day of transfer (linear, p=0.004). However, there was no significant day x genotype x temperature interaction (p=0.100). This initial analysis used a rolling 5 day average on spikelet weight difference to identify potential periods in the experiment where there was an effect due to heat stress. Whilst this process was useful to identify possible trends in the data quickly, the variability of the data was greater than that of the grain yield.

Grain yield per pot indicated a three factor interaction between day of transfer, temperature and cultivar ($P = 0.002$; deviation from quartic $P = 0.007$; fig. 2.2a, b). Savannah appeared to consistently perform better than Renesansa, having a higher grain yield in both stressed and control environments (fig. 2.2a, b). Most of the interaction was due to changes in grain number per pot ($P < 0.001$ for the three factor interaction; deviation from quartic $P < 0.001$), with some modification through partial compensatory increases in mean grain weight, particularly after some of the earlier transfers (e.g. $P < 0.001$ for cubic.Day x Cultivar).

The two genotypes also varied in time taken to reach anthesis, Renesansa reached mid anthesis (GS65) approximately 10 days before Savannah. This observation is supported by a day x line interaction ($p<0.001$) when comparing GS in to the cabinets. There is also an apparent decline in yield ($p = 0.038$) for both temperatures and genotypes later in to the experiment. This is likely an effect of transferring the pots from outside to the cabinets.

Growth stage data provided in Fig 2.2 e, f from the main stems of the plants show the differences in developmental rates. Renesansa was not only more advanced in its development compared to Savannah, but also less synchronous in its development, as shown by the increased spread of the box plots. There were clear reductions in grain yield from the whole pot data (Fig2.2 a, b) and main stem data on its own (Fig 2.2 c, d), although when presented in this format it becomes difficult to compare genotypes on a certain day due to differences in development rates. Therefore, adjusting the x axis relative to growth stage allows for a much more direct comparison when analysing the fertility data.

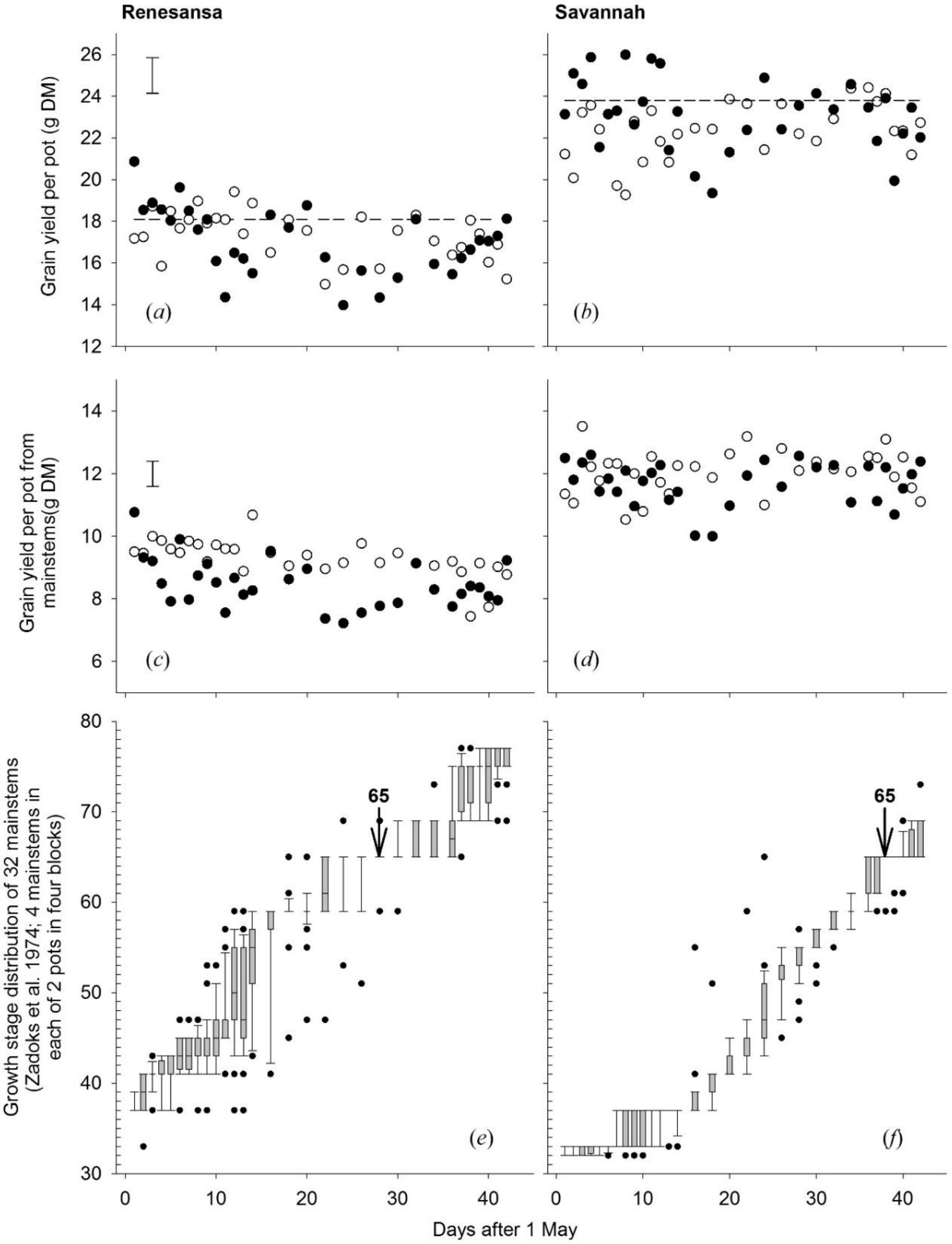

Figure 2.2: Effects of wheat cultivar and successive 1-day transfers to controlled environment cabinets at 20/15 (open) and 35/30°C (filled) day/night temperature (16h day) on grain yield per pot from all stems (a,b) or only mainstems (c,d). Panels e and f give the growth stage distributions of the mainstems at the time of transfer in to the cabinets (boxes are limited by 25 and 75 percentiles, whiskers by 10 and 90 percentiles; points are outliers beyond 10 and 90 percentiles, and the line within the box is the median where appropriate). S.E.D. (358 d.f.) in a and c is for comparing temperatures within day and cultivar for both cultivars. Arrows in e and f denote the assumed timing of growth stage (GS) 65 (Zadoks et al. 1974). Dashed lines in a and b are the mean yields from eight pots per cultivar left outside.

Initial modelling of the response involved drawing individual lines of fit for each yield parameter. However, there was no evidence to suggest that the timing of the peaks differed for each genotype ($p<0.05$) or that the width of each peak differed ($p<0.05$). Therefore, a more parsimonious approach was undertaken where the model was constrained to fit multiple lines at the same time, which did not differ for the timing or width of the two peaks.

Whole pot data shows two clear peaks of susceptibility to heat stress (Fig2.3; Table 2.1). The peaks are centred 17 days and 1 day before anthesis. While there is a clear indication of what has happened, overall fit can be improved, as the R^2 value is low (0.19). This is likely due to the fact that combining main stem and tiller data, where growth stages differ within the same plant (Barber et al., 2015) increases variability. Separating main stems from tillers should provide better fits.

Table 2.1: Parameter values for simultaneous double Gaussian fit (Fig. 2) to the effects of increasing day temperature from 20°C to 35°C over successive single days for grain yield components on whole pot data for two cultivars of winter wheat.

			estimate	s.e.
Gaussian shape factor (S, days)			2.25	0.52
Peak position (days relative to GS 65)		Peak 1	-17.1	0.59
		Peak 2	-1.06	1.01
Grain number	Renesansa	Peak 1	-430	130
		Peak 2	-427	174
	Savannah	Peak 1	-529	178
		Peak 2	-233	131
Mean grain weight (mg)	Renesansa	Peak 1	7.23	7.44
		Peak 2	4.6	10.4
	Savannah	Peak 1	24.4	10.8
		Peak 2	5.93	7.94

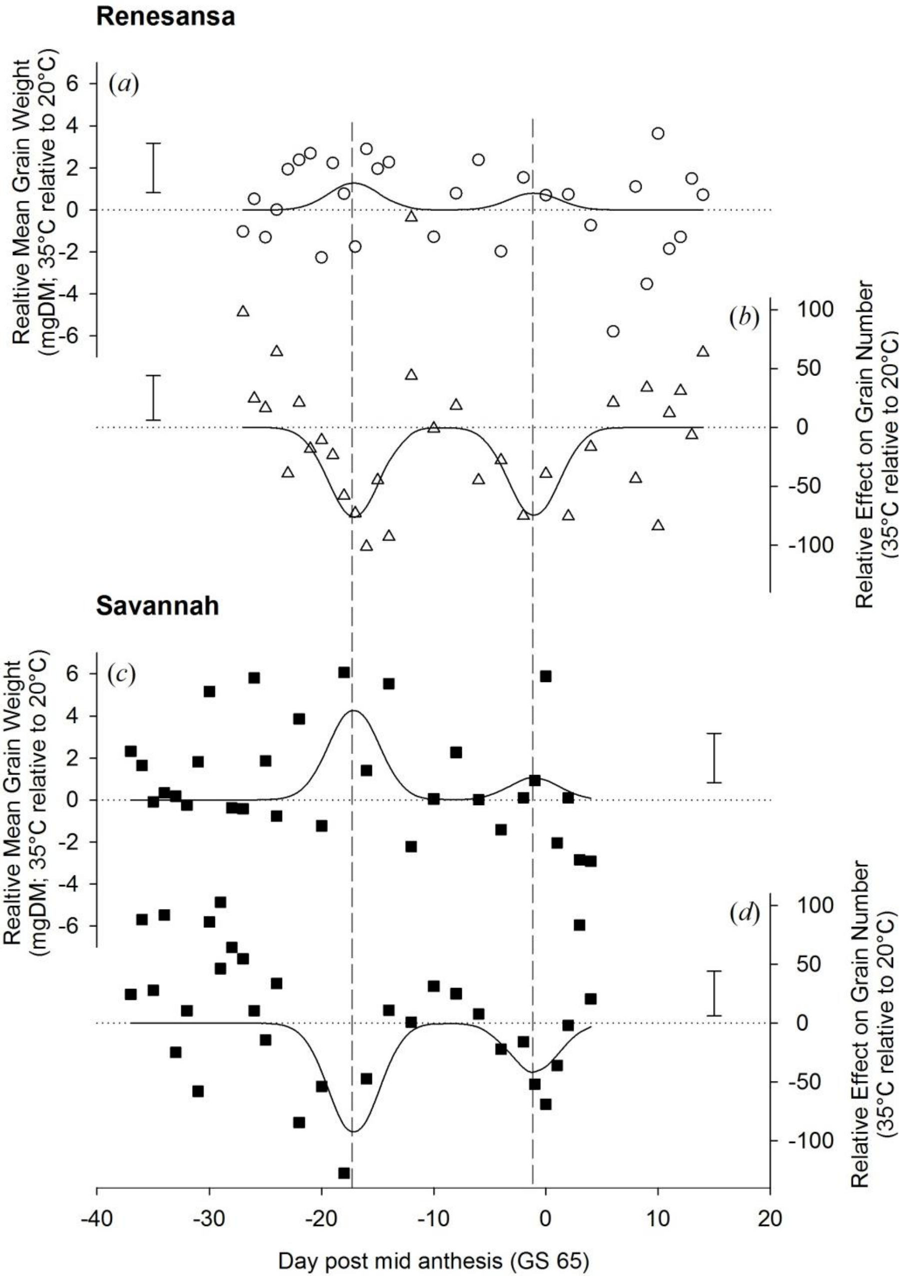

Figure 2.3: Effects of increasing day temperature from 20°C to 35°C in successive 1-day transfers to controlled environment cabinets on yield components per pot of two cultivars, Renesansa (open triangles) and Savannah (Filled squares), of winter wheat. Whole pot data consists of all of the ears from each pot being used in this analysis. Fits are double Gaussian (1) constrained for peaks to have the same shape (Gaussian S, eqn 1) and timings for the different components and varieties. Error bars are 1 S.E.D. (358 d.f.) for comparison of individual points with the y=0 line.

Even with the greater spectrum of growth stages in the whole pot data, visible effects of heat stress can still be seen (Figure 2.3). There are two critical periods where fertility and grain size are affected, one around 1-2 days before mid anthesis of the mainstems and the other around 18 days before mid anthesis.

Similar to the grain yield dips seen in Fig. 2.3. There is still quite a lot of variability around the lines, likely caused by the variation in growth stages across the pot when the pots were transferred. This can be reduced somewhat by splitting the data in to mainstems and tillers, as main stem development tends to be at a more advanced stage relative to the tillers (Zadoks et al., 1974b; Barber et al., 2015).

With regards to timing of susceptibility to heat stress, the grain yields from the main stems provided better clarity than the yields from the whole plot, presumably because of the broader spectrum of the growth stages deriving from the tillers (Jones et al., 2016) and as growth stage assessments focussed primarily on main stems.

On the main stems, grain yields of Renesansa appeared to be repeatedly compromised by day transfers to the higher temperature from 6-12 May, and again from 22-30 May (Fig. 2.2c). In Savannah there was a significant period of susceptibility from the 17-21 May, and possibly a second period from 4-9 June (Fig. 2.2d). Variation in growth stage amongst mainstems appeared to be greater for Renesansa (Fig. 2.2e) than for Savannah (Fig. 2.2 f). Nonetheless, on average, for much of the period of transfers, the growth stage development of Savannah appeared to be about 10 days later than that for Renesansa. This difference could be identified with accuracy at mid anthesis as over 80% of mainstems were scored as at GS 65 on 28 May for Renesansa and on 7 June for Savannah.

When Day of transfer was expressed as relative to GS 65, there was strong evidence for two peak timings of susceptibility, but there was no evidence that timing of the peaks for susceptibility varied for the two cultivars, or that the standard deviation of the two peaks varied (Gaussian s; Table 2.2).

Table 2.2: Parameter values for simultaneous double Gaussian fit (Fig. 2) to the effects of increasing day temperature from 20°C to 35°C over successive single days for grain yield components on main stems of two cultivars of winter wheat.

			estimate	s.e.
Gaussian shape factor (S, days)			3.71	0.416
Peak position (days relative to GS 65)		Peak 1	-18.2	0.55
		Peak 2	-3.0	0.82
Grain number	Renesansa	Peak 1	-359	66.7
		Peak 2	-491	92.1
	Savannah	Peak 1	-555	92.4
		Peak 2	-231	77.6
Mean grain weight (mg)	Renesansa	Peak 1	17.5	8.5
		Peak 2	2.3	11.8
	Savannah	Peak 1	45.3	12.0
		Peak 2	12.2	10.2

With regards to grain numbers on the mainstem (Table 2.2; Fig. 2.4), the first peak was centred about 18 days before GS 65 when 50% of Renesansa mainstems were at GS 43-45, and 50% of Savannah mainstems were at GS 41-43 (Fig. 2.3). Both cultivars appeared comparatively tolerant of the heat stress during late booting and ear emergence. A second period of susceptibility, however, was detected during late ear emergence and early phases of anthesis, centred 3 days before GS 65 (Table 2.2;Fig. 2.4), when most of the ears would have been at GS 61. Grain set in Renesansa appeared equally susceptible to the heat stress during booting and anthesis (Table 2.2; Fig. 2.4). Grain set in Savannah was significantly more susceptible during booting than at anthesis, but the only time when grain set was significantly compensated by increased mean grain weight was at the earlier timing (Table 2.2; Fig. 2.4).

There was no statistical evidence ($p>0.05$) for compensation for grain set failure through mean grain weight by Renesansa during either period of susceptibility. It is also clear that analysing mainstem fertility provides a clearer view of what has occurred compared to the whole pot data, demonstrated by the increased R^2 value (0.50).

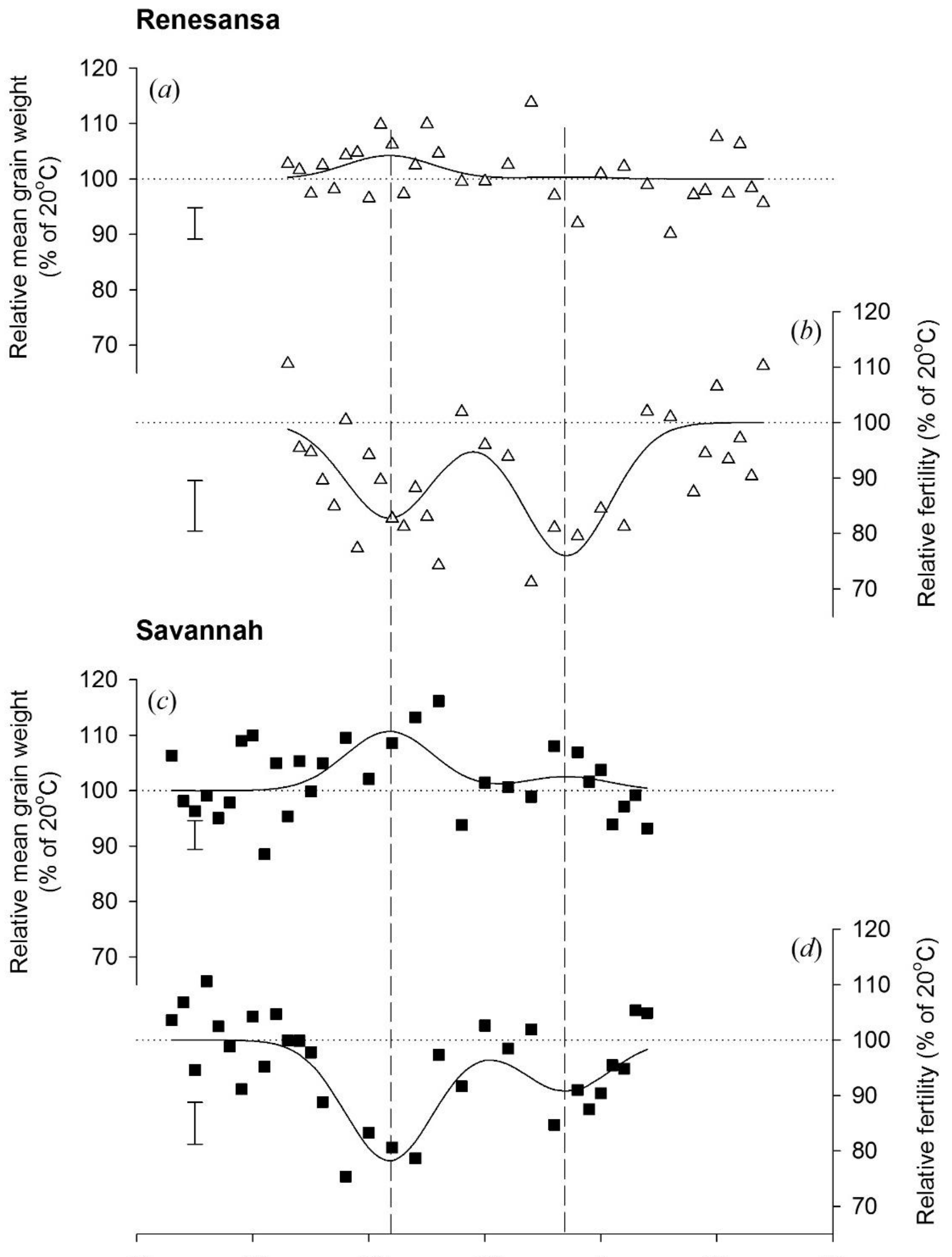

Figure 2.4: Effects of increasing day temperature from 20°C to 35°C in successive 1-day transfers to controlled environment cabinets on yield components per pot from main stems only of two cultivars of winter wheat, Renesansa (open triangles) and Savannah (filled squares). Fits are double Gaussian (Table 1) constrained for peaks to have the same shape (Gaussian S, eqn 1) and timings for the different components and varieties. Error bars are 1 S.E.D. (358 d.f.) for comparison of individual points with the *y*=0 line.

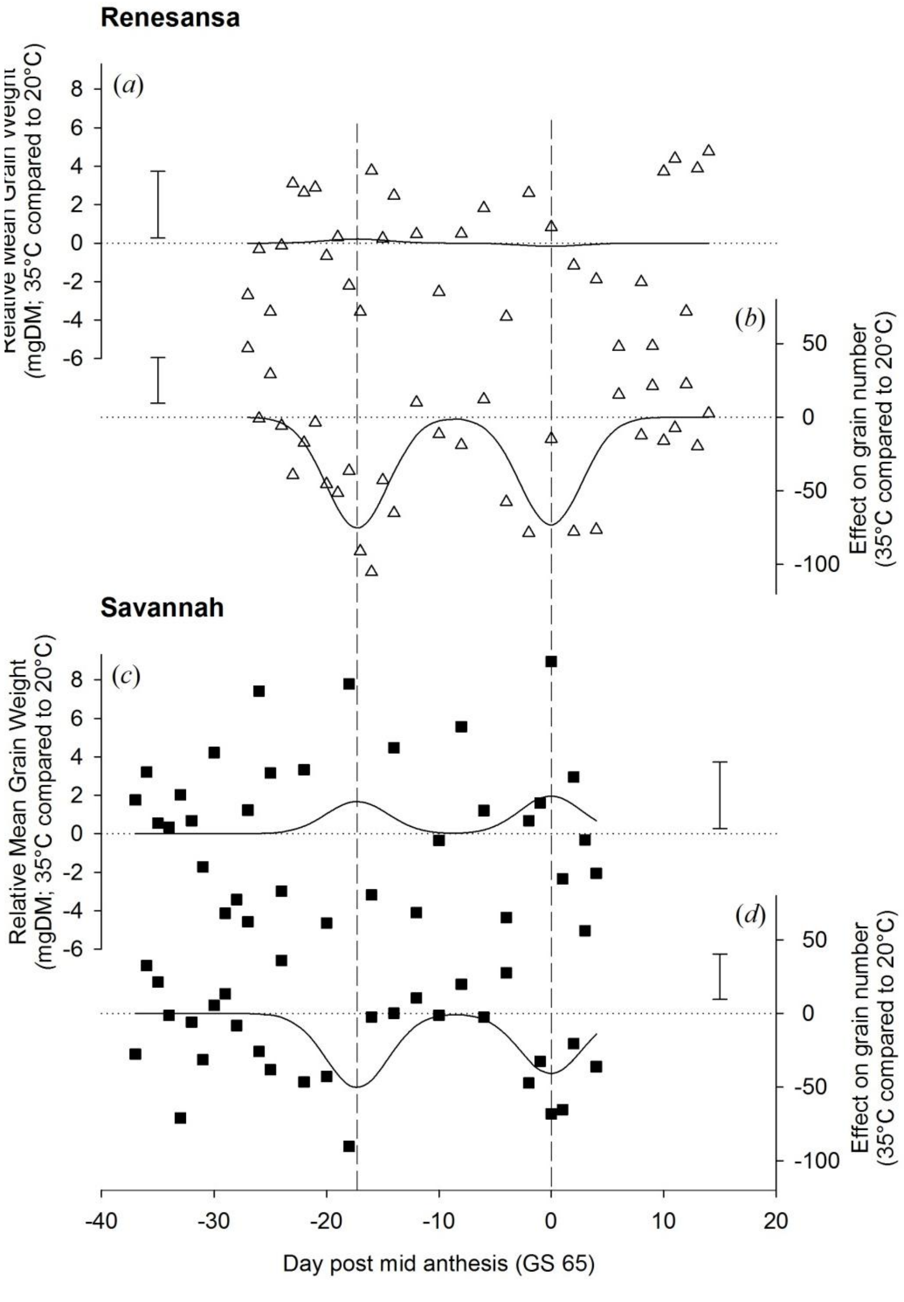

Figure 2.5: Effects of increasing day temperature from 20°C to 35°C in successive 1-day transfers to controlled environment cabinets on yield components per pot from secondary tillers only of two cultivars of winter wheat, Renesansa (open triangles) and Savannah (filled squares). Fits are double Gaussian (Table 1) constrained for peaks to have the same shape (Gaussian S, eqn 1) and timings for the different components and varieties. Error bars are 1 S.E.D. (358 d.f.) for comparison of individual points with the *y*=0 line.

The last step of the analysis involved isolating data from the secondary tillers (Fig 2.5; Table 2.3). Compared to the main stem data, the size of the peaks (S; 2.68) appear lower, as does the overall fit ($R^2 = 0.29$). There are still two clear peaks, however both appear later than the main stem peaks (Table 2.3), likely due to the delayed development of tiller stems (Zadoks et al., 1974b).

It is apparent that data from the main stems provides the clearest analysis of heat stress susceptibility, due to the focus of growth stage assessments on the main stems as well as the smaller variation within a pot of the main stems compared to the secondary tillers.

Table 2.3: Parameter values for simultaneous double Gaussian fit (Fig. 2) to the effects of increasing day temperature from 20°C to 35°C over successive single days for grain yield components on secondary tillers of two cultivars of winter wheat.

			estimate	s.e.
Gaussian shape factor (S, days)			2.68	0.55
Peak position (days relative to GS 65)		Peak 1	-17.28	0.68
		Peak 2	-0.042	0.85
Grain number	Renesansa	Peak 1	-522	113
		Peak 2	-508	145
	Savannah	Peak 1	-350	141
		Peak 2	-283	106
Mean grain weight (mg)	Renesansa	Peak 1	1.5	10.9
		Peak 2	-1.1	15.3
	Savannah	Peak 1	11.6	15.3
		Peak 2	13.6	11.4

2.4: Discussion

This study sheds light on the effect of heat stress on wheat yield during reproductive development, identifying two discrete periods at which grain set in wheat is susceptible to high temperature: the first in early to mid-booting presumably commensurate with susceptible meiotic stages (Barber et al., 2015) and the second during the early phases of anthesis. However, it is worth acknowledging the possible influence of floret abortion on grain number due to stress at these timings. Without a detailed inspection of florets it is not possible to definitively say what is causing this reduction in grain number, either damage to meiotic processes or floret abortion due to heat stress.

Reduced fertility and decreased grain number as a result of heat stress was in agreement with previous work (Saini and Aspinall, 1982; Ferris et al., 1998; Dolferus et al., 2011; Liu et al., 2016a).

There is some evidence to suggest that grain size can increase and partially compensate for losses caused by abiotic stresses (Semenov et al., 2014), however this is mostly confined to the booting period of susceptibility and was not consistently observed across genotypes. Grain size increases found at booting but not at anthesis support the lack of grain size compensation found by (Liu et al., 2016a). This variation in compensatory increases in mean grain weight over genotype and growth stage should be accounted for when attempting to improve the response of crop models to abiotic stress (Stratonovitch and Semenov, 2015; Liu et al., 2016a).

Consistent with previous literature, the peak periods of susceptibility appear to be early to mid-booting (Saini and Aspinall, 1982; Alghabari et al., 2014) and early flowering (Ferris et al., 1998; Craufurd et al., 2013; Prasad and Djanaguiraman, 2014). The period between meiosis and anthesis appears to be relatively tolerant to short-duration heat stress, an effect previously observed in rice (Satake and Yoshida, 1978, 1981; Craufurd et al., 2013), with indications that this could also be true in wheat (Prasad and Djanaguiraman, 2014). Responses to heat stress are strongly influenced by genotype, as shown by variation within these experiments, especially between Savannah and Renesansa. Genotypic differences, especially at anthesis, as observed here, have been identified previously (Stone and Nicolas, 1994; Alghabari et al., 2014; Lobell et al., 2015; Liu et al., 2016a).

This suggests that there is potential for identifying heat tolerant traits within the current genetic diversity of wheat, which will be crucial for crop production in future climates (Godfray et al., 2010; Semenov et al., 2014).

These pot experiments do not allow for assessment of plant traits expressed in field conditions, such as more efficient root architecture (Semenov et al., 2014), or acclimatisation as a drought develops. Here, no evidence was found that greater diversity in flowering time improved resilience as suggested by (Lukac et al., 2012; Jones et al., 2016), rather the reverse.

Future research should put greater focus on reducing the impact of other influencing factors on the results, namely relative humidity and water availability during treatment. There was a large amount of variation in the yield of the control pots, which was somewhat unexpected. There was therefore a clear transfer effect of the pots, which needs to be controlled more efficiently in future experiments.

2.5: Conclusions

- The key phases susceptible to heat stress at booting and anthesis in wheat are discrete and that genotypes vary with regards to the most susceptible growth stage.
- Periods of susceptibility are repeatedly observed during GS 41-45 and again from GS 61-65.
- In the prevailing conditions (mean daily temperature 14.3°C) periods of peak susceptibility could be separated by 15 days.
- Main stem data provided the clearest indication of any susceptibility to heat stress compared to whole pot or tiller data.

Chapter 3: Investigating the heat stress tolerance of *Rht8*

Content from this Chapter (data and text) appears in:

Barber HM, Lukac M, Simmonds J, Semenov MA and Gooding MJ (2017). Temporally and Genetically Discrete Periods of Wheat Sensitivity to High Temperature. Front.PlantSci. 8:51. doi: 10.3389/fpls.2017.00051 **(Appendix 1)**

3.1: Introduction

Chapter 2 added further clarity on the discrete relationships between heat stress, growth stage and genotype. Genotypes which differed for *Rht* alleles were seen to respond to short duration heat stress events differently. Here, the influence of *Rht8* on heat stress tolerance is directly investigated in an exploratory study, to gauge whether differences in response between those genotypes in Chapter two were possibly due to the presence of *Rht8*.

There are several semi dwarfing alleles used worldwide in wheat populations, introduced during the green revolution to improve crop yields (Hedden, 2003). Additionally, different regions have adapted their genotypes to best suit the local climate, such as by the inclusion of *Ppd* alleles in southern areas of Europe (Snape et al., 2001). Currently there are 22 catalogued major alleles that effect the height of wheat plants: designated as *Rht* genes (Worland et al., 1998b; McIntosh and Yamazaki, 2008).

Notable inclusions, as mentioned in Chapter 1, include *Rht-B1b* (*Rht1*), *Rht-D1b* (*Rht2*) and *Rht8*. *Rht8*, a gibberellin sensitive semi dwarfing allele (Worland et al., 2001), located on chromosome 2D (Gasperini et al., 2012) is prevalent around the Mediterranean and appears to contribute to adaptation to that environment (Worland et al., 1988).

Rht8 is deployed to promote improved establishment due to its effect on increasing coleoptile length compared to GA-insensitive *Rht* alleles (Rebetzke et al., 2007; Addisu et al., 2009). However the benefits of *Rht8* appear to be regional. In more temperate conditions in the UK, *Rht8* has been associated with reduced yield and grain quality (Casebow et al., 2016; Kowalski et al., 2016).

Rht8 is linked to the photoperiod gene *Ppd-D1a* (Worland et al., 1988; Gasperini et al., 2012; Langer et al., 2014), which is commonly associated with reduced time to anthesis, a common method of escaping abiotic stresses in warmer climates (Jones et al., 2016). There is some evidence that *Rht8* increases tolerance to heat and drought stress around the reproductive phases of growth, when studied in a Mercia background, with the close linkage to *Ppd-D1a* present (Alghabari et al., 2014). Clearly more work is needed to assess the actual tolerance of *Rht8*, rather than just the closely linked escape characteristics, in order to identify its potential use in future Mediterranean and Northern European climates.

In this experiment, the tolerance of *Rht8* to heat stress begins to be explored. Using a population of Paragon, which contains neither *Rht-D1b* nor *Rht8,* along with two NILs (+*Rht8*, “Tall”) a small scale potted experiment was conducted using controlled environment heat stress at the Plant Environment Laboratory, University of Reading.

This experiment sought to explore any possible heat stress tolerance traits of *Rht8* around key reproductive phases of growth (Barber et al., 2015) using short duration heat stress events.

3.2: Materials and Methods

3.2.1: Plant material and growing conditions

Paragon and two near isogenic lines (NILs), one containing *Rht8* and the other being "tall", were used in this experiment. The production of the two NILs was described by Kowalski et al (2016): "A 2D recombinant inbred line (RIL) in the Cappelle-Desprez background, carrying the Mara (Akakomugi-derived) semi- dwarfing allele at Rht8 (Korzun et al., 1998) was crossed to Paragon. Paragon is a high-quality, bread-making UK spring wheat, does not contain the GA insensitive Rht-B1b or Rht-D1b alleles and is photoperiod sensitive. The population was developed to BC3F2, producing NILs contrasting for the Akakomugi derived Rht8 allele (short phenotype) and wild-type rht8 allele (tall phenotype). The presence or absence of the Rht8 introgression was determined using the Rht8-flanking microsatellite markers Xgwm261 and Xcfd53 (Korzun et al., 1998; Gasperini et al., 2012). The NIL population was multiplied in the field in 2011 and subsequently one Rht8 NIL and one rht8 NIL (herein called 'tall') were selected at the BC3F3 stage for further multi-environment field experiments. The selection was made on the basis of preliminary height analysis to identify one semi-dwarf line (Rht8 NIL) and one tall line (rht8 NIL)."

Paragon was a mainstay of the UK recommended list, originally being listed in 1999 and being removed after the 2015/16 growing season (AHDB, 2016). Paragon does not contain either of the two main semi dwarfing alleles present in the UK, *Rht-B1b* or *Rht-D1b* (Kowalski et al., 2016), therefore is an ideal background for studying the effects of particular *Rht* alleles. The experiment was conducted at the Plant Environment Laboratory at the University of Reading, UK (51 27’ N latitude, 00 56’ W longitude), under the same growing conditions described in Chapter 2.

3.2.2: Experimental design and heat stress conditions

The treatment structure comprised a complete factorial design of: three genotypes (Paragon, *Rht8* NIL and Tall NIL (Kowalski et al., 2016)); day of transfer to Saxil growth cabinets (5 separate days between 19th May and 10th June 2014; targeted for early booting (timing 1), mid booting (timing 2), ear emergence (timing 3), early anthesis (timing 4) and mid anthesis (timing 5)) and the two temperature regimes within growth cabinets. Confounding effects associated with temperature included water loss. The mean weight of pots on entry was the same as Chapter 2 (3.40kg), as the experiment was carried out in parallel with that experiment. Treatments and measurements of growth stage were the same as described in Chapter 2.

3.2.3: Post harvest and statistical analysis

Main stems and tillers were harvested separately after physiological maturity (GS 89) and dried (48h at 80°C). Ears and spikelets per ear were counted, after which grain was threshed from ears, and then re-dried, weighed and counted by a Kirby Lester K18 Tablet counter.

The primary statistical approach was an appropriate factorial analysis of variance with a split plot random model of Block / Cabinet / Pot (GenStat 14th edn. VSN International Ltd). Effects on grain yield and fertility were primarily studied. Pots where there were fewer than 3 main stems present were excluded from analyses.

3.3: Results

The three genotypes differed for a number of phenotypic traits (Table 3.1). There were significant differences in ear number per pot (p<.001), which also differed in size (spikelet number per ear; p<.001). The presence of *Rht8* reduced ear number but increased the size of the ears present. Stem weight per pot also differed (p<.001) between genotypes, although this was not affected by temperature (p>0.05).

Stem weight per ear (defined as stem weight per pot adjusted for ear number per pot) differed between genotypes (p= 0.025) with the “tall” NIL having the largest stem weight per ear, likely due to it being the tallest genotype (Kowalski et al., 2016). Unlike the experiment in Chapter 2, genotypes did not significantly differ for development rate (p= 0.096), with each genotype entering the cabinets at a broadly similar development stage at each treatment time.

Table 3.1: Phenotype data, where significant, for the three NIL genotypes used in this experiment, averaged across all timings and temperatures. Effects of temperature and timing were not significant (P > 0.05) on these measures. SED is standard error of the difference.

Phenotype	**Paragon**	***+Rht8***	**Tall**	**SED**
Ear No. per Pot	10.1	9.6	11.2	0.31
Spikelets per Ear	22.2	23.2	22.1	0.15
Stem Weight per Pot (gDM)	24.1	23.5	28.0	0.61
Stem Weight per Ear (gDM)	2.4	2.5	2.5	0.04

There was a strong genotypic effect (Table 3.2; p<.001) for grain weight per pot, as well as a significant interaction between temperature and timing (p=0.046). There was also a significant three way interaction between genotype, temperature and timing (cubic p= 0.048). Covariate effects were also identified for stem weight (p<.001) and ear number per pot (p=0.002).

The differences in ear number likely caused the covariate effect on grain yield. When comparing genotypes, identifying effects on mainstems rather than the whole pot would lower variation caused by differing ear numbers, as the genotypes do not significantly differ for number of mainstems per pot (p= 0.821), as outlined in Chapter 2.

Table 3.2: Grain weights per pot (gDM) for the three NIL genotypes across two temperatures and five timings. A significant three factor interaction (cubic; p = 0.046) was found.

Timing	Paragon		*+Rht8*		Tall	
	20°C	**35°C**	**20°C**	**35°C**	**20°C**	**35°C**
1	16.3	19.4	20.2	22.9	19.5	19.7
2	19.2	18.2	22.2	19.7	19.3	17.5
3	19.1	20.6	20.4	20.6	18.6	21.2
4	18.1	17.8	20.8	20.4	17.7	20.1
5	17.9	19.1	18.4	20.5	18.6	16.7

There was a significant interaction between the time of transfer and temperature on mainstem grain number (P = 0.005 for Temperature x quadratic Day). As in Chapter 2, a significant reduction in grain numbers from the main stems resulted from a day transfer to 35/30°C rather than 20/15°C, 18 days before mid anthesis (GS 65; Fig. 3.1), whilst the plants were in the early to mid-stages of booting (c. GS 43; Fig. 3.1).

There were smaller reductions in grain numbers following heat stress during late ear-emergence and early anthesis, commensurate with the effects on grain numbers of Savannah at similar timings in Chapter 2. Plants appeared tolerant of the higher temperature at the start of booting (c. GS 40) and by mid anthesis (GS 65; Fig. 3.1). There was no statistical evidence here that reductions in grain numbers were mitigated by increases in mean grain weight.

There was some evidence to suggest that genotype affected tolerance to heat stress in regards to grain number, when comparing all three lines (genotype x temperature; p=0.012).

However, when directly comparing the genotype containing *Rht8* against the other two genotypes (Fig. 3.1), there was no evidence that *Rht8* influenced tolerance to heat stress during booting or anthesis (P = 0.997 for Temperature x Day x Genotype on mainstem grain numbers).

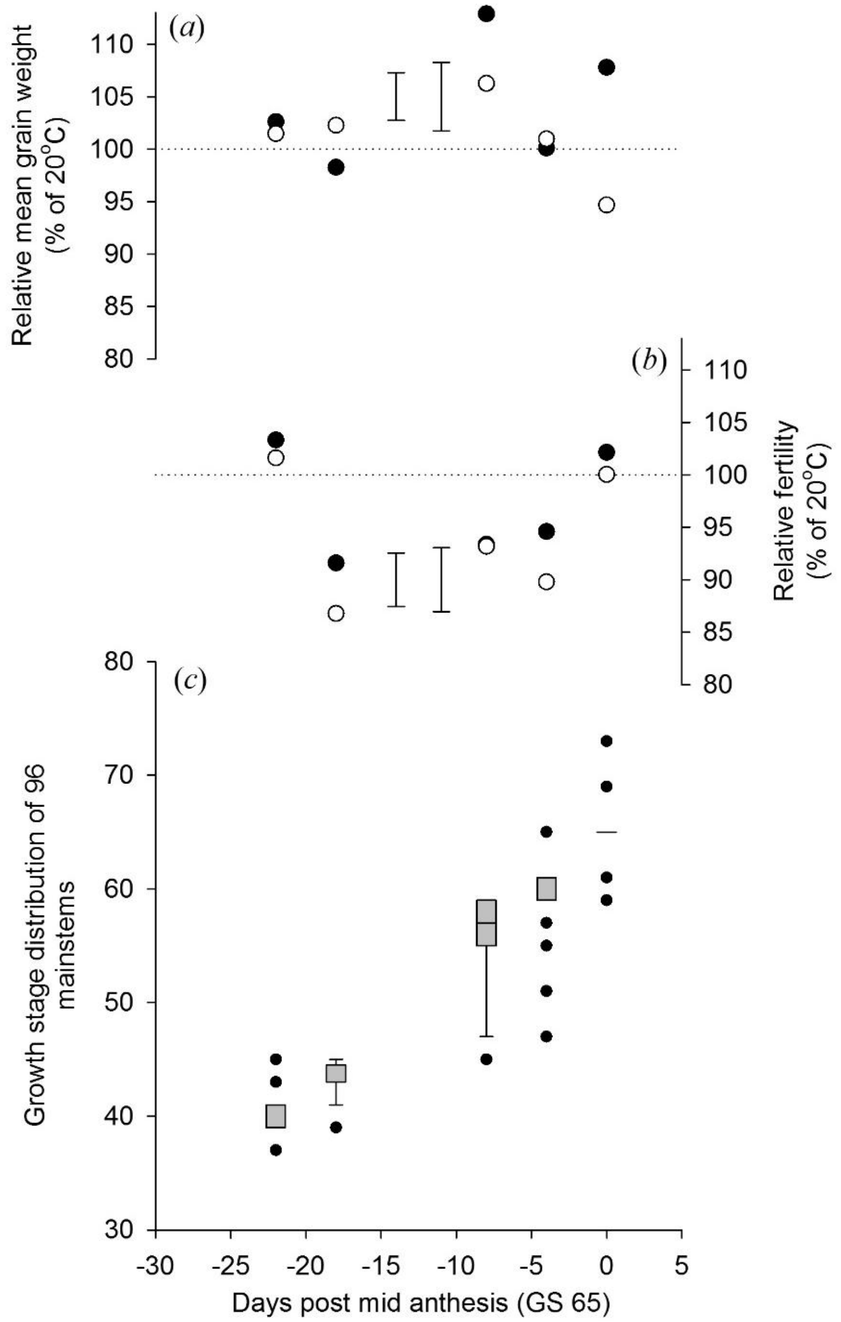

Figure 3.1: Effects of increasing day temperature from 20°C to 35°C in 1-day transfers to controlled environment cabinets on yield components per pot from main stems of near isogenic lines with (●) and without (○) *Rht8* in a Paragon background. Error bars in *a* and *b* are SEDs for comparing points without (left) and with (right) *Rht8* with the 100% line. Box-whisker plots in *c* show growth stage distributions of mainstems on day of transfer.

3.4: Discussion

This experiment was used as an initial study on the possible influence of *Rht8* on heat stress tolerance of NILs in a Paragon background, in a controlled environment. The relationship between heat stress and growth stage once again showed discrete effects as well as genotypic differences in response, although there was no evidence this was due to the presence of *Rht8*.

The reduced fertility seen around mid booting and the onset of anthesis is in agreement with the findings of Chapter 2 as well as other previous work (Saini and Aspinall, 1982; Ferris et al., 1998; Dolferus et al., 2011; Liu et al., 2016a). Although in this experiment there was no evidence of compensation through increases in grain weight, as suggested by Semenov et al. (2014) and Liu et al. (2016). It was harder to distinguish discrete heat stress effects in this experiment compared to the one in Chapter 2. This is mostly because the reduced number of timings here (5 compared to 31) reduced the overall size of the experiment, reducing the spread of growth stages stressed. Still, there were a few observations in regards to *Rht8* that we can draw from this experiment.

The population used in this experiment did not seem to differ for developmental rate although it likely did differ in height (due to observed differences in stem weight per ear). These observations from a potted experiment are in agreement with those from field trials of Kowalski et al. (2016). Here, a difference in tiller number across the genotypes is also observed.

The line containing *Rht8* was also seen to have a higher grain yield in this experiment, which is consistent with low nitrogen based field conditions, although the opposite is seen when nitrogen levels are increased (Casebow et al., 2016). These general phenotypic results suggest that in autumn sown conditions results are broadly repeatable between field and pot conditions.

There has previously been some suggestion that the semi dwarfing allele *Rht8*, commonly found in southern European genotypes of wheat (Worland, 1996; Gasperini et al., 2012), could also increase tolerance to heat and drought stress compared to other semi dwarfing alleles (Alghabari et al., 2014, 2016). However, this fully replicated study found no effect of *Rht8* on susceptibility to heat stress. The presence of *Ppd-D1a* in the Alghabari experiment may give reason to these differences. This suggests that even in future climates, *Rht8* would not be of benefit to northern European genotypes due to its lower yield in comparison to other semi dwarfing alleles in typical UK agronomic conditions, even in more extreme climates (Rebetzke et al., 2007; Casebow et al., 2016; Kowalski et al., 2016).

4.5: Conclusions

- Mid booting and the onset of anthesis once again were found to be particularly susceptible to heat stress
- Heat stress susceptibility once again appeared to be discrete in respect to growth stage, with periods of relative tolerance at certain timings.
- The Paragon population had broadly similar phenology in this potted experiment compared to previous UK field trials
- There was no evidence that the semi dwarfing allele *Rht8* had any effect on the susceptibility of wheat to heat stress

Chapter 4: Identifying variation in heat stress tolerance in Doubled Haploid progeny of Renesansa x Savannah

Data and content in this Chapter appear in:

Barber HM, Lukac M, Simmonds J, Semenov MA and Gooding MJ (2017). Temporally and Genetically Discrete Periods of Wheat Sensitivity to High Temperature. *Front.PlantSci.* 8:51. doi: 10.3389/fpls.2017.00051 **(Appendix 1)**

4.1: Introduction

Experiments in Chapters 2 and 3 have determined that: there are two discrete development periods which are vulnerable to heat stress (booting and anthesis); the booting response can be variable with regards to its precise susceptible timing (likely due to variable meiosis timings in relation to growth stage (Bennett et al., 1971; Zadoks et al., 1974; Barber et al., 2015); the response to stress varies with genotype; and that no evidence has been established to suggest that response to stress is influenced by *Rht8*. It was decided, therefore, to establish the range of heat stress responses between the genotypes of the Savannah x Renesansa DH population and identify possible genetic bases for these differences, with a particular focus on alleles previously associated with crop adaptation to environment.

Differing *Rht* alleles are of interest in this scenario due to the differing mechanisms by which they induce semi-dwarfing in wheat. *Rht-D1b*, common in UK and Northern European genotypes (Gale and Youssefian, 1985), suppresses stem elongation through insensitivity to gibberellins (Peng et al., 1999).

Rht8, a common Southern European allele, suppresses stem elongation through the interference of brassinosteroid signalling (Gasperini et al., 2012). These differing interferences in plant mechanisms are possible reasons for differing responses to heat stress (Alghabari et al., 2014; Semenov et al., 2014). *Rht8* is common in southern European areas due to apparent benefits to establishment through increased coleoptile lengths (Rebetzke et al., 2007), but also due to its close linkage with *Ppd-D1* (Gasperini et al., 2012), which is a common source of early flowering and hence escape from developing terminal heat and drought stresses (Snape et al., 2001; Foulkes et al., 2004). Whilst a lot is known about the relation of these alleles to escape and influences on agronomy in differing climates (Addisu et al., 2009, 2010; Kowalski et al., 2016), a closer examination of their actual heat stress response is needed. Chapter 4 found no evidence to suggest that heat stress response is influenced by *Rht8* but this runs counter to reports of (Alghabari et al., 2014) when *Rht8* conferred greater tolerance to high temperatures at booting in droughted conditions.

In addition to Rht alleles, the *1BL/1RS* translocation (Schlegel and Korzun, 1997) also deserves investigation. The inclusion of the short arm of chromosome 1 from rye introduced a number of race-specific disease resistance genes (Snape et al., 2007), and has also been variously associated with increased above ground biomass, spikelet fertility, delayed senescence and drought tolerance (Villareal et al., 1998; Rajaram, 2001). There is, however, apparently little information as to how 1RS influences on heat stress tolerance.

Although detailed studies on well known alleles are needed, there is also a need to identify less well known alleles and their possible impact on heat stress tolerance.

Using larger, doubled haploid populations allows for QTL analyses to be conducted identifying possible influential traits. This process is already established for traits such as flowering time (Thornsberry et al., 2001) and grain size (Breseghello and Sorrells, 2006).

Although QTL analysis has been attempted for heat stress tolerance (Tripathy et al., 2000; Suzuky Pinto et al., 2010), it can be expanded to explore new, more variable populations. However, in order for this to be a success more focus needs to be given on the methodology of heat stress experimentation to increase throughput to test larger populations. For example, some well known studies in controlled environments use very few control plants for comparison (Saini and Aspinall, 1982; Prasad and Djanaguiraman, 2014) due to limited facilities and space in controlled environments. To observe a larger number of genotypes thoroughly, thought must be given to how to maximise efficiencies in the experiment.

Here, a method is used which enables full replication of a larger population through the use of fewer, more specific stress timings. Sixty-two DH lines from the Savannah x Renesansa population (Snape et al., 2007) will be exposed to heat stress at three different growth stages in controlled environment to assess for genotypic effects on heat stress tolerance. Chapter 2 has established a relative period of tolerance to heat stress during ear emergence so here the number of stress timings will be reduced to three; early booting, mid booting and anthesis, which allows for an increase in the number of genotypes to be studied.

The effect on fertility of main stems will be a point of emphasis as previous Chapters have established these stems as showing particularly clear effects. This focus reflects previous work e.g. Saini and Aspinall, (1982), where main stem fertility was the only characteristic studied.

A comparison of Southern European alleles (*Ppd-D1a, Rht-D1a, Rht8*) against Northern European alleles (*Ppd-D1b* (a photoperiod sensitive allele)*, Rht-D1b, 1BL/1RS*) will occur here, as well as an exploration of other influencing alleles on heat stress tolerance.

4.2: Material and Methods

4.2.1: Plant Material

Savannah had a high yield potential in North West Europe with low bread making quality and was recommended in the UK in 1998. Renesansa, a Serbian winter wheat listed in 1995, has high yield potential and high bread making quality in southern Europe. Sixty-two lines were selected from a recombinant doubled haploid (DH) population of Savannah x Renesansa based on their alleles at Ppd-D1, Rht-D1, *1BL/1RS* and *Rht8* (as marked by Xgwm261) (Simmonds et al., 2006; Snape et al., 2007).

4.2.2: Experimental Design

The experiment was sown on 3rd December 2014. The treatment structure comprised a complete factorial of 62 DH Lines, three growth stages at transfer to Saxil growth cabinets, and two temperature regimes within growth cabinets. The three timings targeted specific stages of growth based on the findings of Chapter 2: early booting (GS 39-41); mid booting (GS 43-45); and early anthesis (GS 63-65).

Due to variable rates of development within a 24 hour period, not all lines were transferred within the target growth stages. Nonetheless, GS at transfer was always recorded. The drastic reduction in the number of timings allowed for a marked increase in genotypes used in this experiment compared to Chapters 2 and 3 (Table 4.1).

Table 4.1: Experimental outline of the three potted heat stress experiments in this thesis, as described previously in Chapters 2 and 3. The experimental structure has stayed the same although adapted in this Chapter to allow for a larger number of genotypes and therefore a large increase in overall size of the experiment.

Factor	Exp.1 Chp.2	Exp.2 Chp.3	Exp.3 Chp.4
Genotypes	2	3	62
Timings	31	5	3
Temperatures	2	2	2
Blocks	4	4	4
Total Pot Number	496	120	1488

4.2.3: Growing conditions and post-harvest analysis

Plants used in this experiment were grown in pots (180 mm diameter) at the Plant Environment Laboratory at the University of Reading, UK (51 27’ N latitude, 00 56’ W longitude). Growing conditions were as described in Chapter 2, in regards to potting mixture and ambient growing conditions.

Table 4.2: Temperature records for the 2014/2015 growing season, recorded by a weather station at Sonning, near Reading, UK.

Month (2014/15)	Mean of Daily Minima (°C)	Mean of Daily Maxima (°C)	Average Mean Temperature (°C)
December	1.2	9.1	5.2
January	0.5	8.4	4.5
February	0.3	7.4	3.8
March	2.4	11.3	6.9
April	3.8	15.9	9.8
May	6.5	16.4	11.5
June	9.5	21.1	15.3
July	11.0	22.7	16.8

All treatments consisted of transfers to Saxil growth cabinets, which began between 10:20h and 11:20h (BST) and remained there for 24h (16h day, night time between 22:00h and 06:00h) before pots being returned outside to their original randomised block position.

Average daily temperature during the treatment period was 13.5°C. Two temperature regimes were used in all experiments and were the same as Chapters 2 and 3. The mean weights of pots upon entry to the cabinets in this year was 3.50kg, whilst the weight of pots post treatment were 3.30kg and 3.18kg (S.E.D. = 0.02) for the 20/15 and 35/30°C treatments respectively. Eight growth cabinets were used which allowed the two temperature treatments to be replicated for the four blocks. On the day of transfer main stems in each pot were tagged and assessed for growth stage (GS; Zadoks et al., 1974).

Main stems and tillers were harvested separately after physiological maturity (GS 89) and dried (48h at 80°C). Ears and spikelets per ear were counted, after which grain was threshed from ears, then re-dried, weighed and counted by a Kirby Lester K18 Tablet counter.

4.2.4: Statistical Analysis

The primary statistical approach was an appropriate factorial analysis of variance (ANOVA) with a blocking structure of Block / Cabinet / Pot (GenStat 14th edn., VSN International Ltd). The ANOVAs contained a treatment structure of Genotype x Target Growth Stage x Temperature. A regression analysis was conducted in an attempt to control the effects of varying growth stages within the target GS cohort. Main and interacting effects of *Rht-D1b*, *Rht8*, *Ppd-D1a* and *1BL/1RS* were tested for their significance in the regression model. In addition, after correcting for the linear effect of GS within target GS cohort, a QTL analysis was conducted from the effects of the high temperature treatment on individual lines within each target GS. A framework genetic map was previously constructed from 93 lines of the population as described by Snape et al. (2007), containing 107 single sequence repeat (SSR) markers and perfect markers for Ppd-D1, Rht-D1 and *1BL/1RS*.

Linkage map construction was previously conducted using JoinMap® 3.0 (Kyazma BV) with default settings (Simmonds et al., 2006; Snape et al., 2007). Linkage groups were determined using a Divergent log-of-odds (LOD) threshold of 3.0 and genetic distances were computed using the Kosambi regression.

The genetic map consisted of 25 linkage groups with 45 unlinked markers. QTL Cartographer 2.5 (North Carolina State University) was used for QTL detection using single marker analysis and composite interval mapping (CIM) (Snape et al., 2007). Estimates of the additive effects and percentage of total variation for identified QTL were calculated using the multiple interval mapping (MIM) function. The QTL analysis was performed by James Simmonds of the John Innes Centre.

4.3: Results

Within the doubled haploid population, when using the ‘target’ growth stages for transfer as a fixed effect there was a very highly significant interaction (P<0.001) between temperature, growth stage and DH line for grain number. When making some allowance for actual growth stages within target stress timings, there was evidence of increasing susceptibility from GS 37 to 41 (Fig. 4.1d) and from GS 59 to 65 (Fig. 4.1f). There was wide variation in susceptibility of lines within the doubled-haploid population. None of this variation was significantly associated with the markers for *Rht8* or the *1BL/1RS* translocation. At anthesis, however, main effect associations with both *Rht-D1b* (P<0.001) and *Ppd-D1a* (P=0.006) were significant. *Rht(tall)* and *Ppd-D1a* were associated with increased susceptibility during anthesis (Fig. 4.1f).

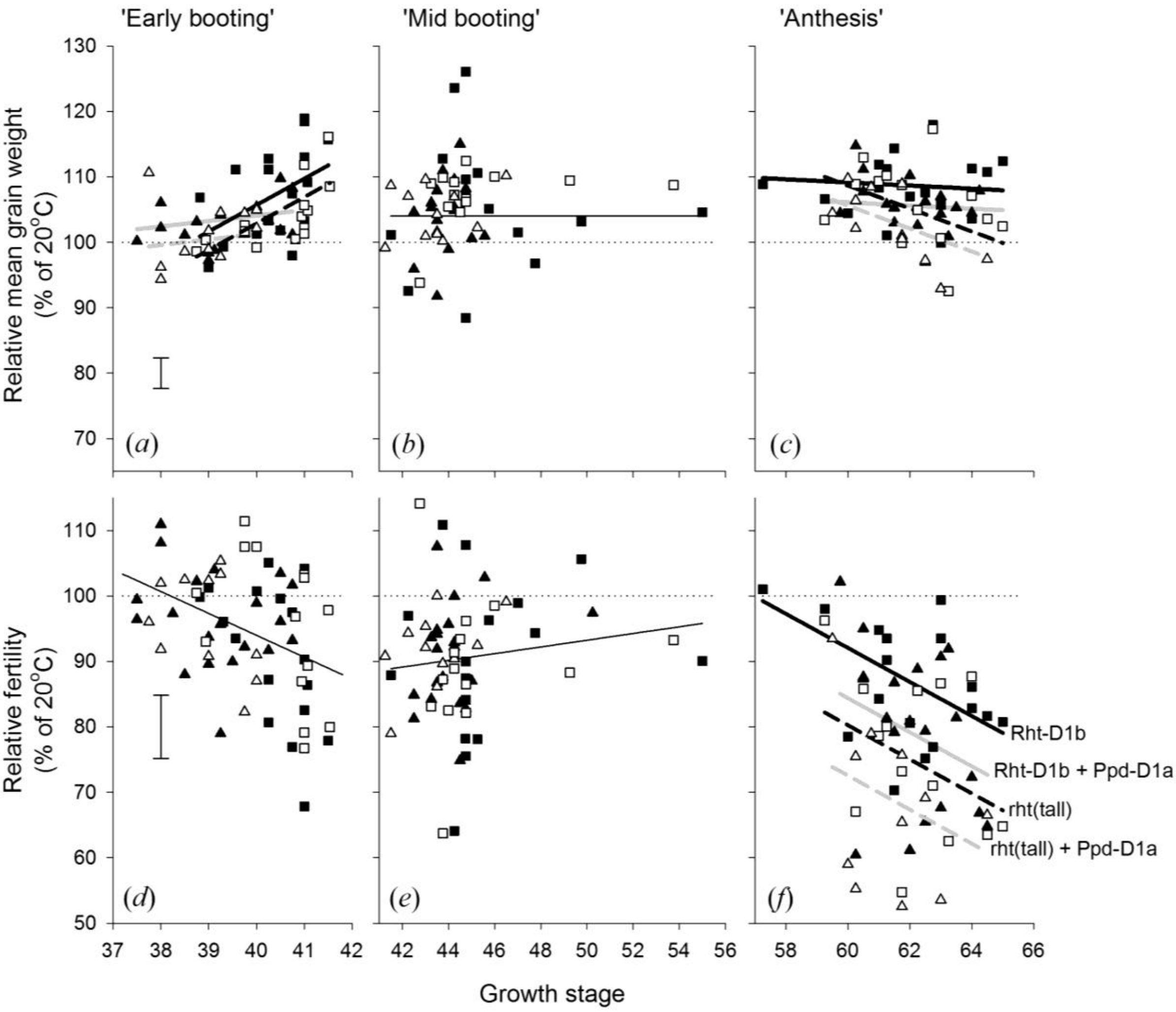

Figure 4.1: Effects of increasing day temperature from 20°C to 35°C in 1-day transfers to controlled environment cabinets and growth stage on yield components from main stems of the doubled haploid progeny of Savannah x Renesansa marked for with (solid symbols) and without (open) *Rht-D1b* and with (triangles) and without (squares) *Ppd-D1a*. Error bars are S.E.D.s for comparing any point with the 100% line. In (a), (c) and (f) lines are fits corresponding to markers as described in F: with (solid) and without (dashed) *Rht-D1b*; and with (light line) and without (heavy line) *Ppd-D1a*.

At anthesis, the QTL analysis confirmed the protective nature of the Savannah alleles (*Rht-D1b* and *Ppd-D1b*), but in addition identified a further, and stronger protective QTL from Renesansa on chromosome 2A (Table 4.3). None of these alleles could be detected as being protective against heat stress applied during either of the booting timings. There was however, a weak protective QTL from Renesansa for heat applied during early booting on 2B (nearest marker = Xgwm120; LOD = 1.85; additive effect = -3.75).

Table 4.3: Quantitative trait loci for relative fertility (%) in response to heat stress during anthesis (grain number following one day transfer to 35°C as a percentage of that achieved at 20°C).

Chromosome	Closest Marker	LOD	Additive Effect	Source of protecting Allele	Effect (%)
2A	*Xgwm448*	7.02	-7.1971	Renesansa	38.1
2D	*Ppd-D1*	2.11	3.7296	Savannah	7.1
4D	*Rht-D1*	3.77	5.2518	Savannah	16.7

In addition to effects on fertility, there was a significant three factor interaction on mean grain weight (P=0.032). Increased mean grain weight at the higher temperature during the early stages of booting (Fig. 4.1a) occurred in the lines not marked for *Ppd-D1a*, and was most evident in lines containing *Rht-D1b*. As anthesis progressed, the higher temperature caused progressively greater reduction in the mean grain weights of lines containing *Ppd-D1a* (Fig. 4.1c).

4.4: Discussion

It is necessary to acknowledge the possible confounding effects between heat stress tolerance and water deficit (Barnabas et al., 2008; Alghabari et al., 2014) in this experiment. However, the deficits below FC reported here at the end of pot transfer and the durations over which significant deficits could have occurred, are considered to be relatively minor compared with the results from experiments with longer periods of stress (Gooding et al., 2003; Alghabari et al., 2014). Nonetheless, booting is known to be a period particularly susceptible to drought (Alghabari et al., 2014; Barber et al., 2015) and future work on identifying tolerant traits to abiotic stresses will require consideration of the combination of drought and heat stress.

This study found no effect of *Rht8* on susceptibility to heat stress, in line with findings reported in Chapter 3. This suggests that even in future climates, *Rht8* would not be of benefit to northern European genotypes due to its lower yield in comparison to other semi dwarfing alleles (Rebetzke et al., 2007). Furthermore, *Ppd-D1a*, to which *Rht8* is closely linked (Gasperini et al., 2012), was shown to increase susceptibility to heat stress. Photoperiod insensitivity caused by the allele *Ppd-D1a*, a mechanism used to avoid abiotic stress (Gomez et al., 2014), is widely considered to be a beneficial trait in future climates due to reducing thermal time to senescence (Barber et al., 2015), thereby avoiding late season heat and drought stress. It was also suggested by Jones et al., (2016) that the increase in flowering duration associated with *Ppd-D1a* would add further resilience by increasing diversity of flowering timing within a field. However, the increase in susceptibility to heat stress associated with this allele, as well as lower overall grain yield in non-stressed seasons (Addisu et al., 2010) casts doubt over the benefits that *Ppd-D1a* might bring under future northern European climates.

Although the introduction of *Rht-D1b* in to Northern European genotypes has increased yield through increased harvest index and reduced lodging in fertile conditions (Flintham et al., 1997), it has also been associated with some negative traits, including decreases in fertility under stress (Law et al., 1981). Preliminary work by Law and Worland (1985) suggested that the decrease in GA sensitivity caused by *Rht-D1b* increases susceptibility to high temperatures. This is supported by later work in other cereals, such as barley, which shows that reducing sensitivity to GA increases susceptibility to heat stress (Vettakkorumakankav et al., 1999; Maestri et al., 2002). However, this study shows evidence to the contrary.

Here, *Rht-D1b* was associated with greater tolerance of high temperatures at anthesis than the other alleles associated with stature. In particular, the tall allele (*Rht-D1a*) was associated with susceptibility to heat stress at anthesis. This contrasts with the effects of *Rht-D1* dwarfing alleles (*-D1b* and *–D1c*) in some, but not all, backgrounds reported by Alghabari et al., (2014). We have found no genetic explanation for the poor performance of the Northern European genotype at booting. However this can likely be attributed to the lack of selection pressure previously on breeding programmes for this trait.

With respect to the QTL analyses, others have also found regions on chromosomes on 2A and 2B to be associated with differential responses to heat stress (Mason et al., 2010; Talukder et al., 2014; Goswami et al., 2016). Further heat tolerant QTL have been identified on chromosome 5D (Draeger and Moore, 2017).

Given the strength of the protective effect associated with the QTL on 2A further investigation is warranted for alleles in the relevant region from Renesansa. What is very clear from this study is that alleles and QTL detected as being associated with heat stress tolerance is highly dependent on the precise growth stage of the plant when excessive heat is experienced.

4.5: Conclusions

- There is a high amount of genetic variability in response to heat stress at both booting and anthesis
- Relationships relative to growth stage closely mirrored those observed in Chapters 2 and 3
- *Rht8* nor *1BL/1RS* were not associated with any influence on response to heat stress
- *Ppd-D1a* was associated with increased sensitivity to heat stress around anthesis, through reductions in grain weight and grain number
- *Rht-D1b* was associated with increased tolerance to heat stress at anthesis (grain number) relative to the tall allele at this loci
- These findings were further confirmed by the QTL analysis, which found a further QTL associated to heat stress around anthesis

Chapter 5: Identifying heat stress tolerant material that also performs well in the field

Data and content from this Chapter appear in:

Barber, H. M., Carney, J., Alghabari, F. and Gooding, M. J. (2015) 'Decimal growth stages for precision wheat production in changing environments?' *Annals of applied Biology*. Blackwell Publishing Ltd, 166(3) pp. 355–371. **(Appendix 2)**

5.1: Introduction

It is likely that tolerance to heat stress will be an important feature in future UK wheat genotypes due to climate change (Semenov et al., 2014). However, it is important to identify traits which will not lower current UK yield potential as a result of better heat stress tolerance, unlike currently pursued escape mechanisms (Jones et al., 2016). Within the Savannah x Renesansa DH population studied in previous Chapters, alleles including *Ppd-D1* and *Rht-D1* have been shown to be of interest in regards to heat stress tolerance.

Since the development of the Savannah x Renesansa DH population (Simmonds et al., 2006), there have been numerous examples of field experiments searching for field phenotypic traits within the population. Examples of field traits identified within this population include the reduction in coleoptile length and early season growth associated with *Rht-D1b*, establishing the importance of early season growth traits (Addisu et al., 2009), which is likely to be of increased importance in future climates (Foulkes et al., 2004; Semenov et al., 2014).

This population has also been studied in the field to identify allelic effects on PAR intercepted and relations with flowering time, notably in relation to *Ppd-D1a* (Addisu et al., 2010). Variations in grain quality, notably Hagberg Falling Number have also been studied (Gooding et al., 2012b; Casebow et al., 2016).

As well as allelic effects on establishment mentioned before, the genotyped alleles within this population have also been found to contribute to other phenotypic and yield traits, which were identified within other populations. Such examples of these include apparent increases in yield potential due to the presence of the *1BL/1RS* translocation (Zhao et al., 2012). Increased yield potential due to the presence of *1BL/1RS* has been found in multiple trials (Reynolds et al., 2009) and has been found to be beneficial in different environmental conditions (Villareal et al., 1998).

Photoperiod insensitivity, largely conferred through the allele *Ppd-D1a* (Langer et al., 2014), has been shown to confer yield penalties in UK field conditions (Foulkes et al., 2004; Addisu et al., 2010). This is mostly due to earlier flowering and senescence through increased development rate between establishment and the onset of stem extension (Foulkes et al., 2004). Varying *Ppd* alleles have also been seen to alter the duration of flowering with *Ppd-D1a* and other *Ppd-D1* alleles extending the flowering period (Jones et al., 2016). This has potential consequences for escaping heat stress, by extending its duration there is the possibility of reducing the number of ears affected by a high temperature period (Lukac et al., 2012).

As well as primary effects on flowering, photoperiod insensitive alleles have also been associated with more phenotypic effects such as reducing tillering, plant height and spikelet numbers (Worland et al., 1998a).

A large quantity of previous work has gone into studying field phenotypic effects of the differing *Rht* alleles; *Rht-D1b* and *Rht8*. As well as the previously mentioned establishment effects in UK conditions, differences between the alleles have also been observed for grain quality traits such as Hagberg Falling Number (HFN), where HFN was reduced by the presence of the gibberellin sensitive *Rht8* (Gooding et al., 2012b; Casebow et al., 2016). Grain Nitrogen concentration was lowered by the presence of dwarfing alleles, which also came with a yield penalty with the presence of *Rht8* (Casebow et al., 2016), this yield penalty caused by *Rht8* has been observed on numerous other occasions in UK field conditions (Addisu et al., 2010; Kowalski et al., 2016).

A series of trials has been conducted at the Crops Research Unit, University of Reading, examining a number of different phenotypic traits in the Savannah x Renesansa DH population. There is still a need to add additional data to this series in terms of yield and their relations to phenotypic traits such as PAR interception and anthesis timings. Genotyped alleles can be studied further, especially the ones associated with heat stress tolerance (*Ppd-D1b, Rht-D1b*; Chapter 4) to identify potential beneficial traits for future northern European climates. Further to this, calibration data for the crop model SIRIUS needs to be obtained for future modelling work in chapter 6. The alleles identified as heat tolerant in this population (*Rht-D1b* and *Ppd-D1b*) also needs to be assessed for their performance in UK field conditions within this population.

Here, a field trial was conducted at the Crops Research Unit, University of Reading, using 62 lines of the Savannah x Renesansa DH population (Simmonds et al., 2006). The trial was conducted over the 2013/2014 growing season and multiple assessments were conducted for PAR interception, R:FR reflectance ratio as well as a number of phenotypic traits. These measurements allow for an investigation in to allelic effects on the relationship between yield and light interception as well as a collation of phenotypic data that can be used to calibrate genotypes from the population in to the crop model, SIRIUS.

5.2: Materials and Methods

5.2.1: Crop establishment and Plant Material

A field trial was conducted at the Crops Research Unit, University of Reading (51°29’N, 0°56’W) during the 2013/2014 growing season, where the soil consists of a free draining sandy loam (Sonning Series; Jarvis, 1968, Addisu et al., 2010). Weather data was collected daily from an automated on site weather station (Table 5.1). The Experiment was a third cereal following a grass ley, having been drilled on the 15th October 2013 in to a previously ploughed and power harrowed seed bed.

Table 5.1: Daily weather collated from the weather station at the Crops Research Unit, Sonning, UK for the 2013/2014 growing season.

Month (2013/14)	Mean of Daily Minima (°C)	Mean of Daily Maxima (°C)	Average Mean Temperature (°C)	Total Precipitation (mm)
October	8.6	16.4	12.5	89
November	2.7	10.0	6.4	53
December	1.9	9.7	5.8	99
January	2.7	9.4	6.1	141
February	3.4	9.8	6.6	111
March	2.9	13.4	8.1	23
April	5.1	15.1	10.1	63
May	7.8	17.1	12.5	77
June	10.5	21.5	16.0	63
July	12.4	25.0	18.7	17
August	10.3	20.9	15.6	84

62 lines from the original 64 lines, including parental genotypes, of the Savannah x Renesansa DH population were used in this experiment. Lines 29 and 65 were then excluded due to an extremely late flowering time and very limited grain filling period, respectively, which reduced the experimental population to 60 lines. Savannah has a high yield potential in North West Europe with low bread making quality and was recommended in the UK in 1998. Renesansa, a Serbian winter wheat listed in 1995, has high yield potential and high bread making quality in southern Europe.

The sixty lines were selected from a recombinant doubled haploid (DH) population of Savannah x Renesansa based on their alleles at *Ppd-D1, Rht-D1, 1BL/1RS* and *Rht8* (Xgwm261) (Simmonds et al., 2006; Snape et al., 2007).

5.2.2: Experimental Design and Assessments

Two Randomized blocks containing 2 x 5m plots of each DH line were sown at a seed rate of 300 seeds m^{-2} consisting of 120mm rows. Agronomic applications included a total of 200kg N/ha and 16kg S/ha, as well as sufficient herbicide and fungicide applications to control weeds and foliar pathogens. Grain yield was determined at harvest from data collected from the combine harvester. A number of phenotypic measurements were recorded throughout the season, including growth stage (Zadoks et al., 1974b), FR:R ratio to determine the end of canopy photosynthetic function (i.e. senescence; described by Addisu et al., 2010) using sensors (SKR 1800, Skye Instruments Ltd, Llandrindod Wells, UK), PAR interception derived from a ceptometer (AccuPAR LP-80; Decagon Devices Inc, Pullman, Washington; described by Addisu et al., 2009, 2010). This data can be combined with daily light radiation levels to calculate the total PAR intercepted by the crop (Kindred and Gooding, 2005; Addisu et al., 2010). Anthesis date for each genotype was collected through daily observations of the plots. Other phenotypic data such as height and ear counts were measured prior to harvest. Leaf area was calculated for the top four leaves for each plot. Ten of each of the top four leaves per plot were sampled before anthesis and scanned. Image analysis using Win-Dias determined the area of each leaf. Specific weight measurements were conducted for each plot after harvest using a chondrometer.

5.2.3: Statistical analysis

All statistical analysis was conducted using GenStat 16th edition. The effects of the genotyped alleles were investigated using Residual Maximum Likelihood (REML) analyses. The fixed effects within the REML analyses were *Ppd_D1* + *Rht8* + *Rht_D1* + *1B_1R* and the random model was block +column +row.

FR:R reflectance data for each double haploid line was used to calculate senescence through the fitting of modified Gompertz curves (Gooding et al., 2000). This can then determine the canopy greenness over time, which can identify the time of senescence, classified as crop greenness below 80% of the maximum (Addisu et al., 2010). Logistic curves were fitted to PAR measurements through the season to derive maximum canopy size and light interception, as described by (Kindred and Gooding, 2005; Addisu et al., 2009, 2010). Thermal time to senescence calculations, derived from the modified Gompertz curves, with PAR interception data, derived from the logistic curves, can be can be combined with local weather data to determine the total light intercepted by the crop throughout the season, as described by (Kindred and Gooding, 2005). Simple linear regressions were conducted to display DH line effects for relationships between thermal time to senescence, PAR interception, above ground biomass and grain yield.

5.3: Results

Phenotypic data collected throughout the experiment is displayed in Table 5.2. As expected, the presence of semi dwarfing alleles significantly affected height. *Rht8* was not significantly associated with any other phenotypic trait observed in this study. *Rht-D1b* was associated with increases in harvest index and ear number per m^2; however it also reduced the specific weight of the grain. The presence of *1BL/1RS* and *Ppd-D1a* were associated with an increase in specific weight. *Ppd-D1a* appeared to lower total leaf area, including a reduction in flag leaf size.

Table 5.2: phenotypic data collected from a 2013/14 field experiment for genotyped alleles of a Savannah x Renesansa DH population. Values are means of each allele when present (+) and absent (-). SED is standard error of difference and was calculated using REML analyses.

Phenotype	***Ppd D1a***			***Rht8***			***Rht-D1b***			***1BL/1RS***		
	+	**-**	**SED**	**+**	**-**	**SED**	**+**	**-**	**SED**	**+**	**-**	**SED**
Plant height (m)	84.8	78.6	2.8	75.5	88.0	3.0	75.2	88.3	2.3	84.0	79.4	2.1
Ear number (m^{-2})	356	364	18.6	356	364	19.0	380	340	14.1	372	347	14.1
Flag leaf size (cm^2)	37.4	42.2	1.8	40.1	39.5	1.8	39.7	39.9	1.3	39.8	39.8	1.3
Top four leaf area (cm^2)	105	116	4.5	108	113	4.6	109	112	3.4	113	108	3.3
Harvest Index	47.1	46.6	2.1	46.5	47.2	2.2	49.0	44.8	1.6	46.8	46.9	1.6
Specific weight ($KgHl^{-1}$)	72.1	69.3	1.5	69.5	71.9	1.6	68.6	72.8	1.2	71.7	69.6	1.2

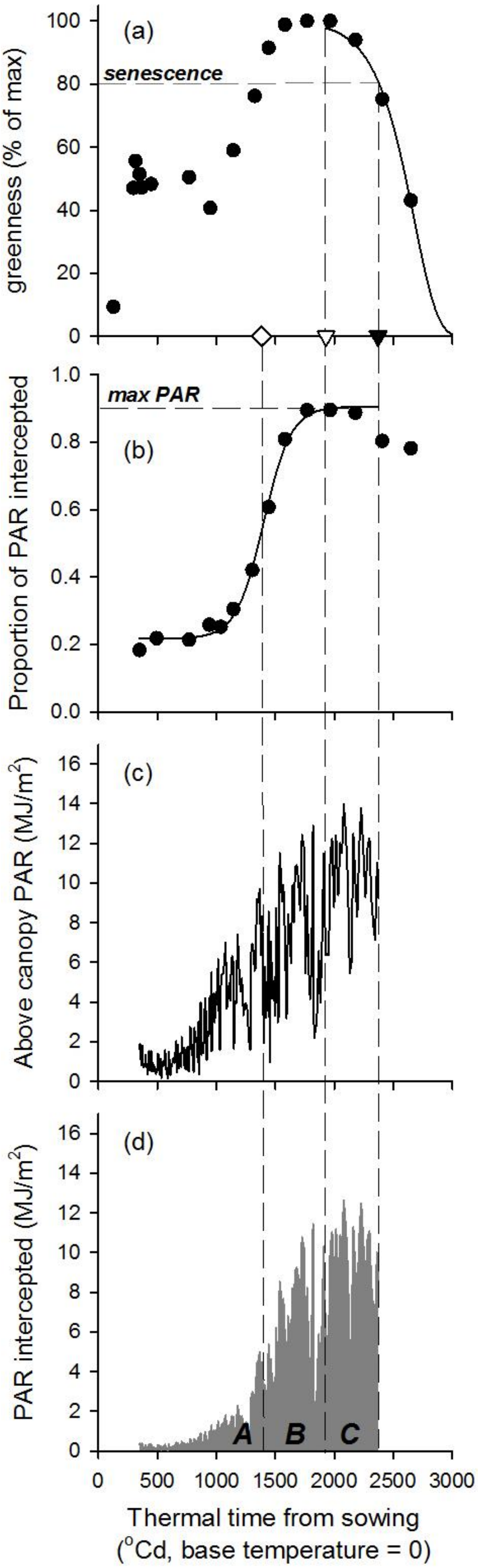

Figure 5.1: Modelled total light interception by the canopy (M^2), displayed here as an average for all genotypes. (a) shows a modified Gompertz curve to identify senescence fitted over individual measurements (points), where senescence is defined as below 80% of the maximum crop greenness. (b) is a logistic curve fitted to PAR intercepted by the canopy, to display where the canopy reaches its maximum. (c) is daily PAR data from a local weather station. (d) combines daily PAR measurements with the proportion of PAR intercepted by the crop as defined by the models in (a) and (b), where the area of the graph is total light intercepted by the canopy. The hashed line denoted by a diamond denotes average flag leaf emergence time for all genotypes, the line denoted by an open triangle shows average anthesis time and the line denoted by a closed triangle shows average senescence time. Therefore in graph (d), area A shows total light interception up to GS39, area B shows total interception between GS39-65 and area C is total light intercepted between 65-senescence.

Combining regular measurements of the canopy throughout the season with local weather station data allowed for the calculation of total light interception throughout the growing season for each genotype. An example of this model is shown in Figure 5.1 as an average for all of the genotypes.

Calculations of PAR interception and Senescence for each genotype are displayed in Appendix 4. The calculation of total light interception (Fig. 5.1), combined with accurate growth stage assessments (Fig 5.2), allows for an investigation of the effect of total light intercepted across different periods of growth (hashed lines; Fig. 5.1). Combining this data with yield and genotypic observations allows for a comparison of the effects of genotype on light interception and yield. A prolonged period of flooding in certain areas of the experiment likely caused the FR:R spike in Fig 5.1a, due to the growth of algae in some areas of the field, which then died away after the flood subsided. Plots which were subjected to prolonged periods of flooding were excluded from further analysis. In total, 38 plots were excluded from thermal time and PAR analyses.

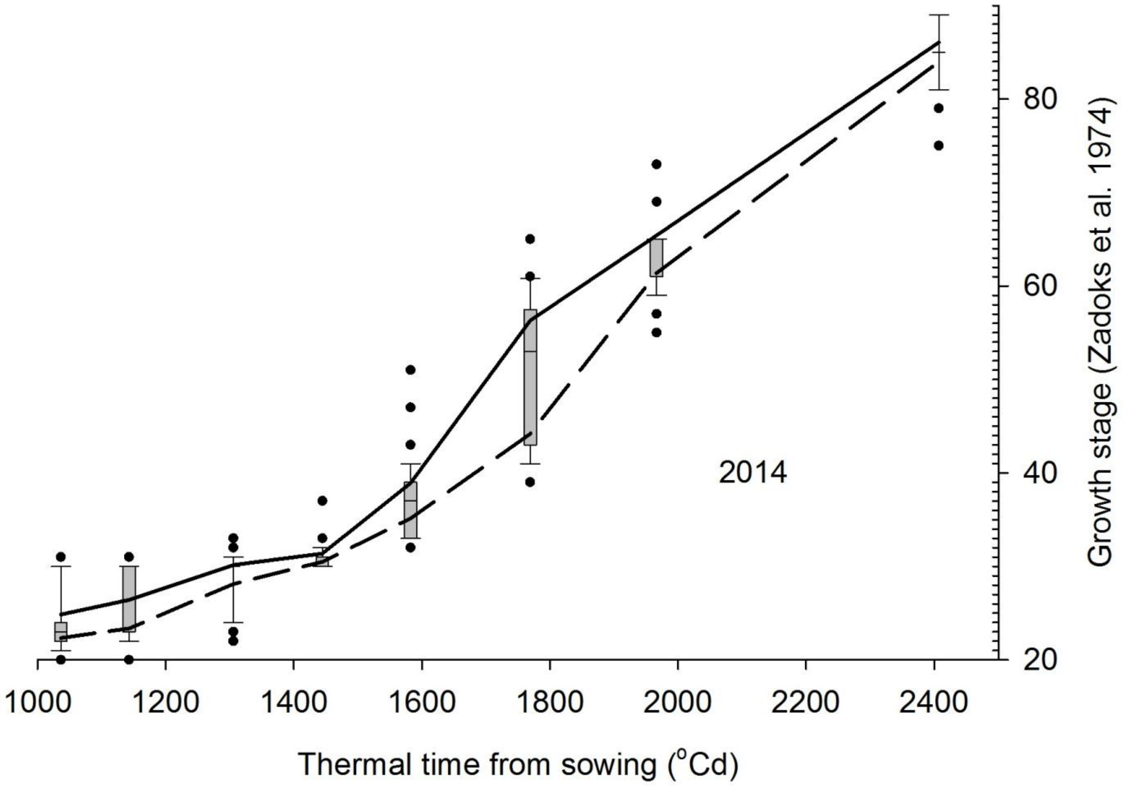

Figure 5.2: Distribution of decimal growth stage (DGS) for 64 doubled haploid progeny of Renesansa and Savannah when field grown in the UK. Boxes are limited by 25 and 75 percentiles, whiskers by 10 and 90 percentiles; points are outliers beyond 10 and 90 percentiles, and the line within the box is the median where appropriate. Heavy solid line connects median for progeny carrying the marker for *Ppd-D1a* (photoperiod insensitive); dashed line is for Ppd-D1b (photoperiod sensitive).

Fig 5.2 draws an initial comparison between the differing alleles of *Ppd-D1* from the two parents. The photoperiod insensitive *D1a* allele from Renesansa shows a more advanced progression through growth stages. The two alleles also show varying rates of development through different phases of growth. Calculating thermal time to key growth stages (Fig 5.2) and combining this with phenotypic and yield data allows for an investigation of the influence of thermal time and PAR on yield, RUE and Harvest index, amongst other traits.

Fig 5.3 shows significant polynomial regression relationships between thermal time and key traits. Polynomial Regressions were conducted up to the cubic level and fitted based on level of significance.

Significant grain yield interactions (Fig 5.3 a-e) with thermal time occurred across all periods of growth, consistently with a quadratic relationship. Where grain yield increased, it was mostly through increases in total biomass (Fig 5.3 k-o) rather than harvest index (Fig 5.3 f-j). Radiation Use Efficiency (RUE) (Fig 5.3 p-t) was rarely significantly linked to thermal time, nor interacted with growth stage. However, there is clear variation for RUE within the population which is likely caused by genetic factors (Addisu et al., 2010).

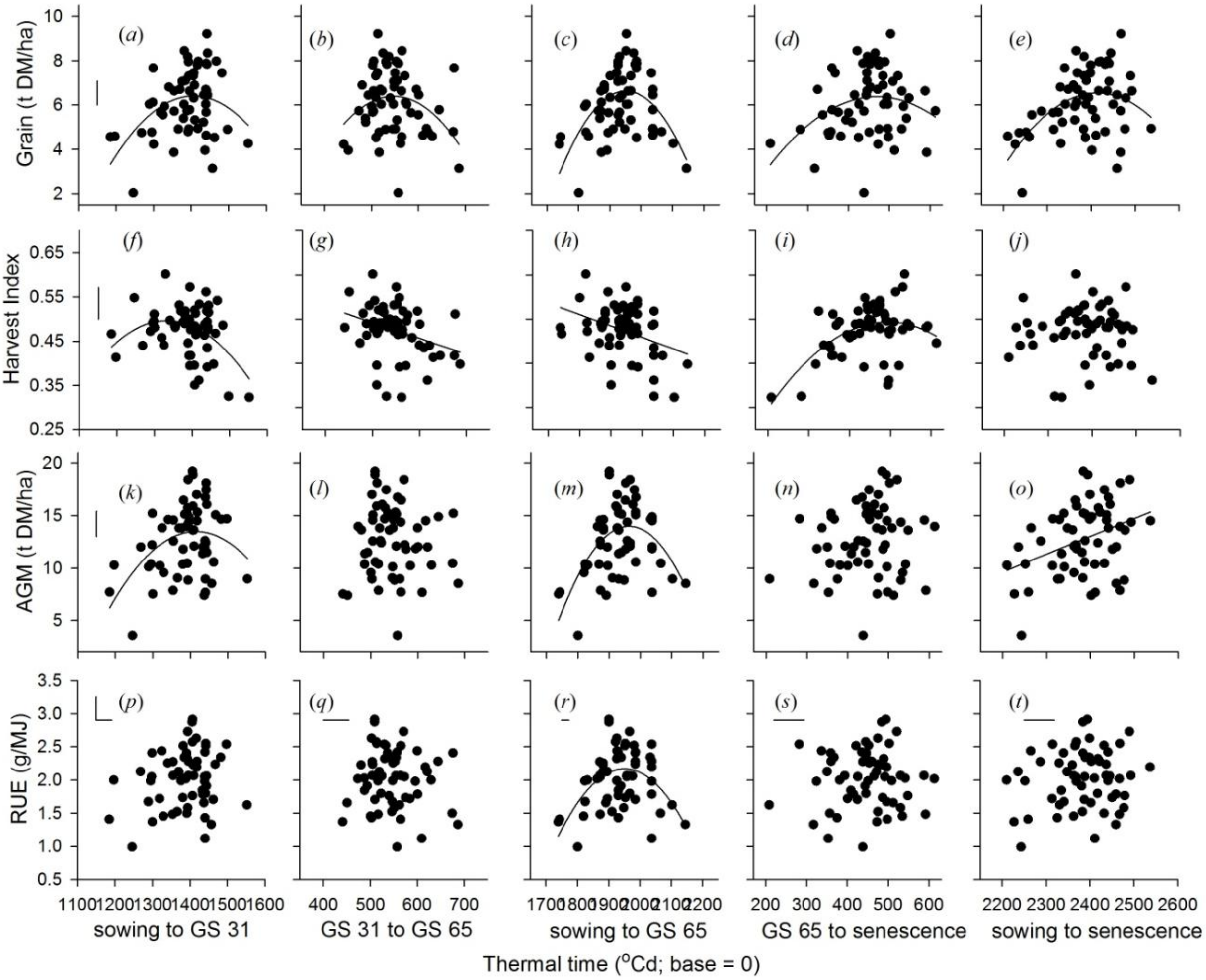

Figure 5.3: Thermal time partitioned between different decimal growth stages (DGS;(Zadoks et al., 1974b)) of 60 doubled haploid progeny of Renesansa and Savannah and associations with radiation-use efficiency (RUE), above ground mass (AGM), and harvest index. Points are means of two replicate field-grown plots in each of three growing seasons. Error bars are SED for comparing points. Fitted lines are polynomial effects when significant (P <0.05).

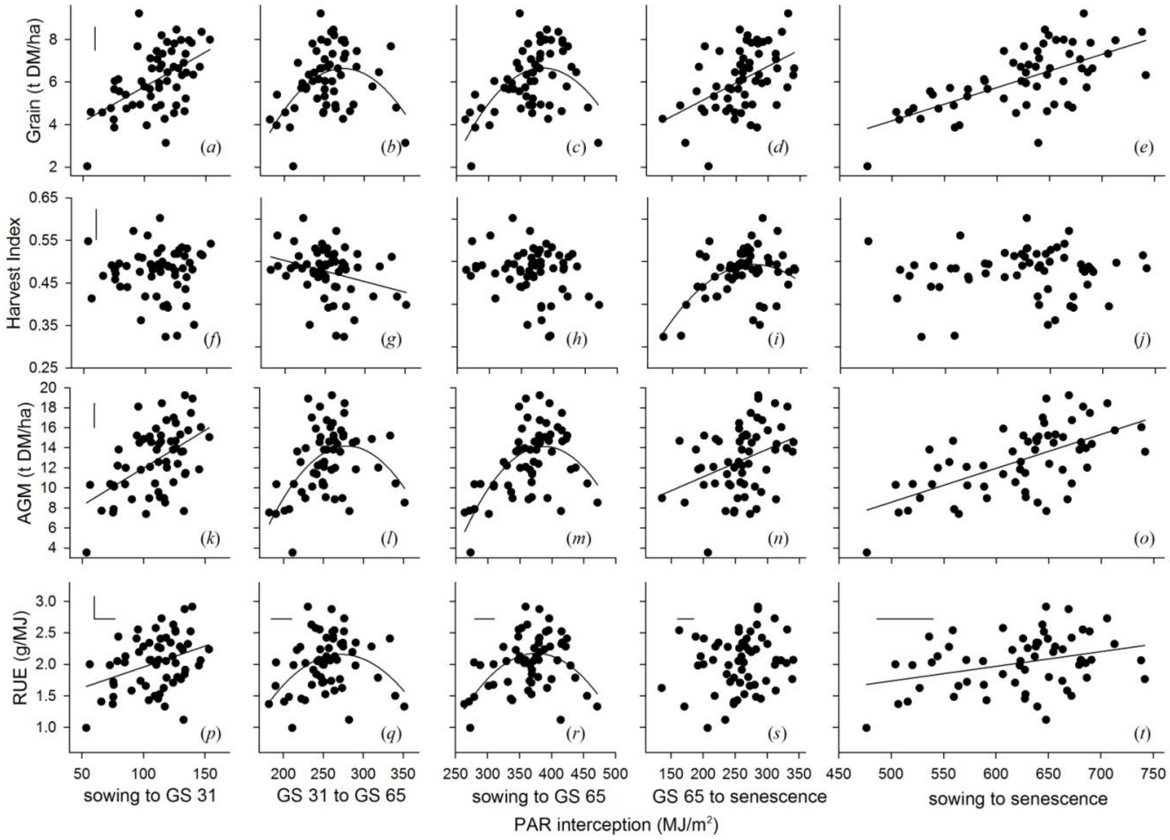

Figure 5.4: Interception of photosynthetically active radiation partitioned between different decimal growth stages (DGS; (Zadoks et al., 1974b)) of 60 doubled haploid progeny of Renesansa and Savannah and associations with radiation-use efficiency (RUE), above ground mass (AGM) and harvest index. Points are means of two replicate field-grown plots in each of three growing seasons. Error bars are SED for comparing points. Fitted lines are polynomial effects when significant (P <0.05).

Significant Interactions ($p < 0.05$) between key traits (grain yield, HI, total biomass and RUE) and PAR interception are shown in Fig 5.4, through the fitting of polynomial regressions. Grain yield (Fig 5.4 a-e) has generally positive correlations with PAR interception at various periods of growth, particularly strong post anthesis (Fig 5.4d), during grain filling. There were fewer significant relationships observed for HI (Fig 5.4 f-j), with the strongest relationship appearing during grain filling. Total biomass was mostly positively related to light interception at all growth stages (Fig 5.4 k-o), although it is worth noting negative relations with high levels of light interception during stem extension, pre anthesis (Fig 5.4 l, m).

RUE relationships with PAR were observed across most phases of growth (Fig 5.4 p-t), quadratic relationships seen before anthesis mirror those of total biomass and grain yield.

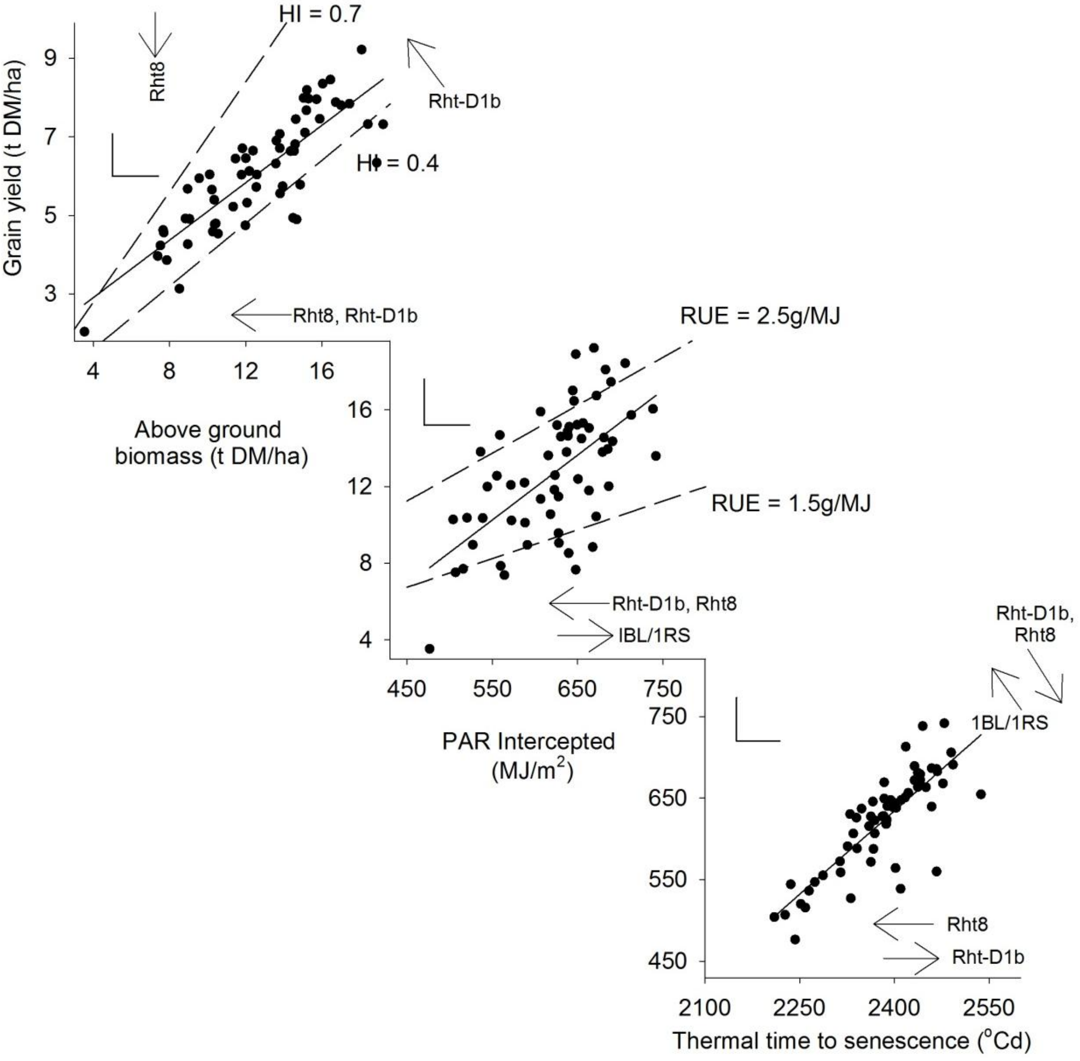

Figure 5.5: Relationships between thermal time to senescence, Photosynthetically Active Radiation (PAR) intercepted, total biomass and grain yield for 62 lines of wheat from the Savannah x Renesansa DH population. Points represent averages for each genotype. Linear regressions were fitted when significant ($p < 0.05$). Error bars represent SED. Arrows are displayed where genotyped alleles have had a significant effect on a relationship, the direction the arrow denotes the effect of the presence of an allele.

Significant linear relationships were identified between thermal time to senescence, total PAR interception, total biomass and grain yield (Fig 5.5). A particularly close fit was observed between thermal time to senescence and total PAR interception (fig 5.5; $R^2 = 0.67$), which is perhaps to be expected. Significant effects of genotyped alleles are indicated on Fig 5.5 as arrows. Surprisingly, there were no significant effects of the heat sensitive allele *Ppd-D1a* on time to senescence, PAR interception or yield (Fig 5.5). RUE and HI was variable amongst the population.

The more heat tolerant semi dwarfing allele, *Rht-D1b*, was associated with increased thermal time to senescence, although reductions in total PAR interception suggest a smaller, but longer living, canopy. Reductions in total biomass associated with *Rht-D1b* in this experiment were offset by HI increases, so no effect on grain yield was associated with this allele. *Rht8* was associated with reduced total biomass and reduced grain yield (fig 5.5). Although there is a large amount of variation for RUE within the population (Fig 5.5), none of this was significantly associated with the genotyped alleles.

5.4: Discussion

This experiment phenotyped the Savannah x Renesansa DH population in field during the 2013-14 growing season, adding more field data to the series already gathered on this population (Addisu et al., 2009, 2010; Gooding et al., 2012b). This experiment focussed on the relationships between thermal time, light interception and yield, focussing on the influence of genotyped alleles, both heat sensitive (*Ppd-D1a*), and heat tolerant (*Rht-D1b*), from both northern (*1BL/1RS*) and southern (*Rht8*) backgrounds.

It is necessary to reflect on the effect prevailing conditions had on this experiment. Extensive flooding occurred at the trial site at Sonning during January 2014 due to a breach in the Thames due to heavy rainfall. The effect on the data can be seen in Fig 5.1a, where FR:R readings have been influenced by the presence of algae on the water saturated soil. Although the trial recovered in the spring, it is possible that fewer allele associations for *Ppd-D1a* have been observed in this experiment due to the flooding affecting early periods of growth. Flooding highlights the importance, where possible, of having repeated trials, especially in the field.

Although this project could not afford repeated field trials, this data can be combined with previous trials at the site in order to provide more dependable conclusions.

This experiment further investigated the relationships between thermal time, PAR interception, growth stage and genotype. Extending the growing season and thus maximising the amount of light intercepted, is shown here to generally increase biomass and grain yield (Fig 5.3, 5.4), as previously suggested (Gooding et al., 2005). Post anthesis light interception appears to be particularly important for final grain yield, which agrees with previous findings (Worland et al., 1998a; Gooding et al., 2005). Quadratic relationships seen with grain yield (Fig 5.3, 5.4), mostly before anthesis, suggest that those with longer durations to anthesis suffered biomass penalties. The quadratic relationships were strongest for the stem extension period (GS 31-65 (Zadoks et al., 1974b)), suggesting the genotypes with particularly late flowering suffered yield and biomass penalties.

There was a particularly close linear relationship between thermal time to senescence and total PAR interception (Fig 5.5), which is not unsurprising. However, *Ppd-D1a* was not seen to significantly influence either of these, which is a surprise as this trait has been well established previously (Worland et al., 1998a; Jones et al., 2016), including within this population (Addisu et al., 2010). There was a large amount of variation for RUE, which was not explained by any of the genotyped alleles, as well as HI which was associated with *Rht-D1b*, but not *Rht8*. Although both semi dwarfing alleles were seen to significantly reduce height (Table 5.1), which suggests that grain yield penalties associated with *Rht8* are due to its lack of adaptation to more temperate climates (Rebetzke et al., 2007), or perhaps its linkage to *Ppd-D1a* (Gasperini et al., 2012).

With respect to identifying heat tolerant genotypes which also perform well in Northern European conditions, *Rht-D1b* was associated with reductions in PAR interception and total biomass, though increases in HI meant that the allele was also associated with increases in grain yield. The QTL associated with heat tolerance seen on chromosome 2A, originating from southern European genotypes, also requires further investigation in the field in UK conditions, which could be carried out through the production of a NIL population.

As stated previously, winter flooding during this experiment highlights the need for repeated experiments, especially in the field where conditions can be so variable. Although time and financial constraints prevented this project from repeating this trial, this population has been grown on site through a series of previous experiments.

Combining these years together will allow for a deeper investigation in to the relationships between PAR interception, allele associations and biomass and would perhaps benefit from a deeper genetic analysis of the population to identify key genetic traits driving grain yield.

5.5: Conclusions

- Thermal time to senescence and total PAR interception are closely linked, with clear importance for grain yield
- The more heat tolerant semi dwarfing allele was associated with increased grain yield through compensations in HI to offset reductions in total biomass
- *Rht8* was associated with reductions in grain yield in UK field conditions
- *Ppd-D1a* was not seen to influence thermal time to senescence or grain yield, possibly a result of the effect of flooding
- Further investigation of the heat tolerant QTL on chromosome 2A need to be conducted in UK field conditions, perhaps through the use of NILs

Chapter 6: An Appraisal of a Crop Models Response to Heat Stress

6.1: Introduction

Crop models are a method of simulating growth, development and yield of crops under various possible future conditions, therefore possibly becoming an essential tool for breeders (Zheng et al., 2016). However, there is a need to improve the accounting for the effects within crop model of abiotic stress, especially of high temperature (Semenov et al., 2014). Here, phenotypic data collected from field experiments (Chapter 5) have been combined with heat stress responses measured for two different genotypes of wheat (Chapter 2) to appraise and attempt to improve the response of the crop model, SIRIUS, to heat stress events.

As previously noted, there is a need to identify heat tolerant traits for future European climates, maintaining yield potential under a higher frequency of stress events. A number of crop models have attempted to quantify the effect of stress on future yield potential of current genotypes. Recently, multiple models have been assessed for simulating the response of two Asian genotypes for anthesis and post anthesis heat stress, finding a large variation between models under the same scenario (Liu et al., 2016a). Crop models have been used to simulate stress at different timings, including anthesis (Stratonovitch and Semenov, 2015) and grain fill (Lobell et al., 2015). Examples of frequently used crop models include APSIM (Keating et al., 2003), CERES-Wheat (Ritchie and Otter, 1985), Nwheat (Asseng et al., 2011) and WheatGrow (Yan et al., 2000).

All of these models simulate growth of wheat and yield in different ways and also use different approaches to account for the effects of stress events differently (Liu et al., 2016a).

For this Chapter, the model used for simulations is SIRIUS. SIRIUS is a wheat simulation model which has been used on numerous occasions to simulate crop development and project yields under future conditions (Jamieson et al., 1998b; Semenov, 2009; Semenov et al., 2014). Whilst the model has been used and updated several times, there is still a need to improve the model response to heat stress events (Stratonovitch and Semenov, 2015), especially around anthesis and other reproductive phases. The most recent improvement made to the SIRIUS crop model involved a dataset from an experiment using the genotype Chinese Spring (Prasad and Djanaguiraman, 2014) used for yield projections in European climates (Stratonovitch and Semenov, 2015). Chinese Spring is a spring wheat variety which is very old, even appearing in agricultural experimentation from the 1970’s (Law et al., 1976) and is therefore not at all representative of the current genotypes grown commercially across Europe currently. There is a clear need to further improve the heat stress response of SIRIUS using European genotypes for European locations, to be able to use the model as a tool to identify potential beneficial traits in these genotypes for future climates.

As mentioned previously, models often differ greatly when attempting to simulate the same heat stress response (Liu et al., 2016a), thereby giving a large spread of projected yields in future climates.

One attempted method to reduce error from the models is to group them together in an ensemble. The most notable crop model ensemble is from the AGMIP project, which combines 27 different crop models (Asseng et al., 2013). There are multiple examples of the ensemble being used to simulate future crop yields on a global scale (Asseng et al., 2013, 2015; Liu et al., 2016b), including the direct response to heat stress (Makowski et al., 2015).

Whilst this approach to modelling can reduce uncertainty of projections, it is a costly and time consuming process that requires a large amount of coordination, highlighting the need for improved single model responses to heat stress to provide a more practical use for smaller scale experiments.

In this Chapter, calibration of two elite European genotypes of wheat, through collected phenotypic data, to the crop model SIRIUS is attempted. Once general phenotypic data for the two genotypes has been calibrated, an appraisal of how the model responds to heat stress will be undertaken. Previous heat stress calibrations using the model (Stratonovitch and Semenov, 2015) have used basic heat stress responses. It is not anticipated that the model will be able to calibrate the response described in chapter 2. However, this exercise will allow for an appraisal of current model limitations and where future work on the model should focus. Yield simulations will then be conducted to assess potential future impacts of heat stress across different sites in Europe.

6.2: Methods

6.2.1: Calibration of the genotypes

The two genotypes used in these simulations are Savannah and Renesansa, the same used in the heat stress experiment of Chapter 2. Phenotypic data was collected from the field experiment described in Chapter 5. To improve the calibration of the genotypes to the model, additional phenotypic data gathered in previous field experiments from 2008 (Addisu et al., 2010) and 2012 (Carney, 2016) was used. The specific phenotypic data required to successfully calibrate a genotype to SIRIUS can be found in Table 6.1. For calibration of the genotypes heat stress response, data from the experiment described in Chapter 2 was used, which provides a detailed description of the specific response of each genotype to heat stress across the whole reproductive phase of growth. The data from Chapter 2 was used to parametize the severity of the yield reduction in the model. Alterations can also be made to the duration of the models response (described below) but not the shape of the period of susceptibility within the model, which stays constant for the whole susceptible period. The response of the model consists of one peak period of susceptibility for a period from 9 days before up to anthesis (Stratonovitch and Semenov, 2015) and will not be altered for this exercise.

Table 6.1: The minimum set of experimental measurements required for calibration to SIRIUS.

	What to measure	Frequency	Priority	Comments
Weather				
	Rainfall, min and max temperature, radiation	IMPORTANT: Daily, 15 days before sowing and 30 days after maturity	High	Vapour pressure and wind are optional. Also see soil temperature measurements in Soil below
Management				
	N and irrigation	As applied	High	
Soil				
	Inorganic N	Once, at or before sowing and at harvest	High	Needs to be measured to depth.
	Organic N	Once, at or before sowing	High	Should be measured in the top 30 cm of soil
	Soil composition: silt, sand, loam	For top soil and hardpan (rest of the soil), or more often if the soil property change with depth	High	
	Saturation moisture content	For top soil and hardpan (rest of the soil), or more often if the soil property change with depth	High	SAT and DUL measurements can be made after harvest by saturating the profile and monitoring moisture each day for a week or 10 days, with some cover to keep off rain
	Drained upper and lower limit	For top soil and hardpan (rest of the soil) , or more often if the soil property change with depth	High	LL estimate can be taken at harvest, but in your weather will probably not be achieved. If necessary, use the 1,5 MPa content.
	Min and max soil temperature at 3 cm	Daily	Low	Bare soil temperature and/or temperature under canopy
Phenology	Sowing date		High	
	Emergence date		High	Cultivar specific
	Anthesis date		High	GS65, Cultivar specific. An alternative is the date of appearance of the flag leaf ligule. Anthesis occurs pretty reliably 300°C days after flag ligule.
	Complete leaf senescence		High	Cultivar specific – could be a problem with this one especially in unirrigated experiments where maturity may be early.
Canopy	Green Leaf Area Index	Stem extension (GS31), flag leaf emergence (SG39), flowering (SG61) and complete leaf senescence	High	Cultivar specific.
	Leaf number	10 days interval, from SD	High	Cultivar specific Might need to vary frequency of measurement. If one cultivar is done in detail, then the others can be compared at specific intervals.
	Final leaf number and culm leaf number	Once	High	Cultivar specific.
	Plant height	At flowering (SG61) + 150 °C days	High	Cultivar specific
	Leaf size	Size of the last 4 fully developed leaves	High	Cultivar specific.
Biomass	Total biomass	At anthesis and maturity	High	If possible, more frequently at 14-days intervals, Cultivar specific
	Grain biomass	At maturity	High	If possible, more frequently at 14-days intervals, Cultivar specific
	Total N uptake	At anthesis and maturity	High	Cultivar specific
	Grain N	At maturity	High	Cultivar specific
	Grain number and size	At maturity	High	Cultivar specific
N storage				
	N concentration in stem	At anthesis	High	Cultivar specific. Compare the well and low fertilised fertility treatments.
	N concentration in leaves	At anthesis	High	Cultivar specific. Also specific leaf weight (mass/unit area) to calculate specific leaf N (N/unit area), for the last three leaves at about flag ligule stage (leaves done individually) compare the well and low fertilised fertility treatments.

6.2.2: The SIRIUS crop model

SIRIUS has been used multiple times to produce future yield projections for winter wheat, using a variety of locations, genotypes and emissions scenarios (Jamieson et al., 1998a; Jamieson et al., 1998b; Lawless et al., 2005; Semenov, 2009; Stratonovitch and Semenov, 2015). A full description of the model was originally produced by Jamieson et al (1998). SIRIUS generates biomass and grain yield data based on the ratio of PAR interception in a season between various times and uses simple partitioning rules of biomass to determine grain yield (Jamieson et al., 1998b).

To produce yield simulations in future climates, 18 GCM's from the CMIP5 ensemble (Taylor et al., 2012; Semenov and Stratonovitch, 2015) are incorporated in to the Long Ashton Research Station Weather Generator (LARS-WG; Semenov and Barrow, 1997) to produce a range of daily weather scenarios up to the year 2090. Simulations were conducted at four locations across Europe, chosen to attempt to represent a range of different climates in Europe. The sites selected were: Rothamsted (UK; RR), Debrecen (Hungary; DC), Clermont-Ferrand (France; CF) and Hamburg (Germany; HA).

The current heat stress response of the crop model reduces grain number when a certain threshold temperature is reached during the period 10 days before anthesis. The severity of stress is determined by the heat reduction factor, which decreases linearly to zero as the severity of the stress event increases.

The predicted grain number is therefore the product of the potential grain number and the heat reduction factor (Stratonovitch and Semenov, 2015). Grain size is then increased to a point in the model to account for potential grain size compensation, although this is not calculated from calibrated data.

6.2.3: Model output

The model provides yield projections and the effect of stress on yields as separate output, for three different periods in time: baseline, 2050 and 2090. Box plots are generated from the 18 different projections from the climate models for each location and genotype. The effect of abiotic stress is displayed through the production of Heat Stress Index HSI95. This is defined as the amount of yield loss that could be expected due to heat stress around the reproductive phases of growth, on average, once every twenty years (Stratonovitch and Semenov, 2015). Drought Stress Index (DSI95) can also be produced and defined in the same way as HSI95 for yield loss due to water limitations (Semenov et al., 2014; Stratonovitch and Semenov, 2015).

6.3: Results

Data for the calibration of anthesis and maturity for the genotypes are displayed in Table 6.2. Root Mean Square Error (RMSE) is an error value used in crop models to assess the accuracy of simulations compared to observed phenotypes, where values closer to zero show a more accurate simulation (Asseng et al., 2015). Here, maturity appears to have been more accurately simulated, whereas the accuracy of simulating anthesis time, particularly for Renesansa, is lower.

Table 6.2 : Observed and simulated days after sowing (DAS) for anthesis and maturity for 2 different genotypes of winter wheat. Calibration was based on multiple years of observed data. RMSE is Root Mean Square Error of observations.

	Renesansa		**Savannah**	
	Observed	**Simulated**	**Observed**	**Simulated**
Anthesis				
2008	210	215	225	226
2012	232	233	237	239
2014	226	221	234	231
RMSE	3.95		2.26	
Maturity				
2012	295	296	301	300
2014	282	282	287	287
RMSE	0.80		0.46	

The calibration data for the heat stress response is outlined in Table 3.3. The observed response of the two genotypes to heat stress around the reproductive phases of growth is outlined in detail in Chapter 2. There are a number of observations to be made in respect of the heat stress calibration. Firstly, severe yield loss due to heat stress only appears 9 days before anthesis (Table 6.3), which is much later than meiosis timings seen in Chapter 2. In fact, the period 9 days before anthesis observed in Chapter 2 appears relatively tolerant to stress. This therefore shows a difference in how the model is able to simulate heat stress compared to actual crop responses to stress. The model does not have a “double dip” response as was previously observed, it instead has one prolonged dip of 9/10 days.

Renesansa also appears to have a greater ability to compensate grain number losses with grain size increases in the model. This again goes against what has been previously been observed. Due to the model automatically compensating for grain number reductions with grain size increases (Stratonovitch and Semenov, 2015), Renesansa has been given a larger compensation response in the model as it has had larger grain number reductions. However, grain size compensation was observed to be variable at best and it was observed that Savannah was the genotype which has the greater ability for grain size compensations, especially around booting. The model generally shows grain number reductions to be very similar for both genotypes, whereas Chapter 2 found that they significantly differ in their response. This shows clear limitations of the models ability to accurately replicate heat stress events. Grain size compensation within the model is intrinsically linked to grain number reduction, which means the model cannot accurately reproduce different grain number/size interactions which are observed, like those in Chapter 2.

Table 6.3: Calibration data of the heat stress response of two genotypes of winter wheat in to the crop model SIRIUS. This is simulated data from SIRIUS attempting to replicate the response shown in Chapter 2.

	Renesansa			Savannah		
Day of stress (relative to GS65)	**Yield Change (%)**	**Grain Number Change (%)**	**Grain Weight Change (%)**	**Yield Change (%)**	**Grain Number Change (%)**	**Grain Weight Change (%)**
-18	-4.6695	-6.0789	1.5006	-2.3575	-2.3884	0.0316
-17	-3.8451	-5.7308	2.0004	-3.5245	-3.5763	0.0538
-16	-2.6525	-4.7018	2.1504	-3.3672	-3.416	0.0505
-15	-3.8639	-5.8961	2.1595	-3.1026	-3.1466	0.0454
-14	-3.8428	-5.7104	1.9807	-3.6971	-3.7516	0.0567
-13	-5.1313	-7.9234	3.0324	-3.4398	-3.4901	0.0521
-12	-3.866	-5.9661	2.2333	-3.7362	-3.7917	0.0577
-11	-3.8523	-5.7911	2.058	-3.7473	-3.803	0.058
-10	-3.8846	-6.0693	2.3259	-4.147	-3.7584	-0.4038
-9	-21.4994	-23.3588	2.4261	-22.6725	-22.9403	0.3475
-8	-21.7904	-23.4295	2.1406	-22.2712	-22.5315	0.336
-7	-21.9573	-23.5326	2.06	-22.4302	-22.6415	0.2732
-6	-21.5881	-23.1612	2.0472	-21.6162	-21.6855	0.0884
-5	-21.7215	-23.245	1.9849	-21.4992	-21.5647	0.0835
-4	-21.9113	-23.5307	2.1178	-22.8904	-22.6238	-0.3445
-3	-21.5586	-23.4384	2.4553	-22.4563	-22.5431	0.1121
-2	-23.4052	-24.379	1.2877	-22.5195	-22.6065	0.1125
-1	-23.2983	-24.2902	1.3101	-21.6291	-21.6968	0.0865

Yield projections for the two genotypes at the four locations are displayed in Figure 6.1. Savannah is projected to have a consistently higher yield across all four locations. Yields are expected to increase from the baseline for these two genotypes at all locations by the year 2090. This raises immediate concerns about the simulations, as numerous attempts at modelling suggest the opposite will occur (Asseng et al., 2015; Liu et al., 2016b). Hamburg is projected as the lowest yielding of the four locations, with Clermont-Ferrand simulated as the highest yielding.

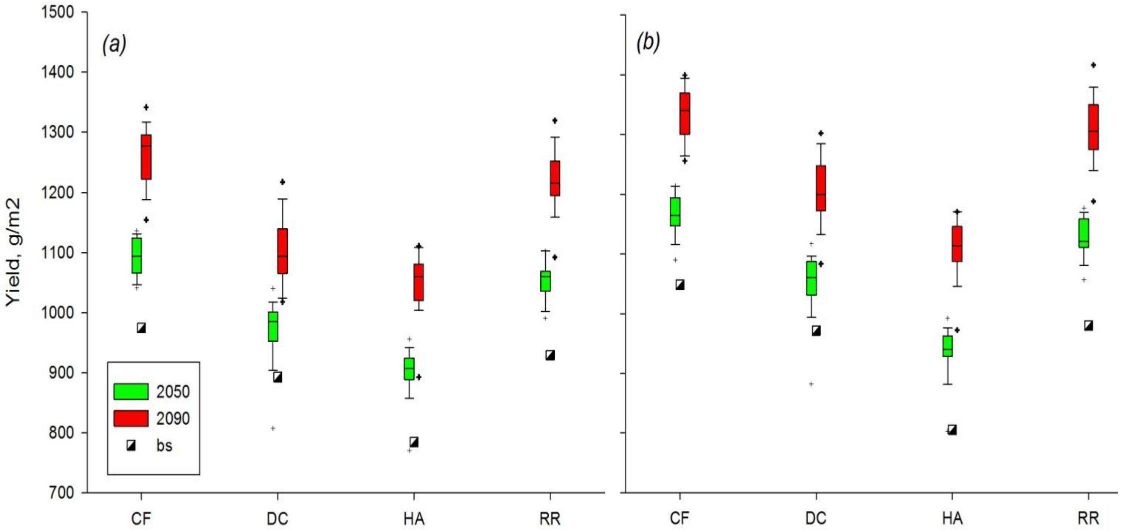

Figure 6.1: Yield projection (grams per metre squared) for Renesansa (A) and Savannah (B) across European Locations for baseline (bs), 2050 and 2090. Box plots are constructed from 18 predictions for future climate scenarios (each consists of 100 yrs of daily weather) based on 18 individual GCMs from the CMIP5 ensemble. Box boundaries indicate the 25 and 75-percentiles, the line within the box marks the median, whiskers below and above the box indicate the 10 and 90-percentiles.

Simulated anthesis dates are displayed in Figure 6.2. As sowing dates varied between the sites (10/10, 10/10, 18/10, 15/11 for RR, HA, DC and CF respectively), data is displayed as days after sowing (DAS). Thermal time to anthesis was different for the two genotypes, calibrated from the field data, although this value was assumed to remain constant for each up to 2090.

For the baseline in the UK, at Rothamsted, there is a predicted 5 day difference in anthesis date between the genotypes. This is shorter than the observed difference between the genotypes, seen to be greater than 7 days (Chapters 2, 5).

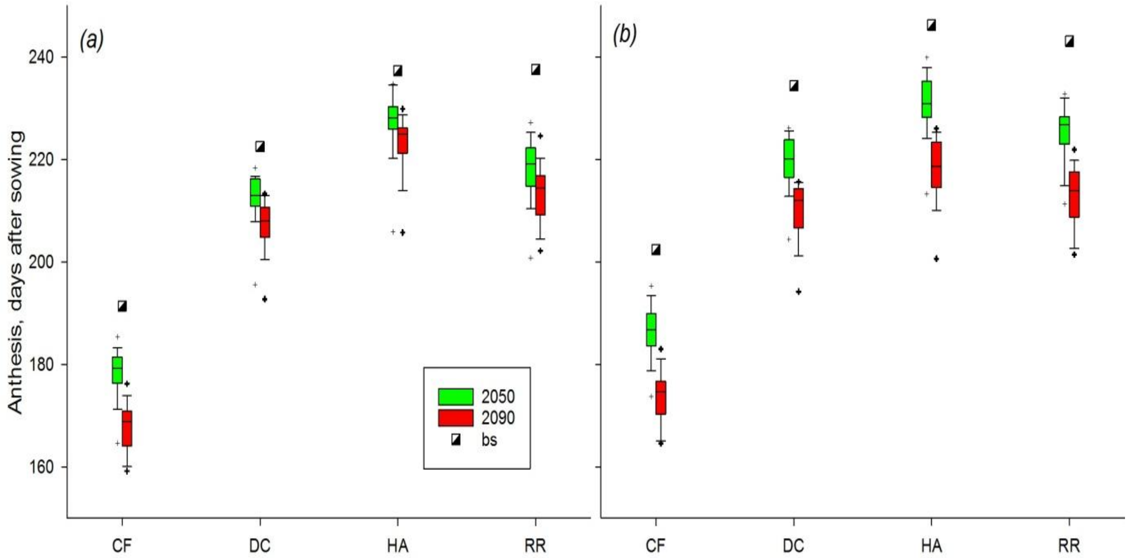

Figure 6.2: Projections of time to anthesis for Renesansa (A) and Savannah (B) across four European Locations for baseline (BS), 2050 and 2090. Box plots are constructed from 18 predictions for future climate scenarios (each consists of 100 yrs of daily weather) based on 18 individual GCMs from the CMIP5 ensemble. Box boundaries indicate the 25 and 75-percentiles, the line within the box marks the median, whiskers below and above the box indicate the 10 and 90-percentiles.

Days to anthesis shortened at all four locations for both genotypes, with the greatest reduction seen at Clermont-Ferrand. As thermal time to anthesis did not change, these reductions are explained by changes in climate anticipated by the 18 GCMs. Clermont-Ferrand was consistently the earliest location to flower, with the other three locations being similar.

Days to anthesis are likely to play a large role in determining the amount of abiotic stress each genotype faces around the reproductive phases of growth and how much it escapes. Yield loss due to abiotic stress is shown in Figure 6.3. There is no expected yield loss due to heat stress around the reproductive phases of growth in the UK, up to 2090.

Debrecen in Hungary is the location expected to experience the most yield loss due to heat stress, with a fourfold increase in yield loss due to heat stress possible there by 2090.

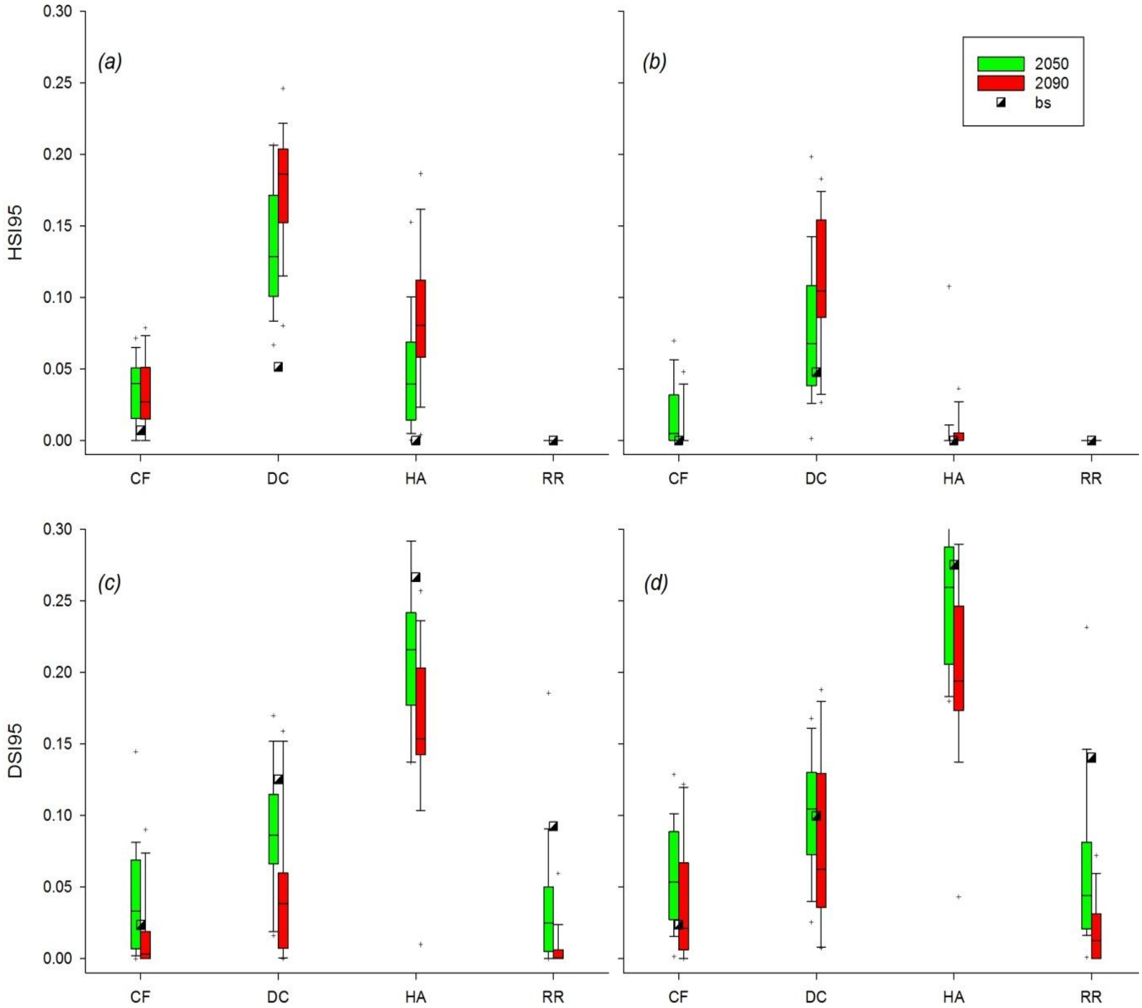

Figure 6.3: Projections of Heat Stress Index (HSI95) and Drought Stress Index (DSI95) for Renesansa (a, c) and Savannah (b, d) across four European Locations for baseline (BS), 2050 and 2090. Box plots are constructed from 18 predictions for future climate scenarios (each consists of 100 yrs of daily weather) based on 18 individual GCMs from the CMIP5 ensemble. Box boundaries indicate the 25 and 75-percentiles, the line within the box marks the median, whiskers below and above the box indicate the 10 and 90-percentiles.

HSI is consistently higher across sites for Renesansa than Savannah. This is likely because of the increased susceptibility to heat stress calibrated for Renesansa. The smaller difference in anthesis date for the two genotypes between observed and simulated means that Renesansa has not escaped as much stress as possible in these simulations. The large spread of the box plots in Figure 6.3 compared to other Figures suggests a bigger discrepancy between the GCMs when predicting high temperatures around anthesis.

Although no data was calibrated for tolerance to drought for the two genotypes, presuming they are both susceptible to drought allows for the simulation of Yield loss due to drought, displayed in Fig. 6.3 c, d. DSI95 is defined as the difference in yield between normal and water limited conditions and is not limited to water loss around the reproductive phase of growth (Semenov et al., 2014). DSI95 is above zero at all locations for both genotypes, with Hamburg being the location where yield loss due to drought is the greatest. There is no obvious difference in DSI95 between the two genotypes. DSI95 is expected to reduce at all locations by 2090, likely due to earlier anthesis times (Fig 6.2) meaning more drought periods are avoided (Semenov et al., 2014).

Consistent timings to anthesis in terms of thermal time are not a certainty. Timing of anthesis is controlled by vernalisation and photoperiod (Langer et al., 2014). These two effects delay development of the crop until their requirements are met. This means that thermal time to anthesis is not a consistent measurement, as a warm winter before vernalisation or day length requirements are met will increase thermal time to anthesis.

This measurement will vary year to year and is likely to change as average temperatures increase towards 2090. This brings in to question the predictions from the model, where thermal time to anthesis remains consistent throughout.

6.4: Discussion

In this Chapter, two European genotypes were calibrated to the SIRIUS crop model using multiple years of phenotypic data. The heat stress responses of the two genotypes were also calibrated to the model so that for the first time SIRIUS simulates European wheat genotypes as susceptible to heat stress. Yield simulations were conducted based on climate simulations from 18 GCMs up to the year 2090 and also included simulations for the amount of yield lost due to abiotic stress. This allows for an appraisal of SIRIUS as to its potential use in identifying stress tolerant genotypes for future European climates and which areas require further improvement to make SIRIUS a useful tool for plant breeders.

One problem with all output data from SIRIUS is that the production of box plots does not provide any meaningful way to statistically compare the two genotypes, relying solely on observing differences in medians and uncertainty levels through the size of the boxes. The phenotypic data collected for calibration of the genotypes to the model all came from the same UK site; however this calibrated data was then used for simulations in different European climates.

While yields were simulated to be consistently higher for Savannah, which was bred in the UK, this might not always be the case in different climates where alleles more suited to warmer climates, like *Ppd-D1a* may provide a comparative yield benefit for Renesansa (Bentley et al., 2011; Langer et al., 2014).

SIRIUS generally predicts that crop yields will increase across Europe by 2090, even with increases in abiotic stress. This generally contradicts the message gathered from other crop model studies, which suggests yields will decrease (Challinor and Wheeler, 2008; Challinor et al., 2014; Asseng et al., 2015; Stratonovitch and Semenov, 2015). This prediction is also based on the use of current genotypes, which means that the model assumes that genotypes are not currently realising their yield potential. This assumption is questionable at best based on the yield stagnation seen over recent periods (Calderini and Slafer, 1998).

This is a common problem across modelling studies where full data sets for calibration are sparse, as seen in the European based modelling study by Stratonovitch and Semenov (2015) who used data from an Asian genotype, phenotyped in the USA (Prasad and Djanaguiraman, 2014). Therefore the use of more regional genotypes in crop models like in this study, but phenotyped across more European locations would prove beneficial.

One of the key phenotypes, both in regards to heat stress and the calibration of genotypes to crop models is anthesis date. The two genotypes have been shown to differ for anthesis date by a margin of up to 10 days in UK conditions (Barber et al., 2017; Chapter 2). Here, simulations for the UK at baseline show the genotypes having a difference in anthesis date of around 4.5 days. Thermal time to anthesis is the phenotype inputted in to the model (Jamieson et al., 1998b), which means that changes in temperatures between locations and years alter the days to anthesis.

This trait becomes important when assessing for abiotic stress impacts around the reproductive phases of growth, as earlier anthesis times are less likely to be impacted by high temperature, thus escaping the stress (Lobell et al., 2015). Therefore, incorrect projections of anthesis times are likely to have an impact on the accuracy of HSI95. This is a key area of improvement needed in future iterations of SIRIUS. Whilst SIRIUS was able to replicate maturity date to a relatively high level of accuracy (RMSE < 1), RMSE for anthesis was high. Median RMSE scores for the AGMIP ensemble were below 0.1 for both anthesis and maturity (Asseng et al., 2015), suggesting that more work needs to be carried out on SIRIUS to improve the accuracy of its simulations for these two important phenotypic traits. This also highlights the importance of photoperiodism and vernalisation and their influence on the timing of anthesis (Langer et al., 2014). This is an important genotype x environment effect, which as previously stated can alter the thermal time to anthesis on a yearly basis. The model currently has no way to distinguish between photoperiod sensitive and insensitive genotypes and therefore cannot adapt genotypes and their anthesis times on a yearly basis. This is a weakness of the model which has not previously been explored in the literature and is perhaps a reason why its replication of anthesis date here, where genotypes differ for photoperiod response, is less accurate (higher RMSE) than the average seen in the AGMIP project.

Whilst the error of HSI95 and DSI95 will likely have been increased due to the smaller difference between the genotypes in anthesis date, there are some issues with the calibration of the actual stress response that require further improvement in the future.

SIRIUS describes a period of time before anthesis as susceptible to heat stress. Unlike the observed period of relative tolerance seen in Chapter 2, SIRIUS considers the whole period to be equally susceptible; where depending on the severity of stress grain number is reduced linearly. This response is consistent throughout the susceptible period in the model, i.e. there are no peaks and troughs like the observed in Chapter 2.

The heat stress response in SIRIUS described previously (Stratonovitch and Semenov, 2015) is much less accurate than the actual observed response in the crop and requires further improvement. Changes to the heat stress response made here increase that period of susceptibility from 10 days (Stratonovitch and Semenov, 2015) to 18 days. The major problem with defining the period of susceptibility as days before anthesis is that the reproductive development period is not constant and is influenced by temperature (Barber et al., 2015), so an observed 18 day period in one climate may be shortened in a different one.

This increases uncertainty in HSI95 simulations in warmer climates at different locations, as the susceptible period is likely to be larger for the simulated compared to the observed. Changing this to a thermal time based period may help in reducing uncertainty.

There was no yield loss due to heat stress observed in the UK for either genotype. Whilst the heat stress response needs further improvement, this finding was likely due to the lack of heat stress events at this location. Whilst a previous study has suggested grain yield might be affected in the UK, it did not provide any simulations which actually showed that this was the case (Semenov, 2009).

The lack of yield loss due to heat stress in the UK has also been seen in other simulations (Stratonovitch and Semenov, 2015). Here, more evidence is added to the suggestion that heat stress is not likely to be a problem in the UK. Other European locations, such as Seville have been predicted to have large amounts of yield loss due to heat stress (Stratonovitch and Semenov, 2015)

The production of DSI95 for each genotype was generated based on the assumption that both genotypes are susceptible to drought. Whilst this is likely true, having drought stress data for each genotype would have helped to improve uncertainty in the model. An issue with the production of DSI in SIRIUS is the separation of the response from heat stress. In SIRIUS, the response to both stresses around anthesis is generated separately, where one response does not affect the other (Stratonovitch and Semenov, 2015). However, multiple observations of drought and heat stress together have shown a strong interaction between the two (Barnabas et al., 2008; Alghabari et al., 2014, 2016). To provide more accurate simulations of yield losses due to heat and drought, it is likely that both responses in SIRIUS will need to interact in the future.

Modelling crop responses to stress and simulating yields is an incredibly complex process with crop models taking years to build and refine. A good example of this is SIRIUS, which was created in the 1990’s (Jamieson et al., 1998b), with responses being improved over time (Semenov, 2009; Semenov et al., 2014; Stratonovitch and Semenov, 2015). One emerging method to reduce uncertainty in simulations is the use of model ensembles (Challinor et al., 2014).

One of the most well known ensembles is the AGMIP project, combining 28 crop models to produce global simulations of yield (Asseng et al., 2013, 2015; Makowski et al., 2015; Liu et al., 2016a). However, ensembles of models require a large amount of collaboration and are not always possible, so in order to make models practical to use for plant breeders and researchers individual models need to be improved.

The prediction here that wheat production is set to increase in these cultivars by 2090 goes against a large amount of work in different models (Asseng et al., 2015) which predict reductions in global production. It also goes against more recent predictions by SIRIUS using different genotypes which suggest overall yield reductions (Semenov et al., 2014; Stratonovitch and Semenov, 2015). These findings likely differ to previous work due to the relatively small geographical area this study was conducted in, where stress events aren't predicted to be that common (Semenov and Stratonovitch, 2010), hence lower yields do not appear. If more locations were to be included in this trial, such as Seville, overall yield predictions may be seen to be lower. In the last simulation run by SIRIUS, Debrecen was the only site used here which was predicted to be largely impacted by stress. Another possible reason for the apparent increase in yields this is the over compensation of grain size for any grain number reductions. The model automatically increases grain size when numbers are reduced (Stratonovitch and Semenov, 2015), however Chapter 2 shows this response is very variable and at best limited in these genotypes. Therefore the effect of heat stress on yield is not as great as it should be. Finally, SIRIUS allows for an increase in biomass when CO_2 concentrations are increased, however no data has been provided from these genotypes to prove this is the case.

This is a fundamental relationship in the model (Jamieson et al., 1998b); without any calibration from phenotypic data from the genotypes, there cannot be any guarantees it is accurate.

For SIRIUS, there are a number of steps which are achievable and can make an improvement to the models simulations under heat stress. Firstly, improved calibration data through detailed heat and drought stress responses, as in Chapter 2, as well as general phenotypic data from different European sites to allow for the model to better simulate a genotype across different locations. Secondly, changing the susceptible period to heat stress from days before anthesis to a range given in thermal time would allow for flexible stress durations as climates differ and improve the accuracy of HSI95 projections. Improving the heat stress response from one period of susceptibility with the same heat stress effect to a stepped approach, with a period of relative tolerance around heading, would also improve the heat stress response. Further understanding within the model of genotype x environment effects, such as vernalisation and photoperiod and their relationship with flowering time is needed in order to improve the accuracy of predicting key phenotypes. Understanding these processes is key in order to then predict how such phenotypes are likely to change in different genotypes in future climates. However, such fundamental alterations to the model in order to improve this will require a large amount of work. Ideally, increasing the number of simulations through increasing site numbers in the model or the number of models used to produce simulations would improve the overall quality of the simulations. However, this would be a costly and lengthy process and wouldn’t be practical to achieve for smaller projects.

6.5: Future work on the model

Following on from the discussion, a number of key areas which require improvement in the model in order for it to become a practical tool for researchers and plant breeders are highlighted. These are areas which could not be addressed in the time frame of this project:

- Improve the accuracy of anthesis date simulations, a key phenotype in the model
- Convert the model from “days after sowing” to thermal time measurements for phenotypes, to allow for betting simulation of seasonal effects
- Allow the model to start simulating different photoperiods, this would allow for much more accurate anthesis date simulations in changing climates
- Further improve the heat stress response to start simulating two discrete periods of susceptibility to heat stress in wheat
- Reduce the compensatory effect of grain size during heat stress events, as it is overstated in the model compared with observations seen in chapter 2

6.6: Conclusions

- Overall grain yield is predicted to increase at all four European locations by 2090, likely due to three key factors:
    - Predominantly Northern European locations being used, where stress events aren't expected to occur as frequently as elsewhere in Europe and globally. Increasing the number of sites used in simulations may have shown a trend where grain yield is reduced
    - Over predictions of grain size compensation within the model increasing yield under heat stress
    - The model over assuming the increase in biomass accumulation in high CO_2 conditions
- Time to anthesis, in terms of calendar days, is predicted to decrease. However, this prediction does not account for the increase in thermal time likely to occur in these genotypes due to warmer winters before vernalisation and photoperiod requirements are met
- Heat stress is not predicted to play a major role in the UK, even by 2090. Heat stress is predicted to play a larger role at other European locations, notably Debrecen
- Yield loss due to drought stress is predicted to reduce across Europe, due to earlier anthesis times
- More work is needed on the heat stress response in SIRIUS and improved calibration data from multiple locations in order to improve accuracy of the model to provide a practical tool to identify beneficial genotypes and QTL in future climates

Chapter 7: Discussion

7.1: Thesis Overview

The main objective of this study was to characterise the timing of discrete growth stages in wheat that are susceptible to heat stress. Further objectives of the research included contrasting responses of different wheat genotypes, alleles and QTL to heat stress at those growth stages; assessment of development, growth and yield characteristics of a Doubled Haploid population and genotyped alleles with parents from northern and southern Europe; and using the SIRIUS crop model to predict yield and yield stability of different genotypes, alleles and QTL in climate change scenarios for the UK but also elsewhere.

The two parent genotypes of the Savannah x Renesansa DH population were heat stressed at 31 separate timings across the whole reproductive phase of growth to identify growth stages susceptible to heat stress and identify whether the genotypes differed in their response to heat stress. Subsequently, the whole DH population was stressed at the timings identified during earlier experimentation to assess for genotypic effects on heat stress tolerance. A field trial was conducted as part of this study to identify tolerant traits that perform well in the field as well as an appraisal of the crop model, SIRIUS, and its ability to simulate heat stress events for the parent genotypes.

The four main hypotheses tested in this project were:

- There are varietal differences in timing and severity of susceptibility to heat stress
- Southern European alleles of relevant adaptive alleles increase tolerance to heat stress
- Southern European alleles have a negative impact on yield in non-stressed, Northern European conditions
- There will be differences in yield predictions and yield loss due to stress between different genotypes from the crop model SIRIUS when predicting yields in future climates across Europe

7.2: Timing of Heat Stress Events

The experiment conducted in Chapter two identified two periods of susceptibility to heat stress across two genotypes, Savannah and Renesansa. The first significant reduction in mainstem fertility was observed at around 18 days prior to mid-anthesis, with a second susceptible period found a day before mid-anthesis. The two genotypes did not differ in the timing of the two periods of susceptibility. There were significant differences between the genotypes in respect of the severity of damage due to heat stress, as well as variation in grain size compensation following damage to the reproductive apparatus. Savannah was found to be most susceptible around booting, while Renesansa was more susceptible at anthesis.

Periods of susceptibility found here are broadly consistent with existing observations for both booting (Saini and Aspinall, 1982; Alghabari et al., 2014) and anthesis (Ferris et al., 1998; Barnabas et al., 2008; Alghabari et al., 2014; Prasad and Djanaguiraman, 2014).

The double dip response that was observed in this study is similar to that described in rice (Satake and Yoshida, 1978, 1981; Craufurd et al., 2013). To the authors' knowledge this is the first time this response has been described in wheat and the first time the response has been parameterised through the use of non-linear models. Whilst numerous studies have conducted heat stress experimentation on wheat at either booting (Saini and Aspinall, 1982; Alghabari et al., 2014) or anthesis (Ferris et al., 1998; Prasad and Djanaguiraman, 2014), this study is unique in its use of multiple, short-duration, stress events to investigate the response across the whole reproductive period of growth. The only other attempt to describe the full reproductive period response to heat stress was by Prasad and Djanaguiraman (2014), however, their study found no clear evidence of fertility reduction around booting. The use of 1 day stress duration allowed for the investigation of more discrete growth stage effects which are often lost when stress periods of 3-5 days are used (Satake and Yoshida, 1978; Prasad and Djanaguiraman, 2014). This finding also highlights the fact that genotypes have different susceptibilities at different stages, making it important that both periods are analysed when assessing genotypes for tolerance, rather than just stressing one timing as numerous previous studies have done (Saini and Aspinall, 1982; Ferris et al., 1998)

The first susceptible period found, occurring around booting, likely coinciding with meiosis (Zadoks et al., 1974b). Data suggests that the peak susceptibility occurred at slightly different booting timings in different genotypes, with the peak aligning at early booting (GS39-41) for Savannah and mid booting for Renesansa (GS43-45). It is possible that meiosis occurs at different growth stages in the genotypes as it has been shown not to align with a specific growth stage (Zadoks et al., 1974b; Barber et al., 2015).

This highlights the importance of short duration stress when aiming to observe discrete differences between genotypes and ensure that meiosis is stressed. Finding a period of susceptibility just prior to mid anthesis, described as 50% of ears extruding anthers (Zadoks et al., 1974b), is in line with other previous research (Ferris et al., 1998; Prasad and Djanaguiraman, 2014). The alignment of the peaks just before this stage was likely due to the self pollination within florets before the anthers extrude, which accounts for the majority of pollination in wheat (Lukac et al., 2012). By showing that genotypes differ in response to heat stress, as well as by confirming the susceptibility of wheat to heat stress at two key stages, we provide justification to explore genotypic reasons for these differences within a DH population of Savannah x Renesansa.

7.3: Comparing Heat-Tolerance of Genotypes from Across Europe

It has previously been suggested that the semi dwarfing allele *Rht8* can increase tolerance to heat and drought conditions, due to its prevalence in Southern Europe (Worland et al., 1998b) and greater establishment and early growth traits in dry conditions (Rebetzke et al., 2007; Wojciechowski et al., 2009).

However, actual evidence for this assertion is limited (Alghabari et al., 2014, 2016). One of the overriding aims of this project was to compare alleles from northern and southern Europe for their influence on heat stress tolerance. This research has found no evidence to support previous suggestions that *Rht8* increases tolerance to heat stress (Law and Worland, 1985). To the contrary, the GA insensitive allele *Rht-D1b* was associated with increases in tolerance to heat stress. Whilst *Ppd-D1a* from Southern Europe was found to increase susceptibility to heat stress, a QTL from Renesansa on chromosome 2A was found to improve heat stress tolerance.

The reductions in the number of heat stress events from 31 in 2013/14 to 3 in 2014/15 allowed for a higher throughput approach in respect of the number of genotypes screened, giving good data for allele associations and the identification of the QTL on 2A. The findings for the two *Rht* alleles contradicts previous studies, including preliminary work by Law and Worland (1985), which suggested the difference in GA sensitivity between *Rht-D1b* and *Rht8* would lead to differences in heat stress tolerance. This finding also contradicts findings in different genetic backgrounds by Alghabari et al (2014) in heat and drought environments and is a unique finding for this study.

For the first time, this study has shown that a key allele from Southern Europe (Bentley et al., 2011) thought to associate with escape from heat stress through reductions in thermal time to anthesis (Gomez et al., 2014; Langer et al., 2014) and increases in flowering duration (thought to reduce risk of all flowers being stressed; Jones et al., 2016), actually increases susceptibility to heat stress.

This finding goes against the original hypothesis, but offers an explanation of the observed differences in heat stress tolerance between Renesansa and Savannah at anthesis seen in Chapter 2.

A QTL has previously been identified on 2A for influencing heat stress tolerance (Mason et al., 2010) and this project adds further evidence that this chromosome is of interest in relation to heat stress tolerance. The protecting allele being from Renesansa does support the original hypothesis, suggesting that even in a southern European genotype that performed poorly overall, there are traits in the genotype that aid with tolerance to heat stress. A new QTL found here adds further evidence to the importance of chromosome 2A for heat stress tolerance, and highlights the need for further analysis of this chromosome to improve our understanding of heat stress response.

7.4: Allele Associations in UK Field Conditions

A field trial was conducted in 2013/14 to assess the Savannah x Renesansa DH population in the field and identify associations of previously genotyped alleles with yield. With respect to the genotyped alleles from southern Europe, *Rht8* was associated with reductions in total PAR interception, biomass and grain yield. There was a lack of allele associations for *Ppd-D1a*. While for the northern European alleles, *1BL/1RS* was associated with increases in total PAR interception, but not increases in total biomass or grain yield. *Rht-D1b* was associated with an increase in HI, but not grain yield.

The observed associations for *Rht8* are in agreement with those observed in a Paragon background (Kowalski et al., 2016). The lack of evidence to show *Ppd-D1a* is associated with reductions in thermal time to senescence or total grain yield was interesting and contrary to previous findings from the same location (Addisu et al., 2010).

The lack of phenotypic associations with *Ppd-D1a* in this trial was possibly due to the flooding encountered in the winter of the field trial, as *Ppd-D1a* has been associated with early plant growth in previous studies (Addisu et al., 2009), meaning that stunted growth made it difficult for differences to be observed. However, growing conditions could have simply suited earlier genotypes, meaning genotypes containing *Ppd-D1a* within the population thrived.

For the first time, this research associates *1BL/1RS* with increased PAR interception, however this study cannot confirm previous findings of an increase in grain yield associated with the allele (Zhao et al., 2012). The lack of yield associations with *Rht-D1b* in the field was interesting as this allele has been observed to increase grain yield in multiple genetic backgrounds and locations (Flintham et al., 1997; Chapman et al., 2007; Addisu et al., 2010), again highlighting the unusual nature of the year for this field trial. However, there is some evidence to suggest that the presence of *Rht-D1b* does not always provide a yield benefit in the UK and these finding provide some evidence to support that.

Overall, the findings support the hypothesis, that *Rht8*, a southern European allele, associates with reduced grain yield in UK field conditions. Findings in this trial, or rather the lack of them in some cases, highlight the importance of repeated field trials, a limitation of the project. This project was limited to one year of field trials for a number of reasons.

Time and financial constraints of conducting a second year of trials during 2014/15 was deemed unmanageable due to the increase in size of the heat stress trials in controlled environments. Further to this, the Savannah x Renesansa DH population had previously been grown at Sonning over a number of seasons (Addisu et al., 2009, 2010; Gooding et al., 2012b).

Further analysis of allele associations with thermal time, PAR interception, growth stage and yield can be conducted by combining data from this project with previous, unpublished, data from Sonning. This will be published as a paper in conjunction with collaborating authors. Assessments of the heat tolerant QTL found on chromosome 2A should also be conducted in the field in future projects to assess whether this QTL would be beneficial in UK field conditions as well as increasing tolerance to heat waves.

7.5: Yield Simulations of Two Genotypes Using the SIRIUS Crop Model

Calibration of the SIRIUS model showed a relatively high Root Mean Square Error (RMSE), a measure of error commonly used in crop modelling (Asseng et al., 2015; Pirttioja et al., 2015), for anthesis date and maturity, compared to the ensemble Figures collected for the AGMIP project (Asseng et al., 2015). The model appeared to struggle to accurately simulate anthesis date for Renesansa in UK conditions.

This raises questions over the validity of simulations in future climates produced by the model. Anthesis date is a key phenotype as the timing of this in the model determines the quantity of heat stress events the crop is subjected to, with later anthesis dates being subjected to more stress events.

The influence of flooding on the data must be acknowledged, although RMSE remained relatively high considering two additional years data, which weren’t subjected to flooding were used to calibrate the genotypes. The heat stress susceptibility period in the model was extended from the original 10 days prior to anthesis (Stratonovitch and Semenov, 2015) to 18 days. Simulations conducted at four European locations suggest that heat stress is unlikely to be a problem this century in northern Europe.

However, yield loss due to heat stress is likely to increase in central and southern Europe. This study has enabled SIRIUS to simulate heat stress susceptibility in European genotypes for the first time, as the model had previously assumed genotypes to be tolerant to heat stress.

There still needs to be a large amount of work conducted on SIRIUS to enable it to be a practical tool for plant breeders and researchers in the future. Although current calibration standards proved to be adequate for general forecasting of long-term trends, improvements need to be made on the model in respect of the accuracy of its representation of anthesis timings and heat stress response. This will enable the model to become a useable tool to directly compare different genotypes in future climates. Currently, the model does not accurately simulate the differences in heat stress response between different genotypes, as shown in this project.

Although the differences between genotypes are often relatively small and discrete, both in terms of timing and severity of stress (Alghabari et al., 2014; Barber et al., 2017), seemingly small differences in heat stress response can often cause large changes in yield at the field level. In doing so, populations such as the DH population used here could be calibrated and output data from the simulations could potentially be used to identify QTL that perform well in future climates within the model.

It is worth noting however, that SIRIUS is not the only model that struggles to simulate small differences between genotypes. Four models compared by Liu et al (2016) differed vastly when attempting to simulate the heat stress response of two genotypes.

The current approach used by modellers is to reduce uncertainty in yield projections by using model ensembles (Asseng et al., 2013, 2015; Liu et al., 2016b). However, in order to provide a practical tool to researchers, more focus should be placed on reducing uncertainty through improved individual model responses rather than ensembles.

7.6: Limitations of the study

One limitation, which is not unique to this study, is its use of controlled environments. The study extrapolates results from pot experiments to field conditions in order to quantify yield loss due to heat stress and identify traits beneficial for future climates. Here, there was clear evidence of an experimental artefact: a transfer effect of moving pots in and out of controlled environment chambers, observed through lower grain yields as the experiment in Chapter two progressed. It would have been beneficial to quantify the response to heat stress in a field environment.

However, unless the heat stress is imposed in field conditions, this is a limitation in the majority of heat stress experimentation conducted globally for a number of crops (Satake and Yoshida, 1978; Alghabari et al., 2014; Prasad and Djanaguiraman, 2014).

Although Ferris et al (1998) managed to conduct an elevated temperature experiment in the field, their methodology is considered very challenging to apply successfully in UK field conditions. This method involved the use of elevated temperatures in polytunnel's placed in the field (Ferris et al., 1998), a costly method when applied to a fully replicated field trial. It would also not be a practical option when screening 62 lines for heat stress tolerance, costing far too much in terms of labour and materials.

Whilst the use of one day stresses provided discrete detail in terms of growth stage interactions with heat stress response, the use of them did not come without their own limitations. The use of one day stresses meant that a high temperature regime had to be implemented. This short, sharp stress is not particularly representative of what would be expected in the field (Lukac et al., 2012), thereby causing some doubt as to how these responses would replicate in field conditions.

Alternatively, a greater understanding of how observed heat stress responses in controlled environment experiments translate in the field would prove to be useful. Even though conducting a heat stress experiment using a large number of genotypes in controlled environments was preferable to the field, it did not come without its limitations. Even by reducing the number of timings to three, the management of such a large scale experiment was at times difficult.

The second timing, mid booting, was particularly difficult to anticipate, as genotypes did differ in time taken to move from early to mid booting, meaning not all genotypes were stressed at that target GS. Furthermore, harvesting and data processing of the grain data proved laborious.

This laborious process limited the amount of detail that could be extrapolated from the experiment for studying heat and drought interactions in the population, which may have been beneficial when studying the crop model output. Although the capability of conducting a drought experiment in conditions where root systems are in a sub optimal state would have been questionable at best. In respect to the field study, the flooding over winter demonstrated the need for repeated field trials in agricultural experiments.

However, while there were financial and time constraints due to the controlled environment experiment, previous experimental series at Sonning using the population provided repeat measurements. This allowed for improved calibration of Renesansa and Savannah to SIRIUS and will enable for improved data when attempting to publish the experiment.

Crop modelling was limited by the number of genotypes that were able to be calibrated to the model. By only calibrating the parents of the DH population, identifying beneficial traits for future climates within the population was not possible.

The modelling was partly limited by the difficulty in replicating phenotypes such as heat stress response and anthesis date (as discussed previously), but also by the length of time it takes to successfully calibrate a genotype to the model.

Calibrating the parents to the model took approximately one week, so calibrating the entire DH population (60 lines), was not practical.

7.7: Future work

There is potential for the crop model SIRIUS to become a useful tool for both researchers and plant breeders in the future, to identify beneficial ideotypes and genotypes in future climates across Europe. There is a clear need for calibration data for genotypes to be provided at different locations across Europe, to improve the accuracy of simulations across Europe as the model is currently unable to accurately predict how genotypes will change under different conditions. There is still a need for the heat stress response of the model to be improved. Here, the model was not able to simulate a detailed dataset accurately. In order to be used as a tool to identify beneficial genotypic traits in the future, this will need further improvement.

Converting the susceptible period in the model from its current format of days before anthesis (Stratonovitch and Semenov, 2015) to thermal time, would enable the model to shorten the susceptible period, in day terms, as the climate warms, which would be expected. Refining the model to show discrete differences between genotypes, in terms of general phenotypes like anthesis date, as well as heat stress responses would allow for the model to be used to identify potential beneficial attributes for the future (Semenov, 2009; Stratonovitch and Semenov, 2015).

Whilst *Rht8* has not shown any influence on heat stress tolerance, a new QTL identified here on 2A warrants further study, both for its heat stress traits and performance in UK field conditions.

This QTL adds further evidence to the importance of 2A for heat stress tolerance and could potentially be studied in a smaller NIL population, allowing for a direct analysis of the effect of the QTL on yield in a future project. Work could then be focussed both in controlled environments for heat stress response, as well as how the QTL performs in a smaller field trial.

Using the higher throughput heat stress method developed here, using shorter, more focussed stresses, would enable future projects to screen larger populations for heat stress tolerance. This method was only able to be developed due to the detailed work mapping the discrete periods of stress in chapter 2.

7.8: Concluding Remarks

This project adds clarity to the susceptibility of wheat to heat stress at different growth stages, identifies key alleles and QTL associated with heat stress tolerance, identifies their performance in UK field conditions and attempts to quantify the effect of heat stress using these genotypes in future climates. In doing so, this project has added information aimed at improving food security in an uncertain future.

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Appendix 1

The following pages contain the publication:

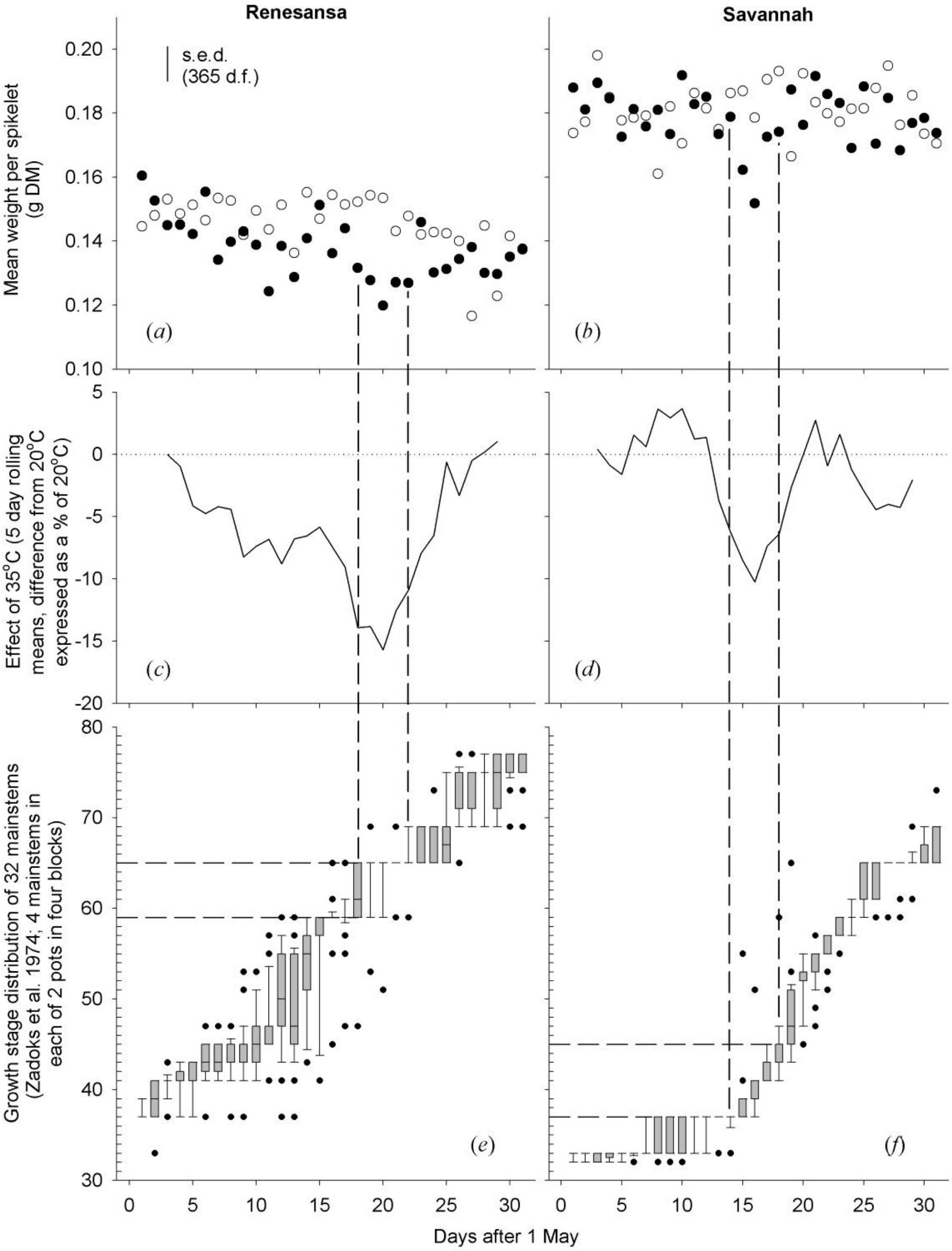
Barber HM, Lukac M, Simmonds J, Semenov MA and Gooding MJ (2017). Temporally and Genetically Discrete Periods of Wheat Sensitivity to High Temperature. *Front.PlantSci.* 8:51. doi: 10.3389/fpls.2017.00051

Appendix 2

The following pages contain the publication:

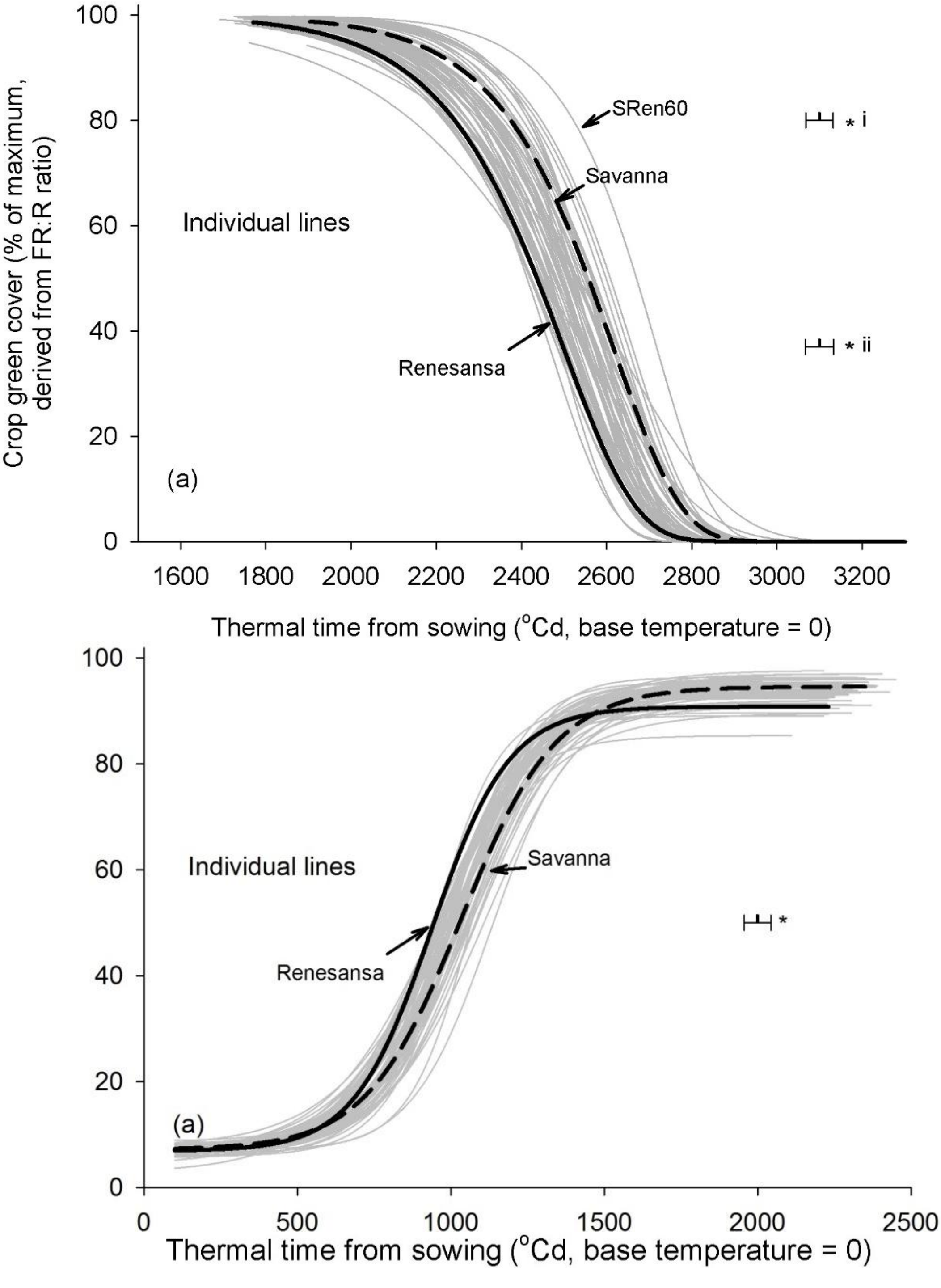
Barber, H. M., Carney, J., Alghabari, F., & Gooding, M. J. (2015). Decimal growth stages for precision wheat production in changing environments? *Annals of Applied Biology*, *166*(3), 355–371.

Appendix 3



Effects of wheat cultivar and successive 1-day transfers to controlled environment cabinets at 20/15°C (Clear circles) and 35/30°C (filled circles) day/night temperature (16 h day) on mean weight per spikelet of main stems. (E) and (F) give the growth stage distributions of the main stems at the time of transfer in to the cabinets (boxes are limited by 25 and 75 percentiles, whiskers by 10 and 90 percentiles; points are outliers beyond 10 and 90 percentiles, and the line within the box is the median where appropriate). SED in (A) is for comparing temperatures within day and cultivar for both (A) and (B). Dashed lines correspond to days and growth stages denoting the most susceptible 5-day period to 35◦C for each cultivar. Extracted from Barber et al (2015).

Appendix 4



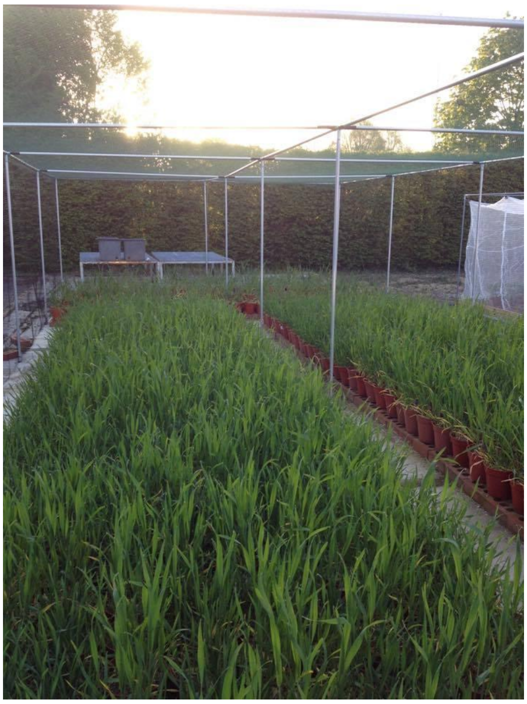
RF:R measurements for all lines showing senescence (modified Gompertz). B) PAR measurements for all lines until senescence (logistic curve). Each line on both graphs represent one of the genotypes of the population, with the parent lines highlighted in bold. S.e.d in a and b is used for comparing individual lines thermal time to max PAR interception. Graphs are averages for lines over 3 seasons and are provided as a representation of the model described in Chapter 6. Data from 2013/14 was extrapolated and used in Chapter 6 to identify PAR and TT relationships as well as allele associations.

Appendix 5

The following contains images taken from throughout the project.

A Swan highlights the extent of the flooding during the 2013/14 field trial at Sonning, UK.

2014/15 pot experiment at sowing, split in to four blocks, protected by a net against birds.



Dawn rises over the 2013/14 Savannah Renesansa Controlled environment experiment.

Pots after washing ready to be filled with potting mixture.

The 2014/15 heat stress experiment after sowing.

The extent of the flooding damage over winter at Sonning, early 2014. This image is looking towards the river Thames.

Sowing the Savannah Renesansa heat stress experiment during a typical British day, December 2013.

Harvesting the 2013/2014 heat stress experiment, July 2014. Pictured (L-R): Henry Barber, Caroline Hadley, and Jake Bishop.