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Exploiting resource use efficiency and resilience traits in ancient wheat species

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1. Abstract

Globally, 590 million metric tonnes of wheat are produced each year, however by 2025, the estimated requirement is projected to be 840 million tonnes (Murchie *et al*., 2009). Over the course of the 20th century, wheat yields notably increased due to improved cultivars from breeding schemes. However, these plants are often reliant upon the established environmental conditions in which they were bred (Calderini & Slafer, 1998; Frederick & Bauer, 1999). Further genetic enhancements in modern wheat are thought to be somewhat limited, since it has been hypothesised that early domestication and focussed breeding schemes have reduced the allelic variance within the modern wheat gene pool (Peleg *et al*., 2005). This makes it increasingly difficult to select for novel plant types that are able to resist biotic and abiotic stresses (Sparkes, 2010), consequently reducing overall productivity through drought, salinity, temperature and nutrient imbalances (Trethowan and Mujeeb-Kazi, 2008). It is therefore imperative that the modern bread wheat (MBW) gene pool is supplemented with more variance, and it is thought that this will most likely derive from external sources (Trethowan & Mujeeb-Kazi, 2008).

Ancient wheat species form a conduit between wild ancient wheat and cultivated *Triticum* species, and may harbour the genetic variation to supplement the modern bread wheat gene pool. The current work investigated a range of morphological and physiological aspects of several ancient wheat species including several representatives of spelt, emmer and einkorn. These were compared to modern bread wheat in the two field and three glasshouse experiments with the aim to investigate their resource use efficiency, where radiation use and water use formed the crux. The main components of the current work relate to 1) canopy interception characteristics 2) leaf photosynthetic capabilities and 3) water use. There was variation for a number of traits across the cultivars assessed and all species displayed favourable characteristics with respect to resource acquisition of radiation and water. Spelt genotypes demonstrated increased WUE and green area longevity compared with modern bread wheat. Emmer displayed increased WUE, assessed on three scales using instantaneous transpiration efficiency (ITE), biomass to water uptake ratios, and carbon isotope discrimination ($\Delta^{13}C$). In addition, the mechanisms whereby emmer, einkorn and spelt maintained ITE differed. Emmer was observed to increase photosynthetic rates, whereas spelt maintained low transpiration as a result of low stomatal conductance. Einkorn however, maintained ITE through an intermediate of both of these mechanisms. This was further supported by species differences for maximum photosynthetic rates (A_{sat}) which, for emmer and einkorn, were comparable with modern bread wheat. Investigation of WUE through $\Delta^{13}C$ and biomass production to water uptake ratios ranked species similarly, showing emmer and spelt to have superior WUE during grain filling. Additionally, spelt was observed to produce biomass comparable to modern bread wheat, thought to be due to enhanced RUE (observed in one field trial) or increased green area longevity rather than increased assimilation capability. In field experiments, biomass production and light interception was relatively high for einkorn species, however this was

believed to derive from excessive tiller production due to poor emergence. Overall, ancient species did partition a larger proportion of assimilates toward tillers. Modern bread wheat produced fewer tillers, but directed more biomass towards the ear, and therefore had greater harvest indices (HI) compared to all ancient species.

Despite this broad analysis, further investigation of the mechanisms responsible for these traits is required. The current work however does suggest that stability, survival and duration may be prioritised over productivity in the ancient wheat growth cycle, and that there is sufficient variation for these traits with some genotypes indicating increased RUE, WUE and leaf longevity; these genotypes therefore warrant further exploration. With further investigation, resource capture and utilisation efficiency, and the morphological traits that confer these advantages in these genotypes, genetic markers could be identified with the aim to introduce valuable traits for the production of novel modern bread wheat varieties. The differences observed highlight potential successful adaptations conferring resource use efficiency between these ancient wheat species and modern bread wheat which could provide an opportunity through which modern wheat gene pools may be supplemented to improve yield productivity and stability, particularly in sub-optimal environmental conditions, thus increasing biomass production per unit resource, thereby enhancing the productivity and the efficiency of crop systems.

2. Introduction

2.1 Global food demands and changing agricultural requirements

Recent projections indicate that global populations will grow from 6.9 billion to over 9.0 billion by 2050 (Lutz *et al.*, 2001). Provision for this growing population is heavily dependent on the agricultural sector (Peleg *et al.*, 2005) and so far, cereal productivity has kept pace with population growth (Araus *et al.*, 2002). Globally, 590 million metric tonnes of wheat are produced each year, however by 2025, Murchie *et al*. (2009) estimates that this requirement will increase to 840 million tonnes. In many regions, the demand for wheat currently outweighs the gains made through breeding by targeting genetic yield potentials; currently these gains are estimated to be 1% per annum, highlighting the requirement to shift the current genetic frontiers in wheat breeding (Reynolds *et al*., 1999).

Throughout the 20th century wheat production has increased a great deal (Araus *et al.*, 2002) however, current rates of yield progress are not sufficient if the demands of expanding global populations are to be met. Further complications arise as a result of increasing energy, water and climate crises, which mean that the production efficiencies of agriculture must increase, particularly relating to the transduction of externally acquired resources such as radiation, water and nutrients into biological yields (Murchie *et al.*, 2009) as these can be a major constraint to plant productivity, accounting for a large proportion of crop losses (Kramer & Turner, 1980; Peleg *et al.*, 2005). The situation is further exacerbated by unpredictable weather associated with changes in the physical global climate (Ewert & Pleijel, 1999) as well significant reductions in crop-land per capita (Hoisington *et al.*, 1999) and soil losses (Davies *et al.*, 2009; Murchie *et al.*, 2009). In the past, high production requirements have encouraged intensified cropping systems that typically deplete water and soil resources (Hardin, 2008), however the focus is shifting towards ‘sustainable intensification’, which aims to achieve intensive production whilst also minimising the environmental impacts. A plausible avenue through which to do this would be to increase crop yields per unit area, whilst also reducing input resources such as fertiliser, water and energy (Cassman, 1999; Hoisington *et al.*, 1999). Therefore identification and understanding of the complex processes that regulate yield will aid the selection of higher-yielding cultivars which possess an increased proficiency to mine available resources is essential (Cassman, 1999; Frederick & Bauer, 1999).

2.2 Wheat production and agriculture

Triticum aestivum (modern bread wheat) production accounts for 200 million hectares of agricultural land and 21% of caloric intake for global populations (Ortiz *et al.*, 2008). Wheat yields have increased remarkably throughout the past century with average gains of 250% (Calderini & Slafer, 1998). Breeding improvements have primarily focused on plant morphology, achieved through the manipulation of several major genes (most notably *Rht*, *Ppd* and *Vrn* genes to alter plant height, photoperiod and growth habit, respectively) (Figure 2.1) (Fischer & Edmeades, 2010; Skovmand *et al.*, 2001). Collectively, these altered partitioning, boosted light interception and Harvest Index (HI), which refers to the ratio of economic yield or harvestable yield, relative to the total biomass produced (Hibberd *et al.*, 2008). This has momentously improved productivity and yield stability (Araus *et al.*, 2002; Murchie *et al.*, 2009). Additionally, these changes have improved plant responsiveness to agricultural inputs, incidentally also allowing greater fertiliser applications. The exact genetic gain in grain yield potential is unknown although it is likely that breeding has dramatically altered the physiological processes that impact yields (Frederick & Bauer, 1999).

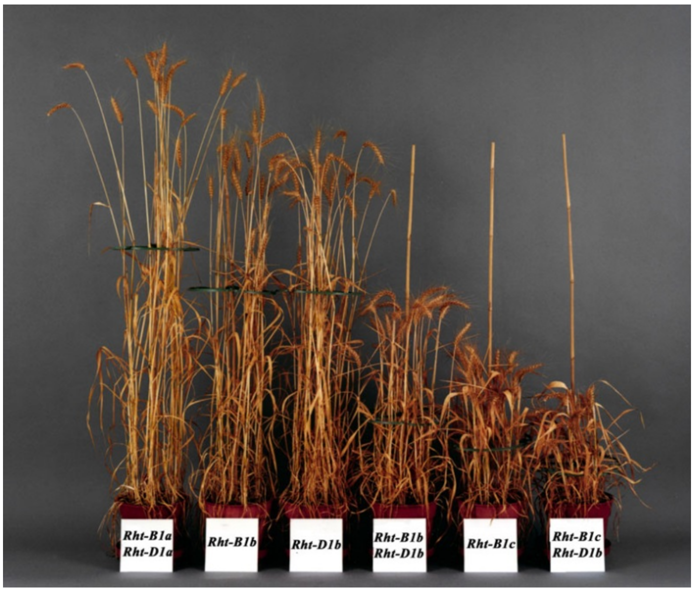


Figure 2.1. The effects of different *Rht* alleles on plant height in wheat (cv. April Bearded). The wild-type contains *Rht-B1a* and *Rht-D1a*, which are homologous (corresponding) genes on the B and D genomes. *Rht-B1c* is a more severe allele at the *Rht-B1* locus. Image origin unknown, however the wheat mutants *Rht-B1b* and *Rht-D1b* were described in Peng *et al.* (1999).

By identifying and targeting those factors that impact yield, such as nutrient uptake, metabolism, photosynthesis and respiration, carbon partitioning, leaf senescence and plant water relations, physiologists have been able to boost yield production and stability (Frederick & Bauer, 1999).

2.3 Ancient wheat: a historical perspective

Bread wheat (*Triticum eastivum* L.) has derived from several progenitors with varying cytogenic classifications (from diploid to hexaploid) originating from the Fertile Crescent, all of which contributed to the hexaploid AABBDD genome. It is this hexaploid (*Triticum aestivum*) that forms the crux of wheat cultivation today (Salmini *et al*., 2002). The first wheat species to be successfully cultivated (a diploid) was *T. monococcum* (einkorn) (initially derived from *T. boeticum*) (Salamini *et al.*, 2002) from which the A genome originates (Campbell, 1997). Einkorn cultivation was abandoned for the tetraploid, *Triticum dicoccoides* (wild emmer) (AABB), later selectively bred to produce *Triticum dicoccum* (emmer) (AABB). *Triticum dicoccum* had traits that made it more suitable for larger scales of production such as free-threshing ears. Emmer was eventually replaced by *Triticum spelta* (spelt), resulting from a cross between *T. turgidum* and *Aegilops squarrosa* (Pagnotta *et al.*, 2005; van der Veen & Palmer, 1997) (Figure 2.2). Finally, following accidental crosses between *Triticum dicoccoides* (domesticated emmer) and *Aegilops tauschii* (a wild diploid species) (D), free-threshing hexaploids (*Tritcum aestivum*) (AABBDD) were produced which marginalised previously cultivated species (Figure 2.3 shows the impact of these genetic alterations made on ear appearance). Today, these form large-scale homogenous systems that produce far greater yields. It is now thought that only 3% of cultivable land accounts for landrace cultivars (Reif *et al.*, 2005). *Triticum aestivum* has no wild progenitors with hexaploid genomes (Salamini *et al.*, 2002). It is this hexaploid (*Triticum aestivum*) that forms the crux of wheat cultivation today (Salamini *et al.*, 2002).

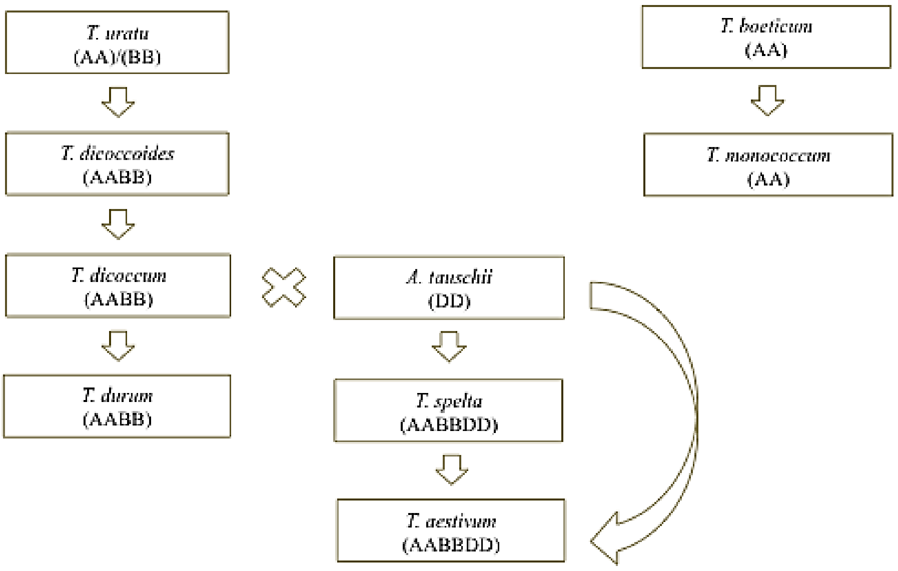


Figure 2.2. Several ploidy alterations through chance hybridisation among *Triticum* species have produced modern bread wheat (*Triticum aestivum*). These can be categorised into three distinctive cytogenic groups; Einkorn (2n), Emmer (4n) and the Vulgare (6n) group (adapted from Hancock (2012) and Salamini *et al.* (2002)).

Figure 2.3. Ear appearance of several wheat species from left to right: *T. monococcum*, *T. boeticum*, *T. dicoccoides*, *T. dicoccum*, *T. durum*, *T. spelta*, and twice *T. aestivum*. Diploid and tetraploid species are characterised by the presence of awns (image origin unknown).

Ploidy changes and selective breeding mean that modern wheat plants are hybrids formulated throughout domestication processes (Salamini *et al.*, 2002) thus many ancient hulled wheat species (including einkorn, emmer and spelt), form an intermediary stage from wild ancient wheat species to the hulled cultivated forms of *Triticum* (Pagnotta *et al.*, 2005); these are generally considered to be underutilised (Troccoli & Codianni, 2005). Subsequent to the early domestication processes described, wheat production proliferated due to seed transportation, which indicated plants to have the capacity to adapt to new environments (Hay & Porter, 2006). Consequently wheat, which was initially adapted to short Mediterranean winters, now produce record yields within cool climates throughout growing seasons typically lasting ten months (Hay & Porter, 2006).

2.3.1 Einkorn wheat (*Triticum monococcum*)

Einkorn, a diploid species, was the first wheat species to be successfully cultivated. The wild related form, *Triticum tauschii,* has been used as a source of simply inherited traits, however there are relatively few reports of this diploid positively impacting yields after introgressions of genetic material although there have been observed advantages through introgression from its wild relative *T. tauschii* (Gororo *et al.*, 2002).

2.3.2 Emmer wheat (*Triticum dicoccum*)

Emmer wheat, a hulled tetraploid species, form a conduit between modern cultivated wheat (bread and durum wheat) and wild wheat species, the main difference between the two being the presence of the Q locus (a single gene on chromosome five) (Pagnotta *et al.*, 2005). Whereas einkorn cultivation is limited to marginal areas mainly within Europe, emmer wheat continues to be cultivated on an economically important scale within Ethiopia and remains a minor crop in India and Italy (Troccoli & Codianni, 2005). Further, it is widely distributed throughout the Near Eastern Crescent (Peleg *et al.*, 2005). Such regions are generally characterised by long, hot, dry summers with short, mild and wet winters. The emmer wheat grown in such environments possess the AABB genome, from which tetraploid and hexaploid wheat were derived (Peng *et al.*, 2000). Emmer reportedly has lower grain yields than durum wheat but is higher yielding compared to einkorn (Troccoli & Codianni, 2005). Wild emmer is thought to be a large source of genetic variation.

2.3.3 Spelt wheat (*Triticum spelta*)

Half way through the first millennium BC, emmer was replaced by *Triticum spelta* (spelt) as the main cultivated wheat species (van der Veen & Palmer, 1997). *Triticum spelta* is still a cultivated species throughout south-eastern Europe, most notably in Germany and Switzerland, although is still underutilised (Bertin *et al.*, 2001; Troccoli & Codianni, 2005) and numerous wheat breeding programmes consider it a large sink of genetic variation (Winzeler *et al.*, 1993). The yields of spelt are reportedly lower than that of bread wheat and emmer however, superior to those of einkorn (Troccoli & Codianni, 2005). Spelt wheat is described to be more robust than modern wheat during early germination and early seedling development (Burgos *et al.*, 2001) and commonly displays greater vigour under adverse conditions (Bertin *et al.*, 2001). The positive impacts of the introduction of spelt genetic material (spelt cv. Oberkulmer and Rouqin) into winter wheat was reported by Winzeler *et al.* (1993) who observed the F_1 generation to have superior yields due to heterosis which improved the number of grains per ear and single grain weight.

2.4 Genetic progress and limitations for modern wheat cultivars

Trends suggest that global wheat yields may be approaching an asymptotic threshold level (Calderini & Slafer, 1998). Further genetic enhancements in modern wheat are thought to be somewhat limited, since it has been hypothesised that early domestication and focussed breeding schemes have much reduced the allelic variation within the gene pool, creating a genetic bottleneck (Peleg *et al*., 2005). Reif *et al.* (2005) state that 86% (excluding China) of spring wheat cultivable area was sown with CIMMYT (International Maize and Wheat Improvement Centre) or CIMMYT related germplasm derived from at least one CIMMYT ancestor, illustrating the narrow genetic basis of modern hexaploid wheat. The limited diversity also increases the susceptibility of wheat to biotic and abiotic stresses (Frankel & Soulé, 1981) and further, makes it increasingly difficult to selectively breed plants that possess traits affording them degrees of resistance to sub-optimal environmental conditions (Sparkes, 2010). Furthermore, genetic uniformity also increases the vulnerability of modern wheat populations to disease epidemics (Gororo *et al*., 2002; Smale, 1997) consequently, reducing productivity through drought, salinity, temperature and nutrient imbalances (Trethowan & Mujeeb-Kazi, 2008). Even new plant types, bred to be highly productive, have negligible harvests in harsh environments, demonstrated by yields being inversely proportional to the environmental stresses imposed (Araus *et al.*, 2002). This is further indicated by yield increments in the United Kingdom being ten times those witnessed in Australia (Araus *et al.*, 2002).

It is therefore imperative that the genetic variance within the modern bread wheat gene pool is supplemented from external sources; these are predicted to occur from 1) synthetic hexaploid wheat, 2) genetic manipulation and 3) related species or landraces (Trethowan & Mujeeb-Kazi, 2008). The latter presents an extraordinary opportunity since most cultivated crop species display the ‘founder effect’; this states that the allelic variation within modern wheat gene pools is diminutive compared to their wild progenitors. Ancient species purportedly have an abundance of allelic variation (Zamir, 2001). In fact, much of the emphasis of crop research institutes is targeted towards the collection, preservation and utilisation of genetic resources (Hoisington *et al.*, 1999). It is now widely accepted that advancements will be most notably achieved through a multi-disciplinary assault to better establish ‘sustainable intensification’ (Davies *et al.*, 2009).

2.5 Ancient wheat species as a tool for wheat improvement

Previous research has indicated that ancient wheat species harbour substantial genetic variation that remains untapped (Skovmand *et al.*, 2001; Trethowan & Mujeeb-Kazi, 2008). Many of these resources include traditional varieties and non-domesticated species (Hoisington *et al.*, 1999); these are relatively underutilised and are a prospective source from which genetically variable alleles can be mined. An example of such a feat within modern breeding relates to the introgression of wild *Avena sterilis* L. germplasm being introgressed into *A. sativa* L. which increased yields by up to 30% (Gororo *et al.*, 2002).

Wild emmer has been observed to harbour a great deal of variation that may be critical for the improvement of modern bread wheat (Peleg *et al.*, 2005). Additionally, *Triticum dicoccoides* is thought to harbour many morpho-physiological traits of high agronomic and economic importance (Carver & Nevo, 1990; Nevo *et al.*, 1991). Furthermore, landraces and varieties of spelt wheat could be of use due to its hexaploid status, increasing the chances of viable crosses (Burgos *et al.*, 2001). Peleg *et al.*, (2005) also highlights the relative ease of crossing between tetraploid emmer and hexaploid wheat species. This is particularly appealing since it provides a sizeable source of genetic variation which can be introgressed utilising conventional breeding techniques (Peleg *et al.*, 2005) and yet yield potential and physiological characterisation for these species has been lacking in any great detail.

Reynolds & Trethowan (2007) stated that assessment for breeding through physiologically based criterion is an advantage due to the increasing occurrences of additive gene actions. Increased understanding of resource capture of radiation and water will improve the efficiency of production and could lead to reduced agricultural inputs in wheat production systems. Fischer & Edmeades (2010) state that the radiation use efficiency (RUE) should remain the focus of any major yield initiative and that the easiest way forward is to assess the natural variation within crop species and their close relatives. In particular, Muurinen & Peltonen-Sainio (2006) found these species to have increased radiation-use efficiencies (RUE), while several authors have reported that emmer has high water use efficiency (for example Peleg *et al*., 2005). Furthermore, Trocolli & Codianni (2005) stated that spelt and emmer also performed favourably under low-input conditions compared to modern bread wheat. Therefore, there is a need to collect robust data relating to resource capture traits for these ancient wheat species. The underlying mechanisms of efficient resource capture traits are highly complex; it is necessary to evaluate these mechanisms in ancient wheat to identify the advantages they confer as well as their genetic basis. Ultimately, this could provide an opportunity whereby wheat yields, particularly in sub-optimal environments, may be improved, increasing production per unit resource, thereby enhancing the productivity and efficiency.

2.6 Increasing agricultural productivity and efficiency to meet growing demands

High yielding, efficient crop systems are reliant upon optimal growth and development (Reynolds *et al.*, 2001). The efficiency of a large scale agricultural system is inherently dependent on its basic components; these include agronomic management practices, resource availability, as well as the genetic material used, the latter ultimately provides the final limitation to productivity. Since productivity is commonly inefficient within many cultivated crop species, enhancement could be key. It is therefore necessary to evaluate traits within a population that has large variation; this increases the chances of identifying combinations of alleles that confer efficiency or tolerance associated traits (Hoisington *et al.*, 1999). Genetic diversity is therefore critical for production of successful, efficient and sustainable systems. Zamir (2001) makes the pertinent statement that: *'plant breeding is the art and science of the genetic improvement of crops to produce new varieties... [and] genetic variation is the engine that propels breeding to meet future challenges'*. As a result, much emphasis within crop breeding is directed towards improvement of cultivars.

2.7 Physiological traits determining yield and resource capture

The resource-capture and conversion efficiency of plants are directly related to biological yield; harvestable yield is determined by the proportion of total biomass that has been directed towards harvestable structures, most notably the grain for wheat. Plants accumulate biomass through the capture of photosynthetically active radiation (PAR) (at a wavelength of 0.4–0.7 µm); the energy contained within this frequency can be used to fix carbon by driving pigment based systems during photosynthesis (Azam-Ali & Squire, 2002). In this process, photons (units of radiation) are absorbed by pigment molecules such as chlorophyll, which transform atmospheric CO_2 into complex carbohydrates (Azam-Ali & Squire, 2002). An informative way of investigating genotypes is the study of the efficiency with which these external resources can be converted into biomass (Hay & Porter, 2006; Miralles & Slafer, 1997). The amount of dry matter accumulated per unit of PAR that has been intercepted is termed radiation use efficiency (RUE) (Monteith & Moss, 1977). This was further described by Mitchell & Sheehy (2006) who stated that harvestable grain yield as dry matter (Y) (g m^{-2}) can be achieved by increasing any one of the following terms; harvest index (HI), radiation conversion factor (RUE) (ε) (g MJ^{-1}), growth duration (days) (n) (days), Q_i is the PAR intercepted on the i^{th} day (MJ m^{-2}) and F_i is the fraction of incident PAR that is intercepted, averaged over the i^{th} day (Equation 1.1).

$$Y = HI\, \varepsilon \sum_{i}^{n} Q_i\, F_i \qquad \text{Equation 2.1}$$

It is widely accepted that there are significant differences in biomass accumulation and RUE within and between species. It is this variation that is critical within breeding programmes (Azam-Ali & Squire, 2002; Mwale *et al.*, 2007). The domestication of ancient wheat species has been well documented however, very little information exists regarding their physiology, growth and development (van der Veen & Palmer, 1997). The following sections review the literature concerning biomass accumulation, canopy architecture and radiation capture.

2.8 Conversion of radiation through photosynthesis

Photosynthesis (in C_3 plants) is the process whereby plants accumulate stored chemical energy in the form of organic carbon from water and carbon dioxide (CO_2), it takes place in mesophyll cells and occurs through two processes; light reactions and the Calvin cycle, both of which have multiple steps (Figure 2.4). In the former, photons are absorbed by chlorophyll (within chloroplast internal membranes), to generate chemical energy in the form of ATP (adenosine triphosphate) and NADPH (nicotinamide adenine dinucleotide phosphate) (Campbell & Reece, 2002). The second stage, termed the light independent reactions through the Calvin cycle (Figure 2.5), begin with 1) carbon fixation, whereby Rubisco (ribulose-1, 5-bisphosphate carboxylase) incorporates CO_2 from the air into organic molecules in the chloroplasts by conjugating it to a five carbon sugar (ribulose bisphosphate) (RuBP) (Beadle & Long, 1985; Campbell & Reece, 2002; Langdale *et al.*, 1988). With the addition of electrons, the products of this process then undergo a 2) Reduction phase, producing complex carbohydrates. In the final steps of the Calvin cycle, ATP is used to rearrange carbon products to 3) Regenerate the CO_2 acceptor, RuBP. It is this concluding step that enables the cycle to continue. The maximum potential for energy conversion through these processes is thought to be 4.6%, although many species rarely achieve this, particularly under natural conditions (Lawson *et al.*, 2012). This reaction can be simply described as the production of two energy rich compounds (oxygen and carbohydrate) that derive from two energy-poor compounds (CO_2 and water), in the presence of solar radiation (Campbell & Reece, 2002):

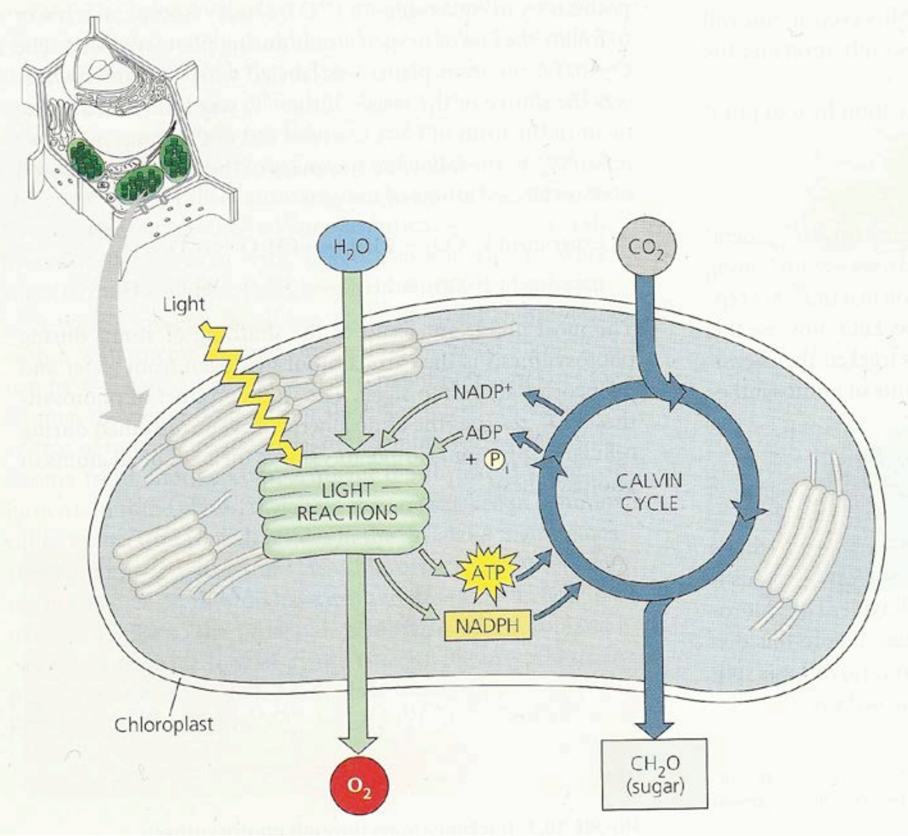


Figure 2.4. An overview of photosynthesis: cooperation of the light reactions and the Calvin cycle. In the chloroplast, the thylakoid membranes are the sites of the light reactions, whereas the Calvin cycle occurs in the stroma. The light reactions use solar energy to make ATP and NADPH, which function as chemcial energy and reducing power, respectively, in the Calvin cycle. The Calvin cycle incorperates CO_2 into organic molecules, which are converted to sugar (Campbell & Reece, 2002).

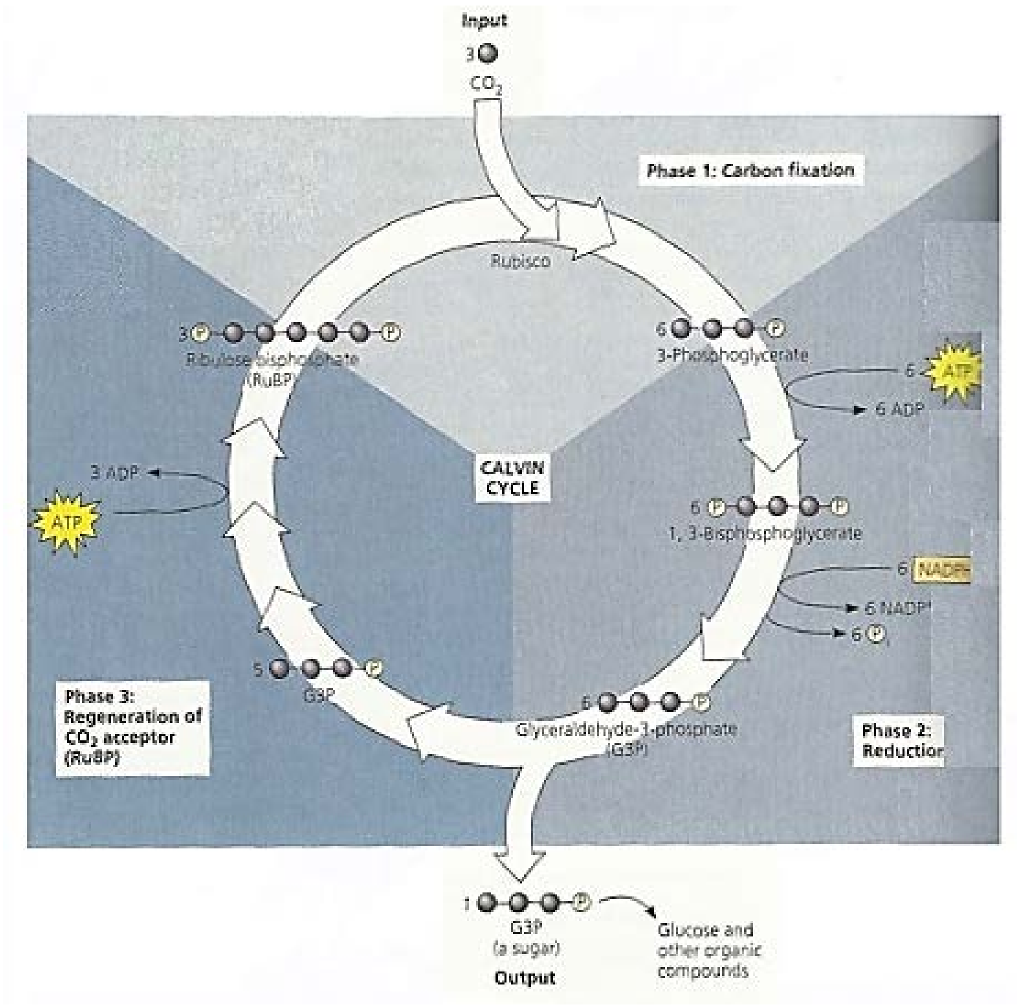


Figure 2.5. The Calvin cycle. The diagram tracks carbon atoms (grey balls) through the Calvin cycle. The three phases of the cycle correspond to the phases discussed in the text. For every three molecules of CO_2 that enter the cycle, the net output is one molecule of glyceraldehyde-3-phosphate (G3P), a three-carbon sugar. For each G3P synthesised, the cycle spends nine molecules of ATP and six molecules of NADPH. The light reactions sustain the Calvin cycle by regenerating ATP and NADPH (Campbell & Reece, 2002).

The productivity of a crop system is ultimately set by the efficiency of absorption light energy, and the efficiency of its transduction into biomass (Murchie *et al.,* 2009). This is heavily influenced by the distribution of quanta over individual elements of the crop foliage, the relationship between photosynthetic rates and irradiance for the various elements of the crop canopy as well as the corresponding rates at which CO_2 is lost (Azam-Ali & Squire, 2004). When light is saturating, the limitation is set by the rate at which atmospheric CO_2 can be fixed and reduced to carbohydrates whereas when irradiance is low, the limitation is set by photo absorption; this linear phase provides a measure of the ‘quantum efficiency’ by measuring the amount of CO_2 absorbed per unit irradiance (Azam-Ali & Squire, 2002). Beyond this phase, with increasingly stronger light incidence, photosynthetic rates typically increase until they become saturated, leading to a plateau in photosynthetic rate (Azam-Ali & Squire, 2002). There has been a great deal of focus directed towards the enzyme Rubisco in order to increase CO_2 assimilation rates. This is because Rubisco is capable of catalysing two distinct reactions; the first leads to the formation of phosphoglycerate, when CO_2 is the substrate, and the second produces phosophoglycollate, which is broken down to release CO_2 in a process called photorespiration (Reece & Campbell, 2002). The net photosynthetic rate of a plant is dependent on the difference between the rate that CO_2 is reduced to carbohydrates and the rate of CO_2 loss through photorespiration (not including mitochondrial respiration in this case). Photorespiration accounts for a substantial proportion of carbon losses; these are thought to occur at approximately 30% of the rate of CO_2 assimilation in temperate climates (Rawsthorne, 1992). At higher temperatures, oxygenase activity is favoured over carboxylase activity, further reducing the efficiency of photosynthesis (Murchie *et al.*, 2009; Rawsthorne, 1992). This imposes limitations on biomass production at the most basic level of plant productivity. Crop yields are indeterminately impacted by the inherent capacity to accumulate carbon through this mechanism since the quantities of dry matter fixed during this process are linearly related to the amount of intercepted radiation (Murchie *et al.*, 2009; Takahashi & Nakaseko, 1990).

As well as photorespiration, all plants also undergo dark respiration (also termed respiration) (R_d), where atmospheric oxygen and carbohydrates are converted into CO_2 and water, producing energy which is used to create even more complex molecules from the comparatively simple products of photosynthesis (Azam-Ali & Squire, 2002). Respiration, both under light and dark conditions, is a critical aspect in the carbon budget of a crop and forms an important aspect of growth. The impact of respiration varies with crop age and weight (Azam-Ali & Squire, 2002). It is the efficiency of light interception and photosynthetic reactions that set the threshold for biomass production (Beadle & Long, 1985). Since the primary determinant of yield is cumulative photosynthetic rate, enhancing photosynthesis in C_3 species has been identified as a potential avenue through which crop yields may be boosted (Lawson *et al.*, 2012).

2.8.1 Photosynthetic response to atmospheric carbon dioxide

Infra-Red Gas Analysis (IRGA) allows leaf level reactions to varying atmospheric CO_2 concentrations to be monitored. Photosynthetic and transpiration rates, as well as a defined leaf-air vapour pressure deficit (D), can be used to calculate internal CO_2 leaf concentrations (C_i) (Long & Bernacchi, 2003). A graphical representation of this response in Figure 2.6 shows three phases; 1) as C_i increases from the lowest of concentrations, the reaction is characterised by Rubisco activity which is termed the maximum ribulose 1-5 bisphosphate carboxylase/oxygenase (Rubisco) carboxylation rate (V_{cmax}) (Miao *et al.*, 2009). Phase 2) occurs during further increases in atmospheric CO_2 and is characterised by limited RuBP regeneration due to electron transport (during CO_2 saturated photosynthesis) (Long & Bernacchi, 2003). The final phase 3) is evident when A plateaus or decreases, even in the presence of increasing C_i. At this point, triose-phosphate utilisation (TPU) restricts any further increases in photosynthetic rates. This response allows differences in biochemical kinetic variables that determine photosynthetic capacity, to be comparatively assessed (Long & Bernacchi, 2003).

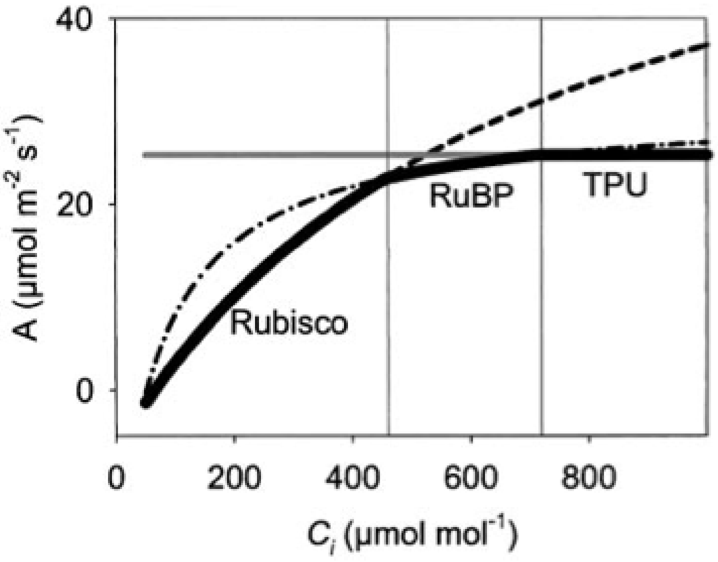


Figure 2.6. Idealised A/C_i response. The rates of photosynthesis that would be achieved are indicated when Rubisco, RuBP or TPU are limiting. The figure also shows the actual photosynthetic rates of a typical response (solid line) at any given $C_{i,}$ the minimum of these three potential limitations. The parameters used were: V_{cmax} = 70 μmol m^{-2} s^{-1}, J_{max} = 130 μmol m^{-2} s^{-1}, TPU = 9.1 μmol m^{-2} s^{-1}, R_d = μmol m^{-2} s^{-1} (Long & Bernacchi, 2003).

2.8.2 Photosynthetic response to photon flux

Whilst exposing a leaf to varying quantum flux, responses may reveal characteristic differences for the underlying photosynthetic mechanism; these relate to light dependent and light independent reactions. This response can be divided into two distinct phases where 1) initial photosynthetic rates are limited by light incidence from which quantum yields (Φ) are obtained, the second phase 2) occurs where light is saturating the leaf, therefore it is the quantity or activity of the enzyme Rubisco or primary substrate CO_2 that becomes limiting (A_{sat}) (Figure 2.7). Different plant species show varying relationships between these parameters (however, the overall shape is uniform), the convexity however (Θ) refers to specific points at which phases apply.

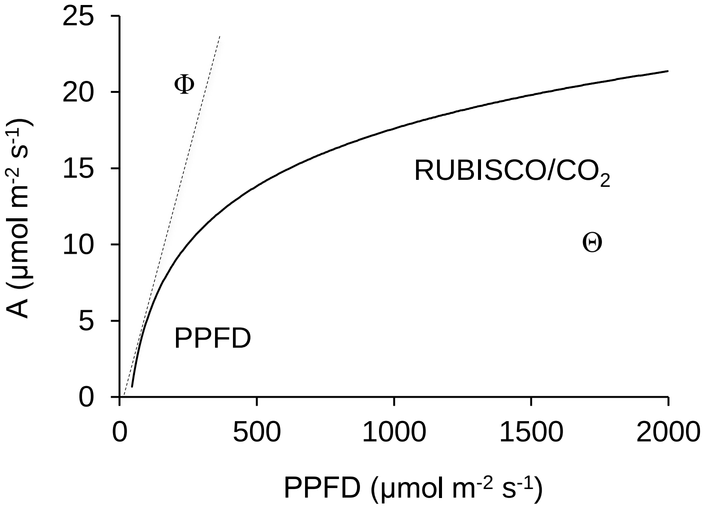


Figure 2.7. Schematic Photosynthetic rate/Quanta (A/Q) response curve. The rates of photosynthesis that would be achieved are indicated when PPFD and RUBISCO/CO_2 are limiting. The broken line represents Quantum yield (Φ) and convexity (Θ) represents the variation between each of the phases. The solid line denotes actual photosynthetic rates at a given light intensity. The parameters derived from this response were: A_{max} = 20.40 µmol m^{-2} s^{-1}, Φ = 0.0437 µmol m^{-2} s^{-1}, Light saturation estimate = 490 and respiration = -1.03 µmol m^{-2} s^{-1}.

2.8.3 Stomatal behaviour during photosynthesis

The amounts of CO_2 reduced to carbohydrate, as well as the decay of compounds broken down to fulfill energy requirements for the metabolic process, is termed the net photosynthetic rate (*A*) (µmol m^{-2} s^{-1}) and can be described as:

$$A = (C_a - C_i) / (r_a - r_s) \qquad \text{Equation 2.2}$$

Where C_a (g of CO_2 per m^3 of air) and C_i are the concentrations of CO_2 in the atmosphere and internal air spaces of the leaf, respectively (g of CO_2 m^{-2} s^{-1}) and r_a and r_s are the leaf boundary resistances created at the leaf surface as well as stomatal resistances (created by the behaviour and distribution of stomatal apertures at the leaf surface) (s m^{-1}) (Equation 2.2). Stomatal resistances are regulated by both physiological and environmental factors and govern CO_2 uptake as well as water loss (Lawson & Blatt, 2014). Stomatal opening is typically stimulated by exposure to PAR, low CO_2 concentrations, and high humidity, whereas stomatal closure is stimulated by the reverse (Lawson & Blatt, 2014; Lawson & Morison, 2006). When closed, stomatal openings are restrictive to carbon diffusion into the leaf, this reduces carbon assimilation and will therefore limit the yields produced (Lawson & Blatt, 2014). Figure 2.8 shows the stomatal pathways which allow diffusion of water and CO_2 from the leaf to the atmosphere and *vice versa* which are subject to resistances (Condon *et al.*, 2004). Stomatal openings must allow sufficient CO_2 to enter the leaf whilst avoiding water losses that may cause dehydration and metabolic disruption (Lawson, 2009;

Lawson *et al.*, 2012; Lawson & Morison, 2006). Stomatal density varies with species and can range from 0 to more than 2000 mm^{-2} (Lawson & Morison, 2006). The importance of stomata is illustrated by Lawson & Blatt, (2014) which outlines a study in which stomatal density was overexpressed in mutants causing an increase of 2/3 greater stomatal densities; this corresponded to a 30% increase in photosynthetic rate and was also linked with an increase in water use as well as a 50% reduction in water use efficiency (WUE) (Lawson & Blatt, 2014).

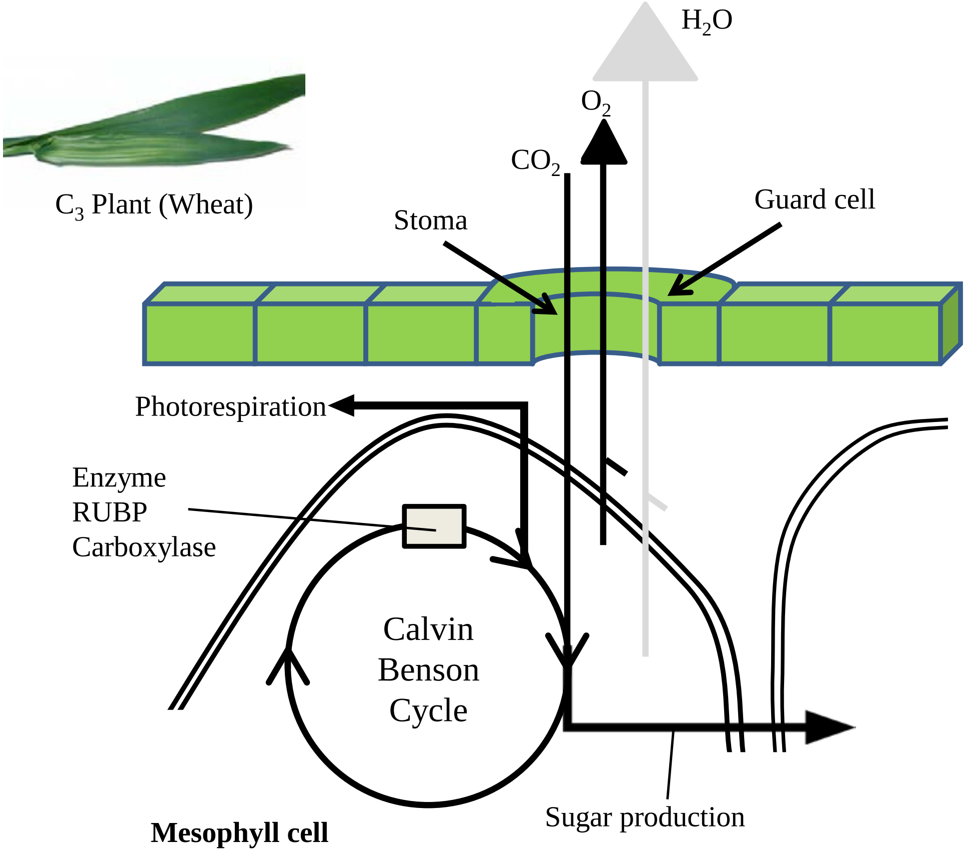


Figure 2.8. Schematic cross-section showing typical gas exchange between the atmosphere and stomatal air spaces within the leaf showing carbon dioxide entry and water vapur loss. The biochemical pathways involved in this process are also shown (adapted from Azam-Ali & Squire (2002)).

The primary biochemical reaction is consistent in all C_3 species; there is considerable variation for photosynthetic rates per unit leaf area, some of which is accounted for by differences in biochemical capacities. One particular study investigated the maximum photosynthetic responses using A/C_i curves of a number of species, under steady state conditions. Maximum photosynthetic rates varied from 29 µmol m^{-2} s^{-1} (for wheat) to 6 µmol m^{-2} s^{-1} in *Picea engelmanni* (Wullschleger, 1993). Wullschleger (1993) illustrated the variation for photosynthetic capacity to derive from biochemical carboxylation (which varied from 6 to 194 µmol m^{-2} s^{-1}), RuBP generation through electron transport rates (which varied from 17 to 232 µmol m^{-2} s^{-1}) and photosynthetic rate within high atmospheric CO_2. There are a range of explanations for these differences: kinetic properties and regulation of enzymes, enzyme expression levels, developmental responses to growth in different environmental conditions, as well as the plasticity of stomatal regulation which can take anything from seconds to hours (Lawson, 2009; Lawson *et al.*, 2012).

2.9 Growth, development and radiation interception

A large degree of crop architecture is determined by genetic lineage (Miralles & Slafer, 1997) however it is also influenced by light incidence. A way of assessing area is through leaf area index (LAI), which Watson (1952) defined as the total one-sided leaf area of leaf tissues per unit ground area; this provides a scale for comparative measure of leaf coverage of a crop. The size and duration of leaf area will affect dry matter accumulation and duration (Azam-Ali & Squire, 2002). The leaf area index (LAI), as well as the angular arrangement of leaves will affect the amount of light that is distributed down the canopy (Azam-Ali & Squire, 2002).

Naturally, the canopy will experience a range of light environments throughout the day due to leaf angle and altering incoming radiation, cloud cover and overshadowing canopy cover; this means that leaves will only reach maximum photosynthetic rates for a small period of the day (Lawson *et al.*, 2012). Also of consequence is the speed at which leaves can respond (termed induction) following increments of PAR, which have the potential to impact daily radiation by as much 10%; increasing the rapidity of this response could provide significant gains in terms of biological yields (Lawson *et al.*, 2012). This may be achieved by altering electron transport processes, Calvin cycle activation, and rates of stomatal responses, all of which affect the adjustment periods to changing light conditions (Lawson *et al.*, 2012). Additionally, partitioning towards components such as leaf area will also influence the proportion of photosynthetically active radiation that are intercepted and converted into organic carbon compounds (Azam-Ali & Squire, 2002). Canopy structure was explored by Monteith (1965) who stated that structure could be described by area, geometric position and size; this typically affects canopy light transmission. Incident light can be assessed relative to light attenuation through fractional interception (*F*) (Equation 2.3), where I denotes incident radiation, r is the reflected radiation and I_0, the Intercepted radiation:

$$F = 1 - (I + r) / I_0 \qquad \text{Equation 1.3}$$

A study by Zhang *et al*. (1998) showed that fractional interception peaked at 148 days after sowing (DAS) and was lower for modern bread wheat when rain-fed rather than when irrigated; *F* also varies with genotype, crop management, irrigation, row spacing and agronomic practices (Singels & Smit, 2002). Additionally, Rajbhandari (2004) highlighted that leaf geometry was responsible for variation for fractional interception, that larger canopy sizes reduced PAR interception and that greater plant density was related to increased interception. Furthermore, Sparkes (2010) found that spelt and emmer cultivars had greater fractional interception through canopy development indicating the potential variance for interception characteristics between modern and ancient wheat species. Other parameters such as the extinction coefficient for radiation (k) can also be obtained empirically using the gradient of the Ln (I/I_0) against LAI using principles detailed in the Monsi-Saeki equation (Azam-Ali & Squire, 2002):

$$F = 1 - e^{-k\mathrm{LAI}} \qquad \text{Equation 2.4}$$

Azam-Ali & Squire (2002) also describe the extinction of light through the canopy; this commonly impacts light distribution, growth rate, yields and radiation use efficiency (RUE). By measuring stratified leaf clippings, the vertical structure of the canopy can be characterised, referred to as the leaf area density (LAD); a measure of the leaf area within a single volume layer of canopy. Mashhadi & Zand (2004) found that light transmission was lower for Iranian winter wheat cultivars in which LAD was greater. The proportion of light intercepted showed that modern cultivars intercepted a greater proportion of light (up to 95% of PAR was intercepted) in the top layer of the canopy, although the study did not indicate whether cultivars differed in size (all being modern wheat cultivars however, it would be assumed that the variation for height would be relatively small).

2.10 Radiation Use Efficiency

Jamieson *et al.* (1998) state that RUE is affected by many factors including nutrient imbalances (Sinclair & Horie, 1989) and temperature (Goyne *et al.*, 1993) although the extent to which the latter affects RUE depends on the leaf response to temperature (Sinclair & Muchow, 1999). The relationship between light intercepted and total crop biomass is linearly related (Kiniry *et al.*, 1989; Monteith & Moss, 1977) although little variance has been observed for RUE as a result of LAI (Sinclair & Muchow, 1999). Many researchers have explored other canopy traits of bread wheat and their influence over RUE; Calderini & Slafer, (1998) reported the morphological characteristics of old and newer wheat cultivars released from 1920–1990 and found that pre-anthesis RUE did not differ widely, however there was variation for post-anthesis RUE. Also, newer cultivars had more consistent RUE from the pre- to post-anthesis period when compared to the older cultivars, which also had lower RUE and produced less biomass (Calderini & Slafer, 1998). Muurinen & Peltonen-Sainio (2006) reported that, at lower altitudes, RUE differed between old and modern cultivars; ancient wheat species had increased RUE. Furthermore, spelt and emmer purportedly perform favourably in low-input conditions (Troccoli & Codianni, 2005) and have been observed to display increased RUE when compared to their modern counterparts (Muurinen & Peltonen-Sainio, 2006). Additionally, Sparkes (2010) investigated a range of ancient wheat characteristics and compared them to modern wheat; spelt was found to have the greatest RUE (1.81 MJ m^{-2}).

Monteith & Moss (1977) estimated that within an optimal environment, approximately 1.4g of dry biomass was accumulated per megajoule (MJ) of intercepted solar radiation. Monteith (1981) demonstrated that this is generally quite consistent across C_3 species, as shown in Figure 2.9, especially when crops are not stressed (Kiniry *et al.*, 1989). Literature commonly states that increased yields are often associated with high RUE (Han *et al.*, 2008; Li *et al.*, 2009) thus enhancing RUE may be central to the improvement of modern wheat yields. Furthermore, Reynolds *et al.* (2000) states that to date, breeding has not substantially improved RUE. Monteith & Moss (1977) suggested that by increasing maximum photosynthetic rates RUE could be significantly boosted, especially where crops have resource limitations. This is further supported by Sinclair & Horie (1989) who state that leaf CO_2 assimilation rates and leaf nitrogen content are both functions of RUE; they demonstrated that RUE remained generally consistent when leaves had high CO_2 assimilation rates but noticeably decreased when photosynthetic rates were low.

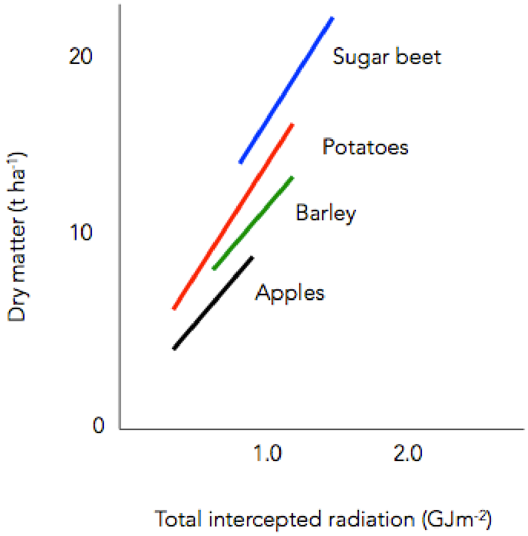


Figure 2.9. The relationship between total dry matter (t ha^{-1}) at harvest and the amount of solar radiation (GJ m^{-2}) intercepted throughout a season for apple, barley, potato and sugar beet plants (Monteith, 1981).

2.11 Green area senescence and persistence

As previously stated, photosynthetic activity, leaf area and green leaf duration are imperative to crop productivity. Senescence is a natural ageing process, whereby chlorophyll, proteins, lipids and nucleic acids degrade (Falqueto *et al.*, 2009). Falqueto *et al*. (2009) also states that photosynthetic efficiency is largely reduced as a result of senescence; the associated chlorophyll catabolism leads to disassembly of photosynthetic apparatus, the timing of which could affect overall efficiency of carbon assimilation. Greater biomass production and yield are associated with functional stay-green traits (Gregersen *et al.*, 2008; Takahashi & Nakaseko, 1990). Information collated about mutant stay-green plants indicate that true green area persistence may be caused by a specific defect in the chlorophyll catabolism pathway (Thomas *et al.*, 2002). Delayed senescence would potentially increase the duration of CO_2 assimilation. However, it may be the case that extended duration may be offset by lower overall photosynthetic capacity. During photosynthesis, highly reactive oxygen species (ROS) are produced when light absorbance exceeds the rate at which the carbon accumulated can be utilised, the accumulation of which can interfere with the electron transport chain to reduce photosynthetic efficiency (Chaves & Oliveira, 2004; Lawson *et al.*, 2012). Increased adaptation to respond to these rapid changes may lead to prolonged survival (Lawson *et al.*, 2012), potentially a factor within green area persistence. Senescence timing is likely to affect the remobilisation of nutrients; a process whereby resources are translocated from old to newer organs, particularly towards the flag leaf, which contributes the largest proportion of assimilates to grain yield (Gregersen *et al.*, 2008). Delayed senescence may also delay remobilisation, potentially leading to reduced grain weight (Gregersen *et al.*, 2008).

The ‘stay green’ persistence trait has been reported to be an advantage when plants are exposed to abiotic stresses. Many authors have reported genetic variation for senescence as a response to water or heat stresses in durum wheat (Falqueto *et al.*, 2009; Hafsi *et al.*, 2007; Srivalli & Khanna-Chopra, 2009). Green area persistence has most often been associated with drought tolerance where the ability to maintain CO_2 assimilation is associated with increased yields, particularly when drought was imposed post-anthesis (Borrell & Hammer, 2000; Christopher *et al.*, 2008; Hafsi *et al.*, 2007). Many other studies have correlated senescence and drought tolerance with yield (Gelang *et al.*, 2000; Gregersen *et al.*, 2008; Verma *et al.*, 2004). Sparkes (2010) suggested that emmer and spelt could harbour ‘stay green’ traits.

2.12 Water Use Efficiency

Although 80% of the world’s allocable water resource is directed towards agriculture, water availability is still a commonly limiting factor within many crop systems (Condon *et al.*, 2004). Crop plants routinely undergo periods where soil and atmospheric water is in deficit (Chaves *et al.*, 2002). It is commonly known that reduced water availability typically reduces plant transpiration rates (Weir & Barraclough, 1986), which reduces leaf carbon fixation (*A*) as a result of stomatal closure (Chaves & Oliveira, 2004; Lawson & Blatt, 2014) both of which will negatively impact biological yield (Murchie *et al.*, 2009). Severe drought has also been observed to reduce Rubisco activity and RuBP (Chaves & Oliveira, 2004). The production of drought-tolerant wheat varieties in the past has been limited due to the complexity of water use efficiency (WUE) traits since these are entwined with morphological and physiological characteristics; furthermore, there is also difficulty in assessing these traits since methods are time consuming and often destructive (Chaves & Oliveira, 2004; McAusland *et al.*, 2013). Literature, however, does state that there is genetic variation for the underlying traits that alter WUE (Condon *et al.*, 2004). An example of which is the development of new Australian wheat cultivars ‘Drysdale’ and ‘Reese’ which both exhibit WUE (Condon *et al.*, 2004). WUE involves a measure of water in exchange for a unit of production (Condon *et al.*, 2004) although there is no definitive description, and it often depends on the specialism in which it is being considered. That which forms the basis for farming and productivity and is perhaps of the most consequence within crop breeding is the agronomists' interpretation, relating carbon gain to cumulative transpiration (Steduto & Albrizio, 2005):

$$\text{Yield} = \text{ET} \times \text{T} / \text{ET} \times \text{W} \times \text{HI} \qquad \text{Equation 2.5}$$

These principles were applied previously to assess WUE of ancient wheat species in a glasshouse study, where plants were grown in columns maintained at 80% water holding capacity (WHC) (considered to be irrigated) and 50% WHC (considered to be under drought). Results indicated that spelt and emmer had potential WUE traits. Furthermore, emmer displayed yield stability, showing an 18% reduction in yield compared to 37, 36 and 62% for einkorn, spelt and modern bread wheat, respectively, from irrigated to drought conditions (Diaz-Alves, 2008).

Equation 2.5 considers yield to be a function of the volume of water utilised by the crop (evapotranspiration) (ET), the fraction of water that was actively transpired by the plant (T/ET), the efficiency term relating to the volume of water utilised relative to the amount of biomass produced (W) and finally how efficient the plant is at translocating this water into the harvestable product (Condon *et al.*, 2004; Passioura, 1977).

Alternatively, at the leaf level, WUE can be defined as a function of photosynthesis (number of moles of carbon gained) relative to water transpired (T) (see equation 2.6). This provides an instantaneous measurement of WUE that may be identified through leaf Infra-Red Gas Analysis (IRGA) (Kramer & Boyer, 1995; Larcher, 2003).

$$\text{ITE mol mol}^{-1} = A\ (\mu\text{mol CO}_2\ \text{m}^{-2}\ \text{s}^{-1}) / \text{T}\ (\text{mmol H}_20\ \text{m}^{-2}\ \text{s}^{-1}) \qquad \text{Equation 2.6}$$

There are a number of factors that affect ITE. Photosynthesis (A) is directly influenced by stomatal conductance ($G_{s\,CO2}$) for CO_2 set by the concentration gradient of CO_2 between the surrounding air and the internal leaf (C_i). Generally, reduced stomatal conductance denotes higher WUE, although this is usually observed in conjunction with a reduction in assimilation rates (A) as well as slower growth rates (Lawson, 2009; Lawson & Blatt, 2014). There is therefore a trade-off between water loss and carbon assimilation; however stomatal behaviour and the relationship between these factors could be manipulated in order to reduce water loss (Chaves & Oliveira, 2004).

Stomatal response is typically much slower than photosynthetic response, creating a disparity (Lawson & Morison, 2006); the removal of stomatal limitation could increase photosynthetic rates by 20% in C_3 species. It is, therefore, a possibility that identifying plants with rapid sensory and/or signalling mechanisms that infer rapid, tuned stomatal control, has the potential to enhance CO_2 gain and water use throughout plant growth and development (Lawson *et al.*, 2012) since a slow response when stomata are required to close, or keeping stomata open to maintain photosynthetic rates, may lead to an unnecessary loss of excess water (Lawson *et al.*, 2012). The relationship between C_i and C_a remains relatively consistent with changing concentrations, indicating the linear relationship between A and stomatal conductance (G_s) (Equation 2.7) (Condon *et al.*, 2002). Other factions of the equation are affected similarly; T is also influenced by stomatal conductance and the water vapour concentration gradients within the leaf (W_i) and in the surrounding atmosphere (W_a) (Equation 2.8) (Condon *et al.*, 2002):

a) $A = G_{s\,C02}\,(C_a - C_i)$ Equation 2.7

b) $T = G_{s\,H20}\,(W_i - W_a)$ Equation 2.8

W_i is typically greater than W_a and the converse is true of CO_2 concentrations (C_i and C_a) (Condon *et al.*, 2002). There is an expanse of recent research that is geared towards stomatal metabolism. It is thought that this area of research might hold the key in the production of drought-tolerant plants (Lawson, 2009; Nilson & Assmann, 2007). All of these physiological principles culminate to form the basis from which instantaneous WUE can be derived:

$$\mathrm{ITE} = A / \mathrm{T} = [G_{s\,CO2}\,(C_a - C_i)] \;/\; [G_{s\,H20}\,(W_i - W_a)] \qquad \text{Equation 2.9}$$

Farquhar & Richards (1984) reported significant amounts of variation for ITE between genotypes. Equation 2.9 shows that there are two avenues through which leaf level transpiration efficiencies may be altered; 1) the ratio of C_i/C_a may be increased, 2) alternatively or in conjunction with reductions in the value of $W_i - W_a$. Both avenues reduce the concentration gradient between the atmospheric air and the leaf internal cavity to lower water loss (Condon *et al.*, 2004), although the latter states that manipulation of the $W_i - W_a$ gradient remains the most straightforward where genetic variation may be used through breeding to increase the ratio of *A* to T; the quest to understand the effects of stomatal control over photosynthesis and water relations is increasingly important in light of more erratic environments (Chaves & Oliveira, 2004; Lawson, 2009). For instance, Lawson & Blatt (2014) state that by manipulation of stomatal densities, gaseous conductance can be modified accordingly. Furthermore, knowledge of stomatal function is critical to determine plant responses to environmental stresses, particularly reduced water availability, and is necessary to identify plants with decreased water use that are capable of high yields (Lawson, 2009). Alteration of stomatal behaviour has the capacity to change response mechanisms according to environmental conditions, and further respond to temporal factors (Lawson, 2009). However, stomatal responses are also variable by species, which further complicates WUE traits at the leaf level (Lawson, 2009). Condon *et al.*, (2004) states that both of these definitions will be required in order to improve of WUE in wheat.

Progress has previously come from experiments that empirically selected for tolerance to drought stress; current genetic progress however is based on the additive variance for yield under drought within current wheat gene pools (Trethowan & Mujeeb-Kazi, 2008). The prospect of increasing the WUE of crops species seems more probable as a result of relatively new genetic methods which, when combined with classical plant physiology, will allow investigation of available germplasm for improvement of cultivated plants (Peleg *et al.*, 2005). Additionally, such analyses will facilitate an understanding of plant adaptive mechanisms and their genetic basis in response to water stress (Peleg *et al.*, 2005; Trethowan & Mujeeb-Kazi, 2008).

2.13 Measuring photosynthesis and WUE: techniques and implications

2.13.1 Gas exchange measurements

The principles discussed so far have related to plant physiology, which address the metabolic processes that typically affect yields. Historically these have been integrated into crop species through breeding. However, it is increasingly evident that yield gains and resource use efficiency will not be achieved by simply using one tool or trait as efficiency traits are, by their very nature, highly complex. Physiological research therefore must utilise a wide range of tools to assess a number of traits.

Infra-Red Gas Analysis measurements allow the biochemical and biophysical aspects of carbon assimilation to be assessed (Long & Bernacchi, 2003). Gas exchange allows parameters such as CO_2 uptake (A), transpiration (T), leaf conductance (G_s) as well as intercellular CO_2 mole fraction (C_i) to be measured in real-time. These can be manipulated to determine a far wider range of characteristics such as photosynthetic efficiencies, capacities, and compensation values *in vivo* (Parsons *et al.*, 1998). Using principles initially outlined by Farquhar *et al.* (1982), gas exchange measurements allow greater insight into the stomatal and biochemical aspects of carbon assimilation and WUE, particularly the limitations imposed on photosynthetic mechanisms that affect these (Long & Bernacchi, 2003) (Figure 2.10).

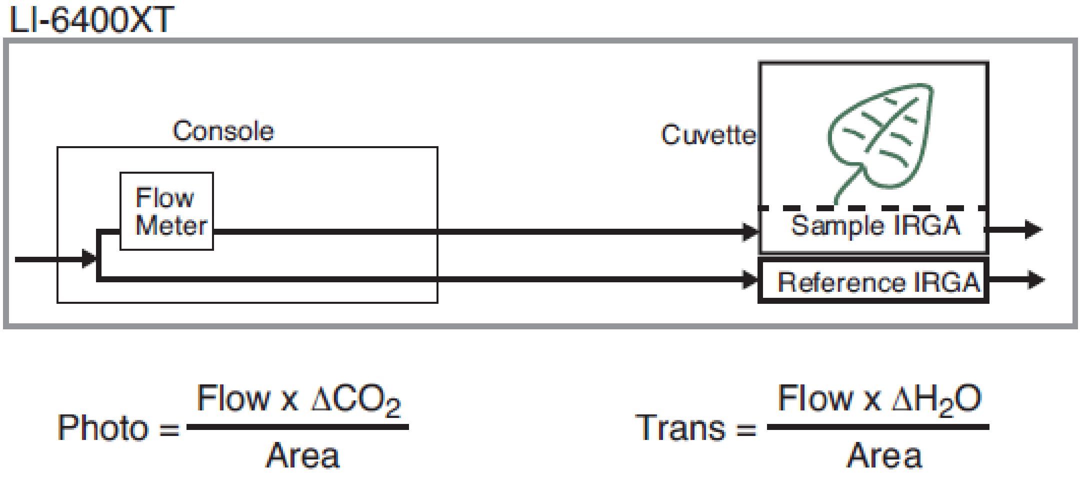


Figure 2.10. Diagram showing the mechanisms by which photosynthetic rates and transpiration rates are attained in an open Infra-Red Gas Analyser. IRGAs typically use the differential carbon dioxide and water vapour concentrations in the chamber (sample line) compared to the reference line in which no photosynthetic activity occurs (Li-Cor Manual, 2004).

2.13.2 Carbon isotope discrimination

Carbon isotope discrimination ($\Delta\,^{13}C$), as a highly heritable trait, can also be used as a means to assess genetic variation for WUE throughout the lifetime of the plant (Condon *et al.*, 1993; Condon *et al.*, 2004). Like gas exchange, carbon isotope discrimination ($\Delta\,^{13}C$) provides an indication of the gradients between C_i/C_a. These are inferred by determining the ^{13}C content of plant dry matter by assuming that Rubisco will discriminate against ^{13}C, which only constitutes for 1.1% of atmospheric CO_2, and for ^{12}C which accounts for 98.9% (Farquhar *et al.*, 1989). The biochemical discrimination derives from the significantly reduced affinity of the primary carboxylating enzyme, ribulose-1, 5-bisphosphate carboxylase against ^{13}C (Condon *et al.*, 2004; Farquhar *et al.*, 1982). $\Delta^{13}C$ refers to the ratio of $^{13}C/^{12}C$ in plant material relative to the ratio of these within atmospheric air (Farquhar & Richards, 1984). Principles of $\Delta^{13}C$ are summarised in Equation 2.10 where δ_p is the $\delta^{13}C$ of the plant material, δ_a is the $\delta^{13}C$ of atmospheric CO_2, taken as -8‰ (Hancock, 2012):

$$\Delta\ (‰) = [(\delta_a - \delta_p)/(1+\delta_p)] \times 1000 \qquad \text{Equation 2.10}$$

Farquhar & Richards, (1984) illustrated that $\Delta^{13}C$ is positively related to C_i/C_a; with the knowledge that A/T is negatively related to C_i/C_a, then $\Delta^{13}C$ should be negatively related to A/T (Condon *et al.*, 2004) (Figure 2.11) although the ^{13}C discrimination method itself does not provide an indication of whether differences in WUE derive from CO_2 assimilation (A) or water loss (T) (Farquhar *et al.*, 1989; McAusland *et al.*, 2013).

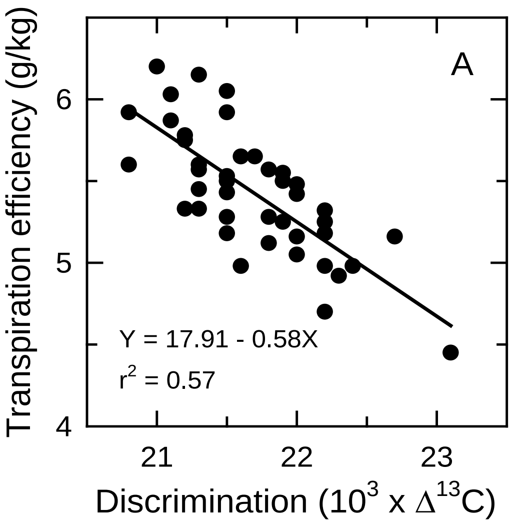


Figure 2.11. The negative correlation between $^{13}C\Delta$ and transpiration efficiency in wheat (Farquhar and Richards, 1984).

$\Delta^{13}C$ is proven to be a robust means of quantifying WUE (Condon *et al.*, 2004). It has been established that there is variation for $\Delta^{13}C$ between species, a prominent example of its use is the production of relatively new wheat cultivars, 'Reese' and 'Drysdale' developed for differing environments in Australia (Condon *et al.*, 2004). Additionally, $\Delta^{13}C$, is closely related to variation seen among plants for C_i/C_a. Indicative measurements of the $\Delta^{13}C$ of one einkorn, emmer, spelt species, as well as modern bread wheat for comparison was performed by Sparkes (2010). The field experiment suggested that emmer had the greatest WUE (indicated by $\Delta^{13}C$ of grain). This was further corroborated by Isaaks (2009) who measured ITE, $\Delta^{13}C$ and biomass production per unit water uptake, to report that emmer had the greatest WUE at each level of measurement.

2.14 Physiological characterisation of resource use in ancient and modern wheat

The current work describes the resource utilisation characteristics of three ancient wheat species with varying cytogentic classifications; namely spelt (*Triticum spelta* L.), emmer (*Triticum dicoccum*) and einkorn (*Triticum monoccoccum* L.). The project was designed to test whether the preliminary data collected at the University of Nottingham, namely concerning architectural characterisation, light interception and conversion (biomass production, radiation use efficiency, harvest index and grain yields) of an assortment of ancient wheat species (einkorn, emmer and spelt), in addition to a number of elite modern wheat varieties, were consistent across growing seasons and conditions. The ultimate aim was to therefore to determine whether these species were more efficient in their use of resource (namely water and radiation). Where previous work included one cultivar of each cytological group, the current project aimed to assess a greater number of representatives in order to observe whether the traits, such as enhanced radiation use efficiency (RUE), water use efficiency (WUE) and green area persistence, observed throughout preliminary analyses, were characteristic of the species or were simply cultivar-associated traits. Data collection from field and glasshouse experiments assessed a broad but fundamental spectrum of resource use efficiency traits. Analyses encompassed three main elements of growth and development, focussing on physiological aspects of water and radiation use efficiency, namely 1) canopy interception, 2) leaf photosynthetic capabilities and 3) water use.

2.14.1 Project overview and objectives

During the first two years of experimentation, data derived from conventionally managed field trials with judicious fertiliser applications so as to optimise canopy development. These provided a catalogue of traits, linked to radiation capture, conversion efficiencies, partitioning and yield formation. Investigation of these traits was also carried forward into the three glasshouse experiments (two are detailed in the current report), in which previous assessments were continued in addition to further measurements that focused down to leaf level traits with the aim to discern the potential basis of the enhanced RUEs observed in preliminary results and within the previous field trials. Glasshouse experiments also aimed to investigate the WUE of these ancient wheat species (using IRGA, carbon isotope discrimination and biomass to water uptake ratios) under irrigated and drought conditions as well as reporting aspects of growth, development and yield. The overall objectives of the work were to:

1) Identify and compare developmental differences, canopy growth, and potential biological yields for modern bread wheat as well as a variety of einkorn, emmer and spelt cultivars.
2) Characterise resource acquisition (of radiation and water) in einkorn, emmer and spelt, and compare these to those of modern bread wheat cultivars under limited and non-limited conditions.
3) Identify the basis of the physiological traits that endow enhanced capture and conversion of resources, scaling from the leaf to the canopy level.

2.14.2 Hypotheses

The specific hypotheses tested in this experiment were:

H_1) The quantities of biomass that einkorn, emmer and spelt accumulate will be comparable to those of modern bread wheat.

H_2) Modern bread wheat will produce fewer tillers and partition fewer assimilates towards tillers than all investigated ancient wheat species.

H_3) Spelt species will display the ‘stay green’ trait through increased green area duration compared to other genotypes.

H_4) Modern bread wheat will partition more carbon towards the ear than ancient species and hence have greater Harvest Index (HI).

H_5) Spelt germplasm will have greater RUE compared to all other genotypes.

H_6) Photosynthetic capacity will not differ between ancient wheat and modern bread wheat.

H_7) Ancient wheat varieties originating from the dry, rain-fed fertile crescent will have increased WUE deriving from lower transpiration rates and stomatal conductance.

3. Plant development, growth and canopy architecture

3.1 Field experiment materials and methods

3.1.1 Seed source and plant material

In the following field experiments it was important to include genotypes that represented all cytological groups (Table 3.1). The grain used was acquired from the seed store at the University of Nottingham in 2009. Each genotype was named so that they could be distinguished from one another rather than the name representing any true detail about their lineage or origins. Table 3.1 also outlines the species, genome group and the typical distribution (although the latter is again, not necessarily specific to these genotypes) as well as any additional details. Table 3.1 also includes a range of modern bread wheat cultivars, which were chosen from the HGCA Recommended List, released in 2008/2009. One modern bread wheat cultivar was chosen from each nabim group; this represents a classification term used by millers to indicate grain quality. These modern wheat cultivars were bought from seed companies, without treatment to mirror the conditions of ancient wheat seed.

Once acquired, seeds were then bulked in the glasshouse. These bulked seeds were used in the 2010 field experiment whereas for the 2012 field experiment, seeds originated from the previous harvest (in 2011). In 2011, grain underwent germination tests prior to drilling.

Table 3.1: Included genotypes in the study detailing species, genome group and general distribution. The table also outlines specific cultivars information when possible.

Variety	Species	Genome Group	Distribution	Details
Emmer	*T. dicoccum*	4n (AABB)	Italy, Ethiopia, India, Fertile crescent	Asturias, North Spain
Emmer SB	*T. dicoccum*	4n (AABB)	Italy, Ethiopia, India, Fertile crescent	
Spelt SB	*T. spelta*	6n (AABBDD)	Italy	
Spelt Tauro	*T. spelta*	6n (AABBDD)	Italy	West stow, UK
Spelt Oberkulmer	*T. spelta*	6n (AABBDD)	Italy	
Einkorn	*T. monococcum*	2n (AA/BB)	Marginal areas in Europe	Kastamonu, Turkey
Einkorn SB	*T. monococcum*	2n (AA/BB)	Marginal areas in Europe	
Einkorn Hungarian	*T. monococcum*	2n (AA/BB)	Marginal areas in Europe	Szazhalombatta, Hungary
Einstein	*T. aestivum*	6n (AABBDD)	UK	
Claire	*T. aestivum*	6n (AABBDD)	UK	
Xi19	*T. aestivum*	6n (AABBDD)	UK	
JB Diego	*T. aestivum*	6n (AABBDD)	UK	

Details: Information was provided by Dr. Amy Bogaard, School of Archaeology, University of Oxford who initially provided these four genotypes.

3.1.2 Field experiment 2010

A field trial, located at the University of Nottingham, Sutton Bonington Campus, south-west Nottinghamshire, England (52°49’53.55”N, 1°15’1.32”W) (2010 – 52° 50’ N, 1°15’) was established in the 2009/2010 winter wheat growing season. The site had a soil indices of P: 3, K: 3, Mg: 4 and a pH of 6.9 with a nitrogen (N) content of 174.1 kg ha-1 to a depth of $90cm^2$ (measured 12 November 2009). The site was managed conventionally, and established as part of a crop rotation on a sandy loam soil (the Dunnington Heath Series). The plots were drilled on 27 October 2009, and measured 24 x 1.63m each, arranged in a randomised block design with four replicates. The field experiment was conventionally managed using best farm practice to minimise weeds, pests and diseases. The experiments were rain-fed, without additional irrigation.

3.1.3 Field experiment 2011

In 2011 experiment, the field site, previously cropped with winter oats, was drilled on 13 October 2010 at 300 seeds m^{-2}. Prior to this, the field was ploughed (16 September 2010) and harrowed (11 October 2010). Soil was rolled after drilling on 14 October 2010. The soil was a sandy loam with soil indices of P: 4, K; 3, Mg: 4 and a pH of 7.2 and N content 32.9 kg ha^{-1}, measured to 90cm on 9 September 2010. The experiment was designed as a randomised block design with four replicates and was managed according to best farm practice as in the previous year.

3.2 Field measurements

3.2.1 Radiation interception, distribution and extinction

Photosynthetically active radiation (PAR), above and below the canopy was measured at varying heights within the canopy using the SunScan Canopy Analysis Systems (Delta-T Devices, Cambridge, UK). Ten measurements were made and the average was recorded. Fractional interception (*f*) was then calculated using the following equation:

$$f = I_0 - I / I_0 \qquad \text{Equation 3.1}$$

Where I_0 refers to the radiation above the crop canopy and I refers to the radiation below the canopy. These measurements were taken at 10cm intervals, beginning at 0cm intervals and moving up within the canopy allowing the average light transmitted through the canopy to be calculated at each horizontal height interval, whereby the measured PAR for positions within the canopy was subtracted from the incident radiation. Distribution curves were obtained by measuring PAR in three random positions along the length of each plot. This enabled the exponential decay of radiation to be calculated. Measurements were performed on 22 June 2010 when canopies were fully mature, with no indication of senescence or lodging. Comparisons of regression were used to determine the differences in light distribution between genotypes.

3.2.2 Crop sampling

During both growing seasons, plots were sampled using 1.2 x 0.6 m^2 ($0.72m^2$) quadrats at various stages of growth and development (detailed in Table 3.2 and 3.3). Sampling was randomised within the plot central strip to minimise edge effects. All plant material was cut at ground level and carefully removed from the field and subsequently stored at 4°C, for no more than seven days before processing. Prior to analysis any soil debris were removed and the sample total fresh weight was recorded. More detailed analyses (according to growth stage) were carried on ~25% sub-sample during which component parts of the canopy were separated and the fresh weight of each faction was recorded.

Table 3.2. Descriptions of wheat growth stage identifiers and benchmarks dates for general reference (HGCA, 2009).

Growth stage	Identifier of GS	Benchmark date
GS 31	First node detectable, stem extension starting.	10 April
GS 39	Flag leaf base all visible, no further leaves emerge.	19 May
GS 61	Start of flowering, little further extension of height occurs.	11 June

Table 3.3. Dates when key growth stages were reached and sampling took place in the 2010 and 2011 winter wheat growing season. Dates stated for 2011 provide average dates across the range of genotypes.

Growth analyses	Sampling date in 2010	Sampling date in 2011
GS 31 (~)	16 May, 2010	18 April, 2011
GS 39 (~)	9 June, 2010	14 May, 2011
GS 61 (~)	28 June, 2010	8 June, 2011

3.2.3 Pre-harvest samples

Samples were also collected directly before harvest when the crop was fully mature and had senesced completely. Quadrats measuring $0.72m^2$ were randomly placed and all above ground plant matter was collected and weighed. The ears of each sample were separated after the peduncle, their numbers counted and fresh weight recorded. So too was straw material and laminar (the latter was only collected in 2011). These samples were then oven dried at 80°C until samples had reached a constant weight. From pre-harvest samples, ear material was threshed, the grain collected and their weight and number recorded, together with the weight of the chaff. The average grain weight was calculated from these values and was used to calculate grain yield per m^2.

3.2.4 Radiation conversion efficiency

Utilising f measurements and total daily incident radiation from a nearby weather station, the cumulative PAR intercepted for each genotype was estimated for a time period throughout the growing season. These data, coupled with dry matter data from growth stage analyses for the same periods (see Crop sampling), quantified by destructive sampling, were used to calculate the conversion efficiency (RUE_{PAR}) of incident PAR (IPAR) to biomass to be calculated (Equation 3.2):

$$\text{DW Biomass} = RUE_{PAR}\, f\, I_0 \qquad \text{Equation 3.2}$$

This calculation allowed the quantities of radiation energy per unit area received and intercepted by the crop to be calculated, providing an indication of productivity relative to light energy flux received. The efficiency of radiation conversion was calculated both pre-anthesis and post-anthesis.

3.2.5 'Stay green' assessment

The 'stay green' of the flag leaf was assessed by indirectly measuring the chlorophyll content of fully expanded flag leaves using a Minolta Chlorophyll Meter SPAD-502 (Minolta Camera Company Limited, UK). SPAD measurements were taken from the flag leaf of ten randomly placed plants and the average of the flag leaf base, mid-section and leaf tip was recorded. The average per plot was used to assess the chlorophyll content of genotypes post-anthesis green area longevity and therefore assess whether green area could still be photosynthetically active.

4. Field Experiment Results

4.1 Establishment

Both ancient and modern wheat seeds alike were untreated which may be an important factor first as to why emergence was significantly different in both experimental years, as well as for the potential growth and development of the canopies. Rates of emergence differed between cultivars across both growing seasons but were more variable in 2011 than 2010 (Table 4.1). MBW cultivars had average emergence rates of 77.5 and 54.1%, in 2010 and 2011, respectively, whereas spelt, emmer and einkorn had significantly lower emergence rates in both years (34.0, 63.3 and 58.6% in 2010 and 15.3, 43.0 and 16.7% in 2011, respectively). As several genotypes had poor emergence (such as spelt Oberkulmer and Tauro), results stated here may not be truly indicative of a closed crop canopy. Further, the low establishment meant that weeds became more invasive, introducing a competitive factor to resource acquisition, potentially hampering the growth of some genotypes.

Table 4.1. Average rates of emergence with the corresponding number of plants per m^2 observed in 2010 and 2011.

	2010		2011	
Cultivar	Emergence (%)	Plants m^{-2}	Emergence (%)	Plants m^{-2}
Claire	88.1	264.2	54.9	164.7
Einstein	70.0	210.1	55.2	164.5
JB Diego	72.7	218.0	53.5	160.0
Xi19	79.3	237.9	52.8	158.4
Einkorn	69.5	208.6	19.0	57.0
Einkorn Hungarian	57.8	173.4	27.5	82.6
Einkorn SB	48.5	145.6	3.5	10.5
Emmer	64.9	194.7	34.2	102.4
Emmer SB	61.7	185.0	51.7	155.0
Spelt Oberkulmer	18.5	55.5	15.4	46.2
Spelt SB	57.8	173.4	6.6	19.9
Spelt Tauro	25.9	77.7	23.9	71.7
P	< 0.001	< 0.001	<0.001	<0.001
SED (33 DF)	4.56	13.69	3.0	9.0

Species averages for emergence in 2010 show MBW to have the highest emergence rates, followed by emmer, einkorn and spelt. In 2011, although species ranked similarly, average emergence was not consistently reduced by the same genotypes. Both ancient and modern wheat seeds were untreated which may be an important factor first as to why emergence was significantly different in both experimental years, as well as for the potential growth and development of the canopies. Seed treatments are primarily used to limit seed-borne diseases but can also be an effective control for foliar diseases such as bunt and *Fusarium* seedling blight (Paveley *et al.*, 2000). Potential pathogens known to affect emergence include fungi, *Microdochium nivale*, *Fusarium graminearum* or *Septoria nodorum,* all three of which cause seedling blight or thinning of the plant stand, often to reduce plant establishment (Clark *et al.*, 2004). Furthermore, these seedling diseases tend to be more severe if poor quality seed is used or if conditions at planting are not favourable for quick germination and establishment, for instance in 2011 the winter was particularly cold and seeds were exposed to prolonged frosts; all of which could have been instructive of the low establishment observed. Another factor was that drilling ancient species was problematic due to the difficulty in removing the glume and awns without damaging the seed, occasionally causing blockages during drilling. This was particularly the case for einkorn but all ancient species had non-threshing ears.

4.2 Plant development

Plant development differed. Observational data of development dates indicate that all modern bread wheat cultivars developed faster than ancient species. This meant that as well as having varying numbers of plants per metre square, respective plots within the experiment reached varying developmental stages in disparate environmental conditions. Although many of the physiological aspects discussed here are comparatively assessed, it is important to note these factors as they may have implications for findings in the following discussions.

4.3 Canopy development, architecture and light interception

4.3.1 Shoot production and tiller economy

When assessed by species, differences between total and fertile tiller numbers were highly significant at all growth stages (P <0.001) (Table 4.2). Species averages, showed fertile tiller numbers to be greatest at growth stage 31, after which production of fertile tillers reduced. This was expected as at later growth stages, shoots often die or production slows (Sparkes *et al.*, 2006). Spelt species carried the largest proportion of shoots carried through to maturity (growth stage 61 or later) whereas einkorn carried the least (72.45 and 65.39, respectively). Total and fertile shoot number followed a similar pattern among all cultivars; fertile shoots initially peaked at growth stage 31, reduced around growth stage 39 and then plateaued by growth stage 61.

Table 4.2. Average shoot number and fertile shoot number (per m^2) according to growth stage by species (including SED) in 2010.

	Total Shoot Number (m^{-2})			Fertile Shoot Number (m^{-2})		
	Growth Stage (approx.)			Growth Stage (approx.)		
Species	31	39	61	31	39	61
Einkorn	1661.8	1344.9	1638.1	1614.1	920.1	1071.3
Emmer	1054.6	777.3	805.9	1051.1	512.4	541.7
Spelt	808.5	722.8	648.5	744.6	549.1	468.6
MBW	1023.0	760.8	809.5	982.9	519.1	562.9
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
SED (44 DF)	146.03	115.22	152.80	139.48	99.30	80.96

In 2011, shoot numbers, analysed according to species averages, showed no particular trend for shoot number per unit area (Table 4.3). In 2011 there was an initial spike in total shoot numbers at growth stage 31, as observed in 2010. However, high tiller production in 2011 was thought to be a result of the low establishment recorded, especially for einkorn species. It is thought that the period in which tiller initiation occurred was extended as a result of low population density (Sparkes *et al.*, 2006). Modern bread wheat, on average, produced some of the highest total shoot and fertile shoot numbers, 70.8% of which were carried through to create productive tillers. Fertile shoot numbers varied significantly between species at growth stage 31 and 61 ($P < 0.001$ and 0.042, respectively), indicating large differences in early canopy expansion. Overall, the number of fertile shoots reduced from growth stage 31 to 39 and increased from growth stage 39 to 61. At growth stage 61, einkorn had the highest number of fertile shoots per unit area (468.2m^{-2}) compared to spelt which had the lowest number of fertile shoots (261.2m^{-2}), potentially a result of the low emergence observed for spelt in 2011. Einkorn and spelt species had the highest and lowest tiller survival, respectively.

Table 4.3. Average species shoot numbers and fertile shoot numbers (per m^2) according to growth stage (including SED) in 2011.

	Total Shoot Number (m^{-2})			Fertile Shoot Number (m^{-2})		
	Growth Stage (approx.)			Growth Stage (approx.)		
Species	31	39	61	31	39	61
Einkorn	586	543.2	541.1	508.1	261.6	468.2
Emmer	969.1	301.8	473.7	796.6	125.0	335.4
MBW	877.5	612.1	505.2	699.8	264.7	360.8
Spelt	389.1	316.3	380.4	317.7	174.8	261.2
P	<0.001	0.017	0.326	<0.001	0.243	0.042
SED (44 DF)	108.9	121.3	95.7	93.3	80.2	73.9

In contrast to data from 2010, these results suggest that tiller production reduced from growth stage 31 to 39 and then increased from growth stage 39 to 61, which may have been indicative of the increased vegetative period for ancient species. In both years MBW cultivars had greater emergence rates with larger numbers of shoots; these were probably attributed to greater plant populations. The described data however is highly limited by establishment that may have skewed the results of ancient species. Spelt had the lowest number of fertile shoots at each growth stage when compared to other species; spelt also had the lowest emergence rate of assessed species.

4.3.2 Green Area Index (GAI)

Leaf area for wheat undergoes seasonal development patterns; initially low temperatures suppress leaf area. As temperatures increase, leaf area also increases after which it then began to decline due to senescence (Hay & Porter, 2006). In 2010, results followed this trend where green area index (GAI) increased from 203 DAS to 227 DAS (which corresponded to growth stages 31 and 39, respectively) after which each genotype either plateaued or reduced in terms of GAI, except for einkorn species (Figure 4.1). The reduction of GAI at this point would potentially limit yield; the reduction of photosynthetically active green area could be purported to decrease the capacity of the canopy to accumulate carbon assimilates when grain filling occurs.

Total green area index (GAI) was significantly different between genotypes on all harvest dates (data not shown) but when averaged across species, no significant differences were found (Figure 4.1).

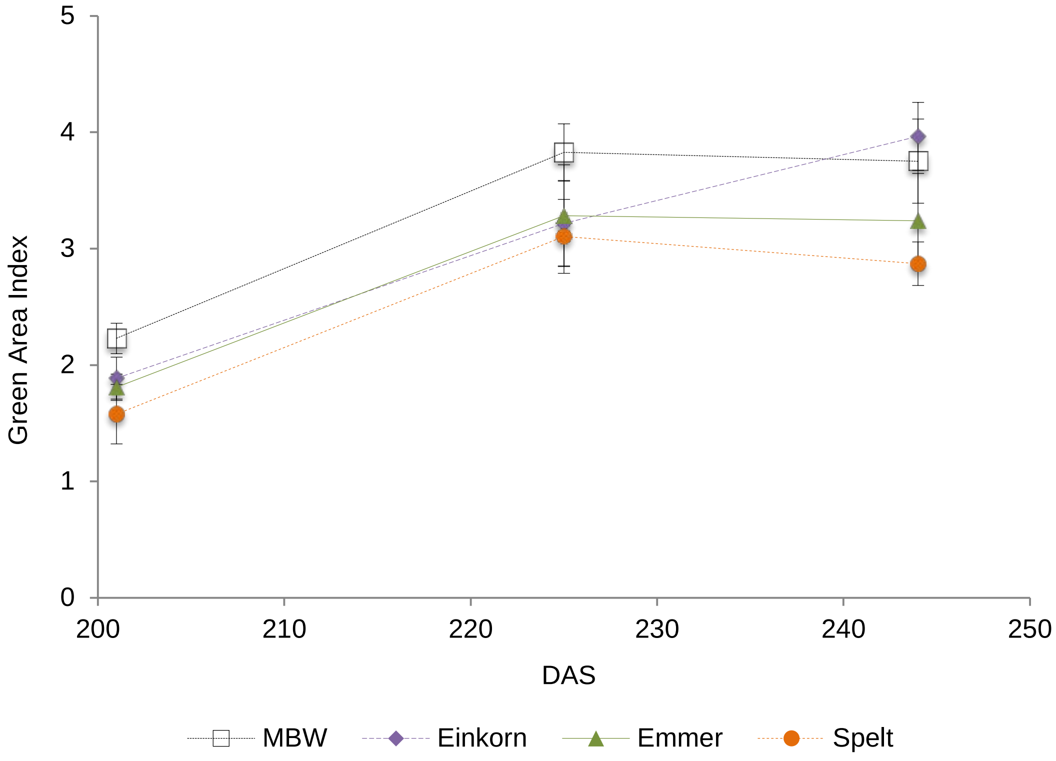


Figure 4.1. Total green area index of spelt, einkorn, emmer and modern bread wheat species averages in 2010 (P = 0.67, 129 DF, SED = 0.4341) (including species SEM).

Trends observed in 2011 were consistent with those observed for GAI in 2010. Differences were significantly different on all sampling dates, which coincided with growth stage 31, 39 and 61 (genotypic data not shown). Differences between species (Figure 4.2) were also significant on the first two sampling dates (P <0.001 and P = 0.002, respectively) but not on the final sampling date. Modern bread wheat had the greatest GAI at 194 and 216 DAS, (corresponding to growth stage 31 and 39) when compared to all ancient wheat species. Modern bread wheat cultivars reached growth stage 61 first, followed by spelt, emmer then einkorn.

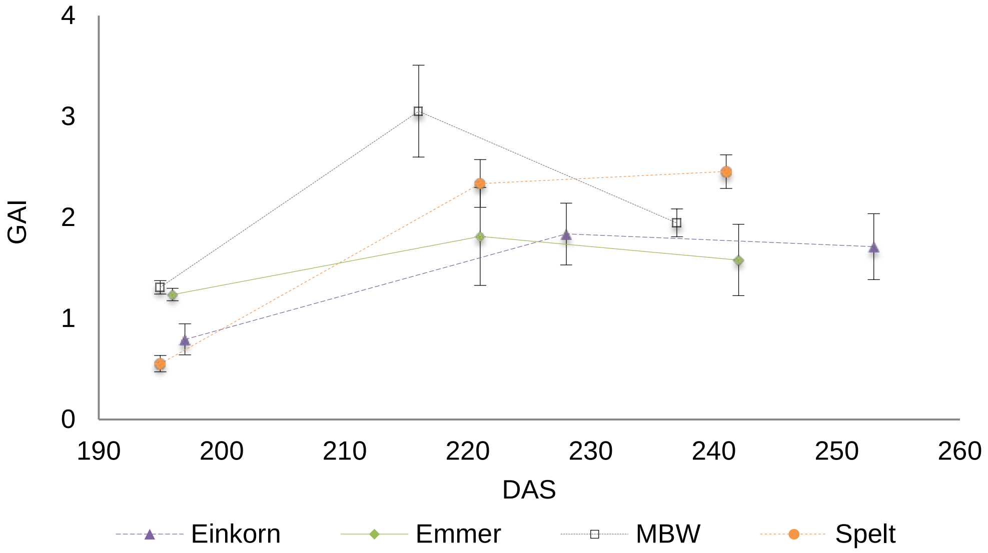


Figure 4.2. Species averages for total green area index of spelt, einkorn, emmer and modern bread wheat (error bars represent species SEM).

Genotypes sampled on the first and second sampling dates were ranked similarly in both 2010 and 2011. Furthermore, in both years, ancient wheat species (einkorn and emmer in 2010, spelt in 2011) 'overtook' modern bread wheat for GAI on the final sampling date.

4.3.3 Radiation interception, distribution and extinction

Regression analyses illustrated significant differences between the light extinction through the crop canopies among different genotypes (P <0.001) and by species (P <0.001). Light transmission for all assessed genotypes were found to fit a logistic curve which described 93% of the variation observed; non-linear regression was used to determine a fitted model for each of the cultivars after which, fitted curves were plotted using the Gaussian equation:

$$A + C\,(1{+}e^{(-B\,(X - M))}) \qquad \text{Equation 4.1}$$

The equation states X to be the height at which the SunScan probe was inserted into the canopy. Predicted curves were attained using parameter estimates from regression analyses (outlined in Table 4.4).

Table 4.4. Estimates of parameters for light distribution fitted curves for four wheat species.

Species	B	M	C	A
Einkorn	0.04960	47.11	1.0060	-0.0140
Emmer	0.04794	52.53	0.9810	-0.0110
MBW	0.06450	36.18	1.0430	-0.0019
Spelt	0.05165	52.87	0.8797	0.0821

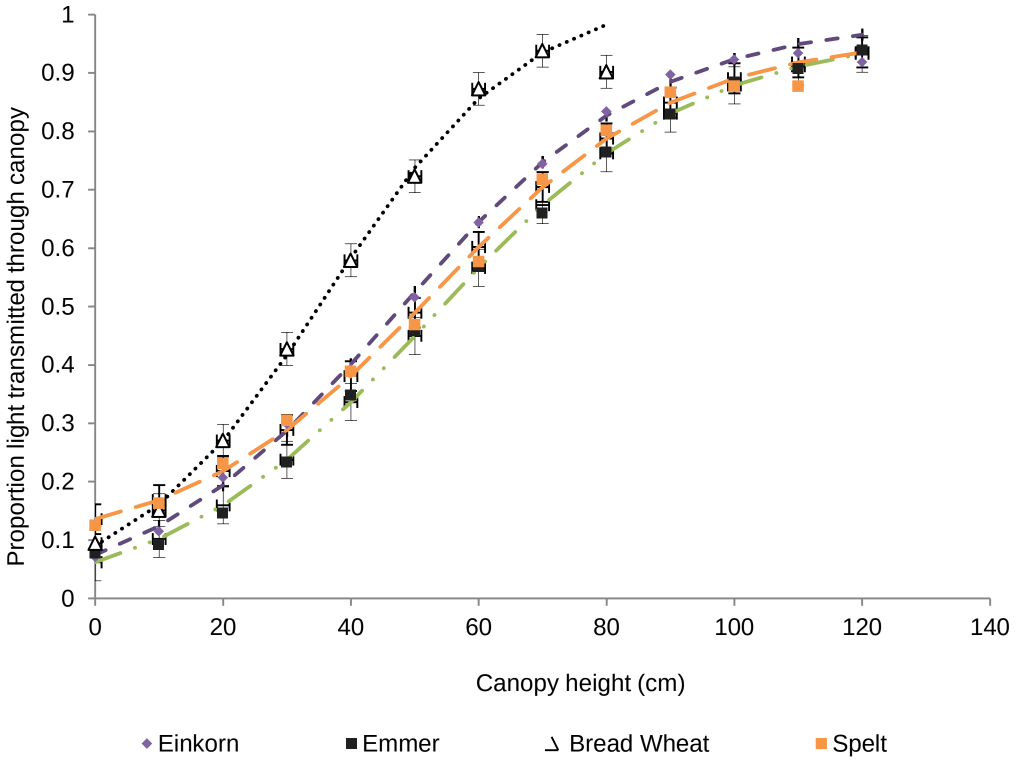


Figure 4.3. Light extinction of four wheat species in 2010 (P <0.001, SED = 0.00315, DF = 15) showing MBW, einkorn, emmer and spelt species averages.

Data indicated that MBW intercepted the largest proportion of light per 10cm leaf layer (Figure 4.3). Einkorn intercepted greatest proportion of light per unit leaf layer of ancient wheat species, followed by spelt and emmer. The canopy arrangement of MBW therefore, allowed less radiation to be transmitted to lower canopy layers meaning that modern wheat species intercepted more ambient light in the upper canopy layers. However, MBW cultivars were also much shorter, therefore, interception started ‘later’. Despite this, modern wheat interception more PAR per leaf layer compared to ancient species; this is indicated by the more staggered extinction of light for ancient species, allowing leaves further down the canopy to intercept ambient light. From observational comparisons of the two canopy types in the field, modern bread wheat had a ‘denser’ canopy structure overall, therefore leaves and ears were more condensed within the area that the canopy occupied, compared to ancient wheat canopies within which leaves and ears were much more spaced. These findings also support that the *Rht* allele introgression reduces height to impact light distribution; the interception of MBW at the uppermost leaf layers, most notably the highly productive flag leaves, intercepted the greatest proportions of light, allowing for an efficient distribution use of radiation, given the spatial arrangement of flag leaves in particular. Reduced height therefore means that middle and bottom layers of the canopy received a smaller proportion of PAR.

4.4 Crop sampling

4.4.1 Biological yield (2010)

The quantities of dry matter produced per m^2 throughout the season were not significant by genotype or by species however data does suggest some fundamental differences between canopy expansion between ancient and modern wheat. Data shown here includes biomass production by each species (genotypic data not shown). MBW cultivars produced the most biomass on all sampling dates except for pre-harvest, at which spelt species produced the greatest biomass (1267g m^{-2}) (Figure 4.4) indicating vigorous canopy expansion, compared to ancient species early in the season. Differences between species and growth stage were significant (P <0.001) however, the combined effects of these factors were not. It is noteworthy that MBW spelt species had increased biomass accumulation late in the growing season when compared to other species, which either declined or plateaued beyond growth stage 61.

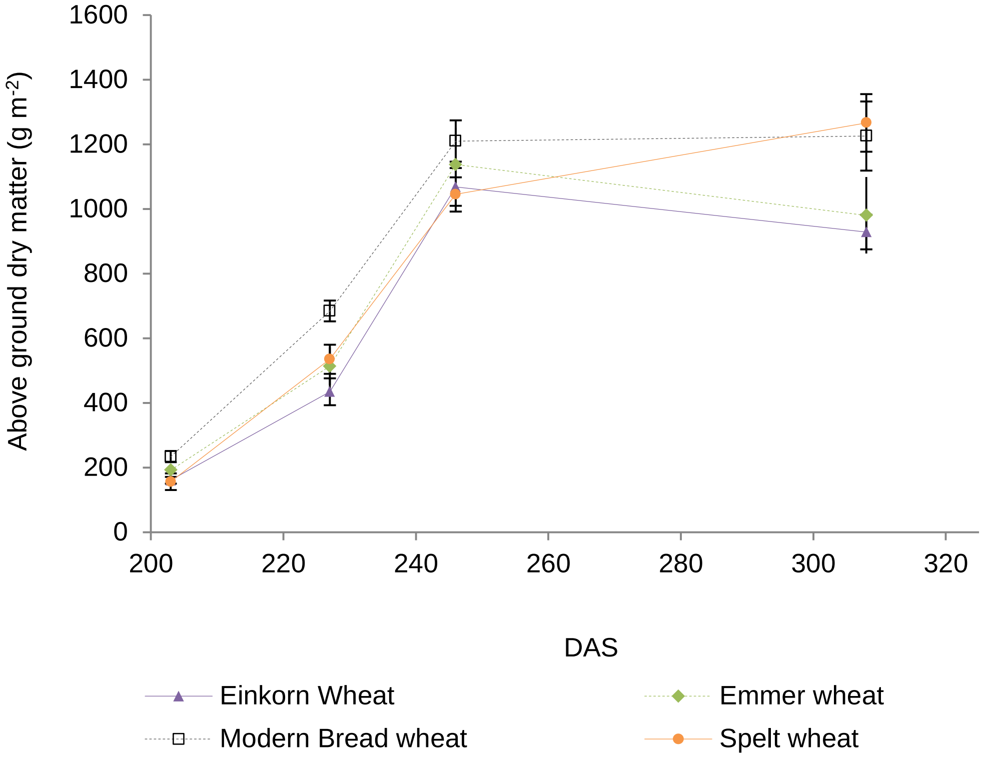


Figure 4.4. Species dry weight accumulated (g/m^2) in 2010 (P = 0.13, 173 DF, SED = 94.9).

4.4.2 Biological yield (2011)

In 2011, MBW cultivars produced the greatest biomass early in the season (ranging from 127.04 to 182.22g m^{-2}) (Figure 4.5). Differences were observed across growth stages (P <0.001). Modern bread wheat and spelt genotypes tended to show similar patterns of biomass accumulation (as was the case in 2010); however, more variation was observed within einkorn and emmer genotypes, potentially a result of the highly variable emergence rates observed. MBW accumulated the greatest quantities of above ground biomass compared to all other species on all sampling dates, except 242 DAS. Overall, einkorn species produced the least biomass at growth stages 39, 61 and pre-harvest. Spelt genotypes produced the greatest amounts of biomass after modern bread wheat across the season which was surprising since it was spelt genotypes that also had the lowest recorded emergence rates in 2011.

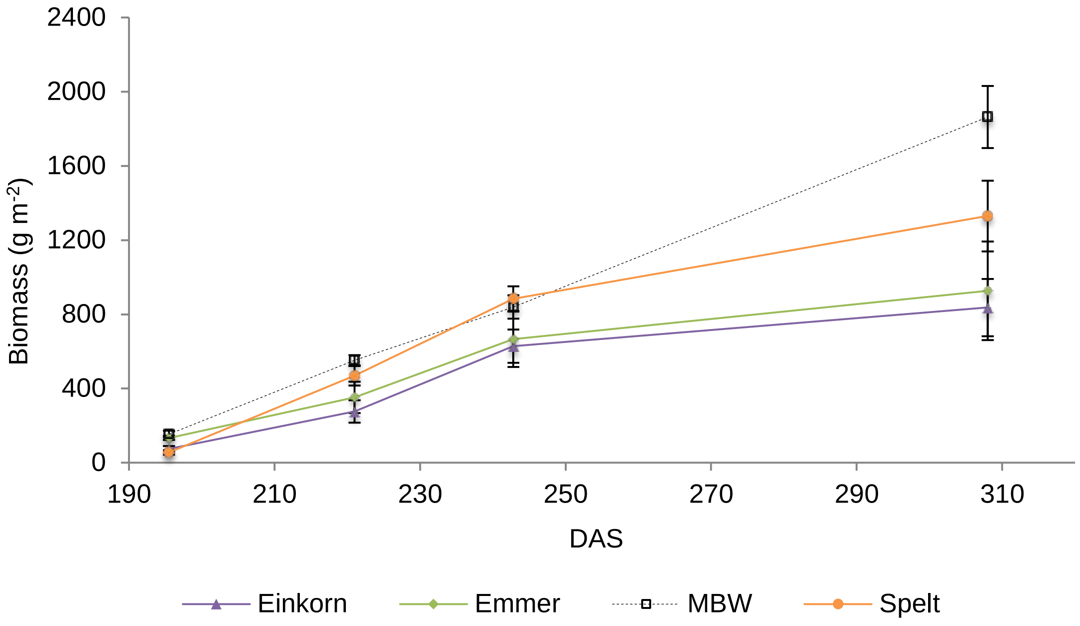


Figure 4.5. Species average for biomass accumulated throughout the 2011 winter wheat growing season (error bars indicate SEM by species) (P <0.001, DF 173, SED = 148.5).

4.4.3 Pre-harvest biomass partitioning

In 2010, analysed by species, MBW cultivars partitioned a larger proportion of assimilates, overall, towards the ear (P <0.001) whereas ancient wheat species, particularly einkorn, were observed to invest more assimilates in fertile shoots as leaf and shoot material (Figure 4.6 A). This trend was observed in 2011 also indicating that modern bread wheat generally partitioned more assimilate towards the ear (P <0.001) whereas ancient genotypes directed the large proportion to shoots (although insignificant) and leaves (P = 0.002) (Figure 4.6 B).

A

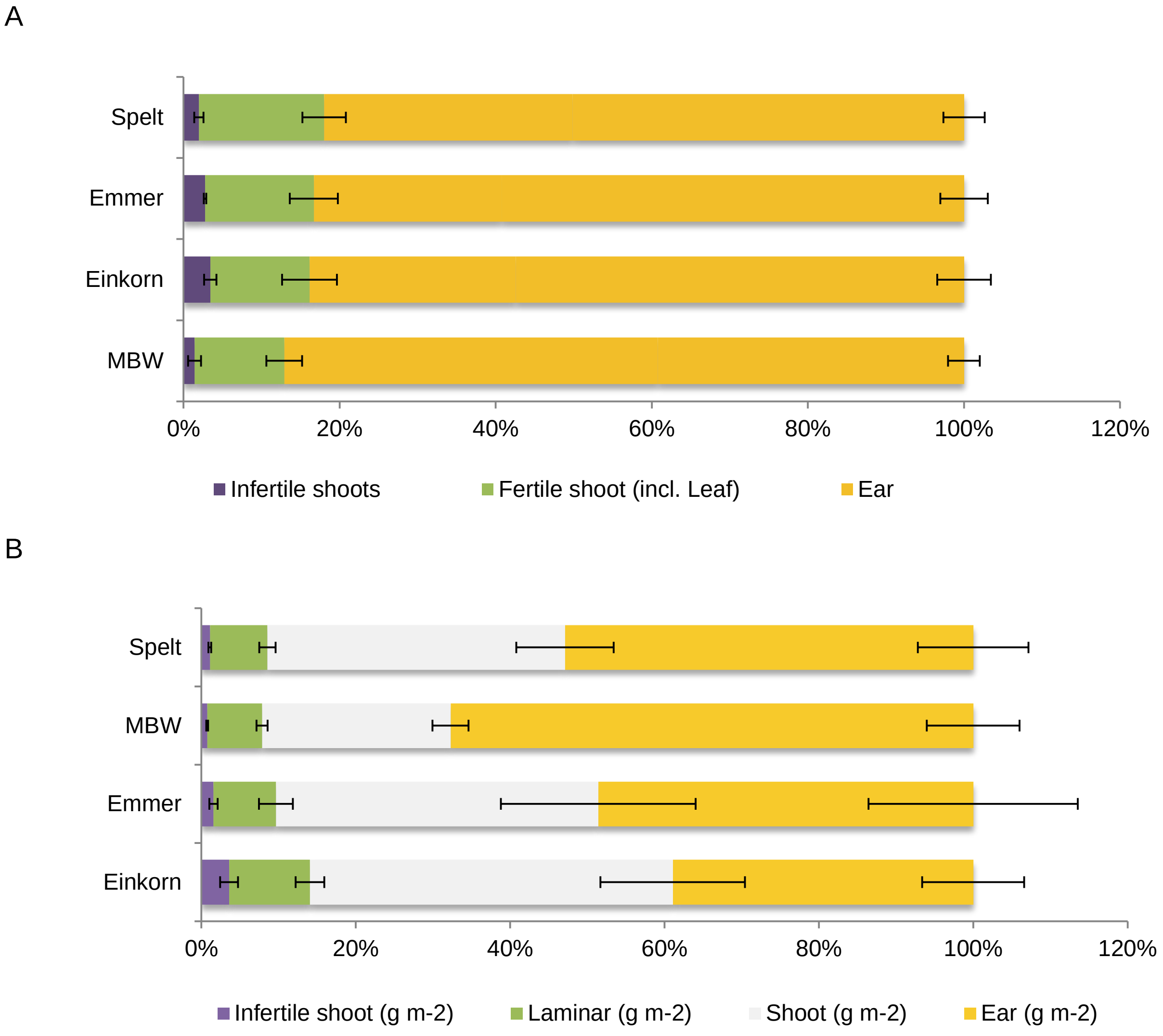


Figure 4.6. Pre-harvest component analysis of assimilates partitioned toward A) infertile shoots (P = 0.06, SED = 0.86) ears (P <0.001, SED = 3.50) and fertile shoots (including leaf and straw) (P <0.001, SED = 3.54) in 2010. B) Pre-harvest partitioning of species towards infertile shoots (P = 0.1, SED= 7.81), lamina (P = 0.002,SED =15.3), shoots (P = 0.297, SED = 75.3) and ear (P <0.001, SED = 98.6) all of which had 41 DF.

4.5 Conversion efficiency of biomass production

4.5.1 Radiation conversion characterisation (2010)

As there was a large amount of variation for the following traits, genotypic data and species data has been displayed. Rather surprisingly, despite low emergence, einkorn and einkorn SB showed the greatest RUE of all genotypes. However, rankings showed that Claire, Einstein and Xi19 also had high RUE which ranged from 1.49 to 1.42 MJ^{-1} (Table 4.5). This corresponds with Siddique *et al.* (1989) who found MBW to have greater RUE compared to older wheat genotypes. Spelt Oberkulmer had the lowest pre-anthesis RUE (1.19g MJ^{-1}) followed by JB Diego (1.20g MJ^{-1}). Also included were analyses of harvest index (HI) which indicated that, as would be expected in light of breeding targets, HI was greatest for modern cultivars. Differences were significantly different between genotypes (P<0.001) as were the amounts of biomass that were directed towards the ear (P = 0.006).

Table 4.5. Ear biomass, ear index, pre-anthesis radiation use efficiency and post-anthesis radiation use efficiency (PAR) for twelve wheat genotypes. Field experiment, 2010.

Genotype	Ear biomass (g/m^2)	Harvest Index	ε_{PAR} ($g\ MJ^{-1}$) (pre-anth.)	ε_{PAR} ($g\ MJ^{-1}$) (post-anth)
Claire	703.5	0.56	1.49	1.00
Einkorn	360.7	0.41	1.61	1.09
Eink. Hungarian	331.1	0.40	1.30	1.28
Einkorn SB	374.0	0.36	1.63	1.00
Einstein	672.3	0.62	1.43	1.12
Emmer	387.6	0.33	1.22	1.00
Emmer SB	376.4	0.43	1.38	1.00
JB Diego	873.6	0.58	1.20	1.36
Spelt Oberkulmer	547.0	0.51	1.19	0.73
Spelt SB	689.2	0.51	1.21	0.73
Spelt Tauro	631.4	0.42	1.41	0.85
Xi19	779.7	0.62	1.49	0.94
P	0.006	<0.001	0.147	0.013
SED (33 DF)	152.6	0.06	0.19	0.15

Overall RUE values were lower than expected. Species RUE averages did not differ significantly pre-anthesis (Table 4.6) however differences did differ post-anthesis (P = 0.012), ranging from 0.84 (spelt) to 1.12 (for einkorn). Overall, einkorn and modern bread wheat displayed the highest RUE. In addition, spelt species produced comparable ear biomass when compared to modern bread wheat.

Table 4.6. Ear biomass, ear index, pre-anthesis RUE and post-anthesis RUE species averages (RUE calculated from intercepted PAR). Field experiment 2010.

Genotype	Ear Biomass (g/m^2)	Ear Index	ε_{PAR} (pre-anth.) ($g\ MJ^{-1}$)	ε_{PAR} (post-anth.) ($g\ MJ^{-1}$)
Einkorn	355.3	0.39	1.39	1.12
Emmer	382.0	0.38	1.42	1.06
MBW	757.3	0.59	1.46	1.10
Spelt	622.5	0.48	1.23	0.84
P	<0.001	<0.001	0.215	0.012
SED (41DF)	87.6	0.04	0.12	0.10

4.5.2 Radiation conversion characterisation (2011)

In 2011, harvest indices for all species were lower than in 2010. Einkorn Hungarian and Xi19 had the greatest HI whereas spelt Tauro had the lowest (Table 4.7). Pre-anthesis radiation use efficiency varied significantly ranging from 0.20g MJ^{-1} recorded for emmer (thought to be an artefact) to 1.78g MJ^{-1} (einkorn SB). The highest observed RUE was for einkorn SB and spelt

Tauro, both pre- and post-anthesis. Consistent with 2010, einkorn and einkorn SB showed comparatively high RUE and high harvest indices. Post-anthesis RUE was highest for einkorn species, potentially due to the low establishment observed; this meant that individual plants could expand into the space without resource limitation or space restraints. However, high pre- and post-anthesis RUE did not necessarily equate to high harvestable product since einkorn SB had among the lowest ear index of all genotypes in 2010. MBW cultivars also had amongst the highest RUEs recorded, except for JB Diego (1.04g MJ^{-1}). Radiation use efficiency for all genotypes reduced from pre- to post-anthesis, except for emmer and JB Diego which had comparatively high RUE post-anthesis. Interestingly, all spelt genotypes maintain relatively high RUE even post-anthesis, this was also true of modern bread wheat Claire, Xi19 and Einstein highlighting a potential to utilise radiation intercepted after anthesis. Such a trait is likely to be of benefit, particularly towards the end of the winter wheat growing season when irradiance levels are likely to be highest.

Table 4.7. Ear biomass, ear index, post- and pre-anthesis radiation use efficiency (intercepted PAR) for twelve modern and ancient wheat cultivars from the field experiment carried out in 2011.

Genotype	Ear biomass (g/m^2)	Harvest Index	ε_{PAR} (pre-anthesis) (g MJ^{-1})	ε_{PAR} (post-anthesis) (g MJ^{-1})
Claire	634.1	0.52	1.45	1.42
Einkorn	519.2	0.54	1.57	1.26
Einkorn Hungarian	752.7	0.57	0.41	0.39
Einkorn SB	767.7	0.56	1.78	1.44
Einstein	601.5	0.56	1.54	1.48
Emmer	373.5	0.52	0.20	0.22
Emmer SB	1123.9	0.54	1.55	1.23
JB Diego	1259.2	0.52	1.04	1.13
Spelt Oberkulmer	1253.7	0.52	1.55	1.31
Spelt SB	438.6	0.49	1.76	1.42
Spelt Tauro	617.5	0.51	1.72	1.46
Xi19	697.0	0.57	1.48	1.37
P	0.298	0.999	<0.002	<0.001
SED (33 DF)	354.3	0.10	0.28	0.18

In 2011, species averages showed spelt genotypes, overall, to have the highest pre-anthesis RUE and emmer to have the lowest (P = 0.033) (Table 4.8). Species maintained the same ranking post-anthesis as pre-anthesis, although overall, modern bread wheat generally declined in terms of RUE by a much lesser degree than the other species. HI did not differ between species and neither did ear biomass produced per meter square, in fact ear biomass produced was comparable not only between spelt and MBW, as in 2011, but also between einkorn and emmer despite these species having the lowest RUEs observed.

Table 4.8. Species averages for ear biomass, ear index, post- and pre-anthesis radiation use efficiency (calculated from intercepted PAR) from the field experiment carried out in 2011.

Genotype	Ear Biomass (g/m^2)	Ear Index	ε_{PAR} (pre-anthesis) (g MJ^{-1})	ε_{PAR} (post-anthesis) (g MJ^{-1})
Einkorn	679.8	0.56	1.26	1.01
Emmer	748.7	0.53	0.87	0.73
MBW	798.0	0.54	1.38	1.35
Spelt	769.5	0.51	1.68	1.40
P	0.952	0.762	0.033	0.003
SED (41DF)	236.1	0.05	0.25	0.18

RUE for all species ranged from 0.87 to 1.68 MJ^{-1} across both years which was below that which was expected; according to a study performed by O'Connell *et al.* (2004), RUE for modern bread wheat ranged between 1.20–2.93g MJ^{-1}. Low RUE observed within the current experiments may have been brought about by assumptions made for the period of time leading up to growth stage 31, which were assumed to be 50% of that measured at GS 31 (the first SunScan sampling date). Although conclusions based on this data are by no means clear cut, the use of cumulative efficiency, rather than dry matter versus intercepted PAR, do provide a more reliable means to assess RUE (Kanton & Dennett, 2008). Therefore, it is reasonable that these data would provide an indication of the true RUE. In the current experiments, root matter was not assessed as a result of time limitation however this is likely to influence RUE also (O'Connell *et al*., 2004).

4.6 Canopy persistence and 'stay green' assessments

4.6.1 Canopy duration (2010)

In 2010, flag leaf persistence was assessed using SPAD measurements over time. SPAD values by species (Figure 4.7) showed significant differences between species and sampling dates (P <0.001) where modern bread wheat had increased chlorophyll content within the flag leaf compared to ancient species, however this declined beyond that for spelt species on the final three sampling dates.

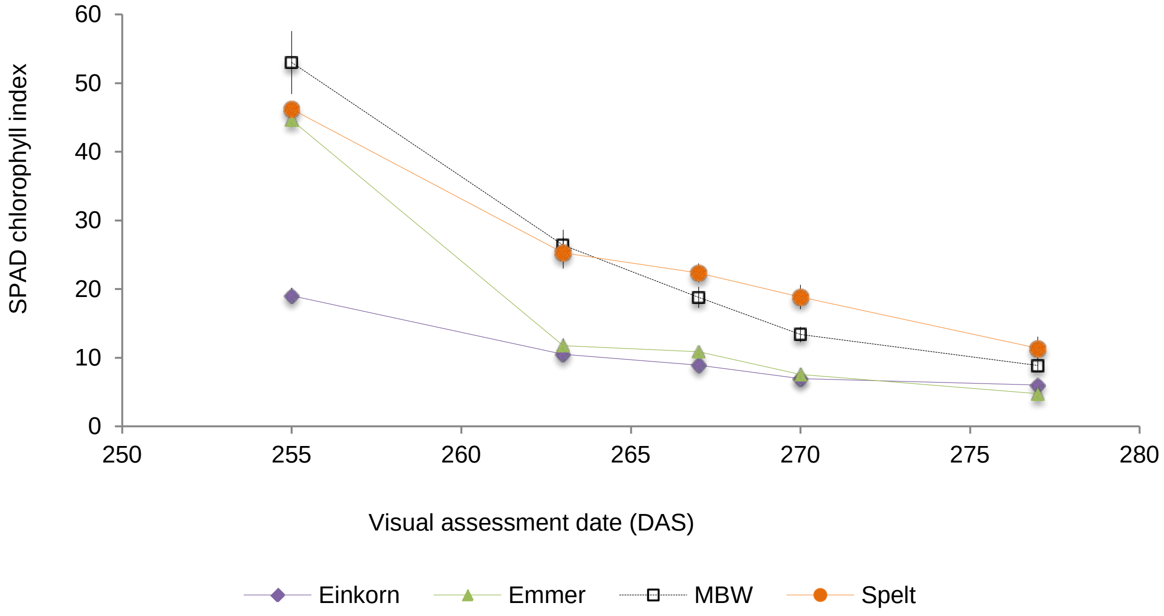


Figure 4.7. Average species SPAD values versus time (2010) (P <0.001, 217 DF, SED = 1.500) (error bars denote SEM) (beginning of DAS axis denotes the onset of anthesis).

Species ‘stay green’ was also assessed using visual scoring methods. Significant differences were observed (P <0.001) on all sampling dates (Figure 4.8). Spelt Oberkulmer and Tauro performed particularly well (genotypic data not shown), however species results mirrored the SPAD measurements, showing spelt to have the slowest decline in leaf greenness throughout the sampling period. Modern bread wheat initially had low recordings, indicating a high proportion of greenness in the flag leaf, however, this declined with time more rapidly when compared to other species. Einkorn species however had comparatively high visual scores on all dates, indicating that these genotypes had senesced to a large degree, even before the onset of sampling. The variation between ancient wheat cultivars was observed to be higher than that of modern wheat cultivars.

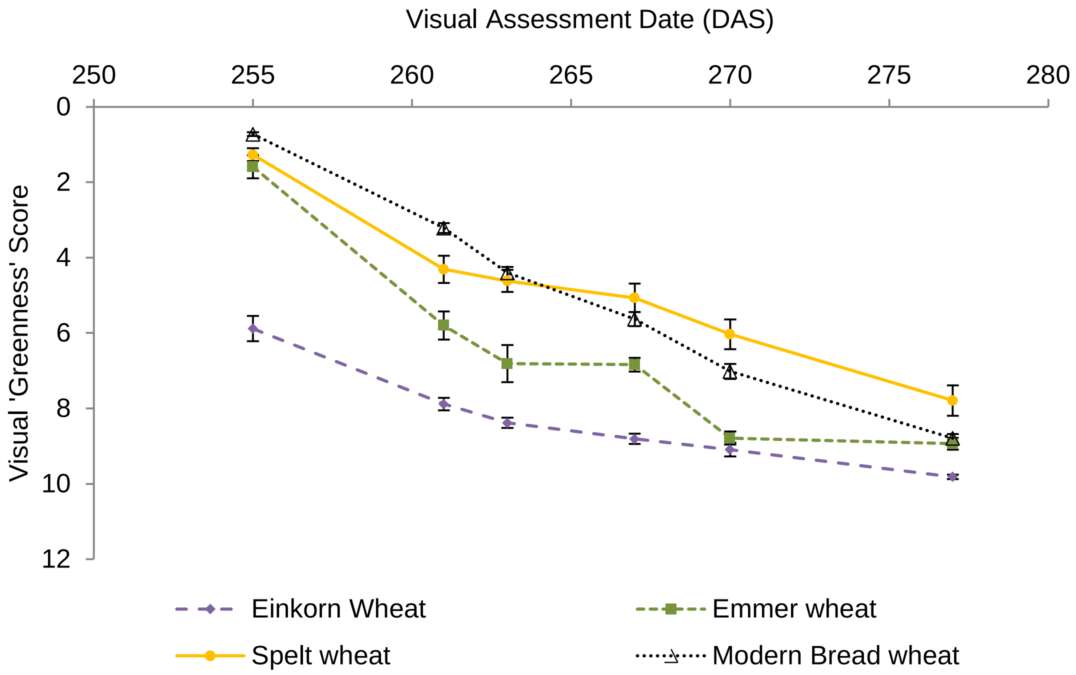


Figure 4.8. Average species visual ‘greenness’ scores versus time (DAS) (P<0.001, 261 DoF, SED = 0.3641) (error bars denote SED).

Linear regression was used to assess the relationship between SPAD scores and visual ratings for the four species (data not shown). For each species, there was a strong, linear relationship between visual score and SPAD value (P <0.001) but the slope of the relationship varied between species.

4.6.2 Canopy duration (2011)

It is thought that increased flag leaf duration correlates with yield (Gregerson *et al*., 2008; Hafsi *et al*., 2007) since longer crop seasons have been identified to positively correlate with yield (Takahashi & Nakaseko, 1990), therefore increasing productivity. Furthermore, it is thought that plants with a capacity to stay green for longer have an increased proficiency to resist drought in certain environmental conditions (Christopher *et al.*, 2008; Borrell & Hammer, 2000). Chlorophyll content measured through SPAD indices showed that all species tended to have high flag leaf ‘greenness’ at the start of sampling which corresponded with the average date at which genotypes reached anthesis, after which flag leaf chlorophyll content then began to decline. MBW had the highest flag leaf chlorophyll content, followed by spelt, emmer and einkorn (Figure 4.9). After this initial measurement however, SPAD readings declined significantly in MBW, einkorn and emmer, at which point spelt wheat had higher chlorophyll content than all other genotypes. Spelt maintained an increased SPAD index until the final measurement date. Einkorn showed the lowest SPAD values on all sampling dates (except for 277 DAS). Although all modern wheat cultivars had high SPAD readings at the outset of sampling, their decline was seen to be more abrupt than ancient species, particularly spelt which was observed to senesce at a slower rate, consistent with results from the 2010 experiment.

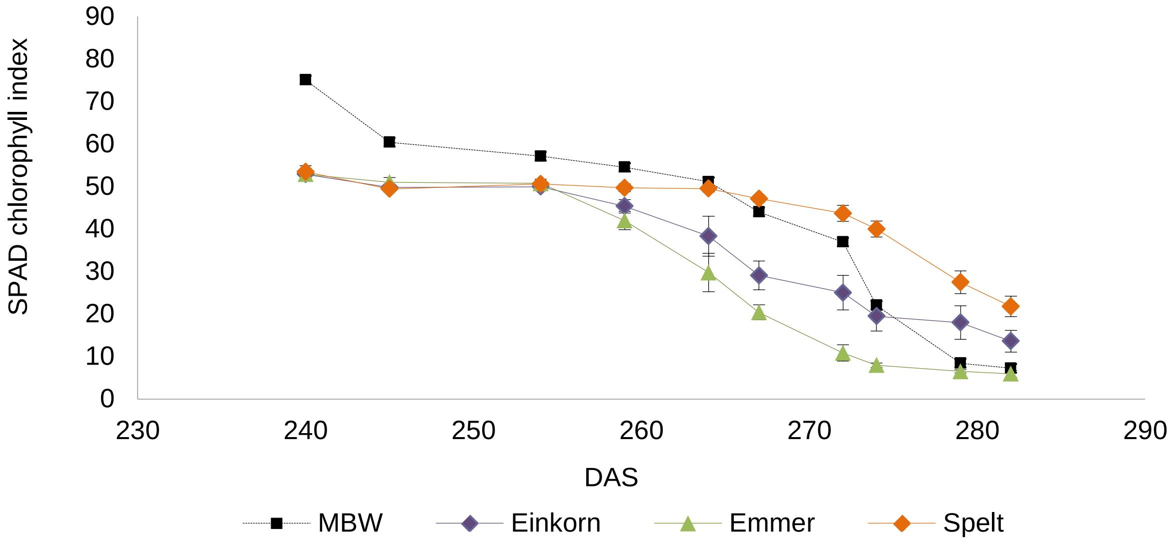


Figure 4.9. SPAD reading of twelve ancient wheat cultivars averaged over four species (spelt, modern bread wheat, emmer and einkorn) against days after sowing in the 2011 field experiments (P <0.001, 437 DF, SED = 3.118) (showing SEM by species).

Data gained from 2010 and 2011 was consistent, observed trends indicate that the flag leaf chlorophyll content of modern cultivars may be intrinsically higher than those of ancient species; therefore these cultivars may have an increased photosynthetic capability early in the season however this was not maintained post-anthesis. So, whereas spelt began with lower chlorophyll content, the species 'compensated' by maintaining the flag leaf for longer. The trends observed here are similar to those observed for GAI where bread wheat initially had larger GAI, which decreased drastically before GS 61. Spelt however maintained green area, corroborating the observation that spelt has higher canopy persistence (Sparkes, 2010). Larbi & Mekliche (2004) state that genotypes reported to have green area persistence during grain filling may be the key to securing wheat yields in semi-arid regions.

4.7 Field experiment conclusions

The field trials highlighted a number of differences between the modern and ancient wheat species. The ancient wheat species produced greater numbers of tillers than modern genotypes in 2010 only, however, they did consistently partition larger proportions of biomass towards shoot production, probably due to differences in developmental rates. MBW produced the greatest yields and consistently had greater HI. The investigations during field experiments also highlighted that although not the case for economic yields, ancient wheat species do have a proficiency to produce comparable biological yields that may be useful in the improvement of MBW (even with highly variable establishment between modern and ancient species). This proficiency to produce biological yield may well be due to green area persistence, consistently observed in spelt, furthermore, this is potentially where the observed high RUE for several ancient species derived. Results therefore indicate that ancient species warrant further investigation particularly in regards to green area persistence, RUE and biomass accumulation.

5. Biochemical and physiological aspects of photosynthesis and WUE

The second component of the current chapter details two glasshouse (GH) experiments, in which analyses were widened to include leaf level water use efficiencies, one under controlled water conditions (2012) and the other within a fully irrigated environment (2013).

5.1 Glasshouse experiment materials and methods

The following section outlines two glasshouse experiments that included the genotypes previously grown in the field experiments. These experiments were maintained in a glasshouse located on-site at the University of Nottingham, Sutton Bonington Campus, Loughborough (52d49’56.04”N, 1° 56.86”W) in 2011. In both experiments, disease and pest controls were applied as necessary. In addition, weeds were removed accordingly to limit resource competition. These measures were maintained throughout the experiment.

5.1.1 Water-limited glasshouse experiment (2012)

Seeds of all listed genotypes were sown in 14 x 20 module trays using John Innes No. 3, a high fertility potting mix, on 15 February 2012. Modules were half filled, one seed placed per module, and subsequently covered with soil. Plant material was transferred to a cold room maintained at 6°C and 12/12 hour light/dark photoperiod and left to vernalise for 54 days. Seedlings were checked on a daily basis and watered accordingly, after which a watering regime was established as described.

5.1.1.1 Column construction and irrigation

Plant material was grown in polyvinyl chloride (PVC) columns that allowed a maximum rooting depth of 1m. A total of 120 columns were constructed using PVC pipe, cut lengthways. Holes with a 5cm diameter were drilled at 20cm intervals into one half of the pipe and covered with gortex (Figure 5.1), after which the pipe was reconstructed, producing columns with a 15cm diameter. The underside of each column was sealed with gortex to allow drainage and filled with a 60/40% soil and sand mixture. Columns were subsequently saturated with water and left to drain and additional soil was added, a process that was repeated twice, after which no more soil consolidation occurred, allowing for compaction to represent natural field conditions. Columns were considered to be at full water holding capacity (WHC) after 48 hours of drainage.

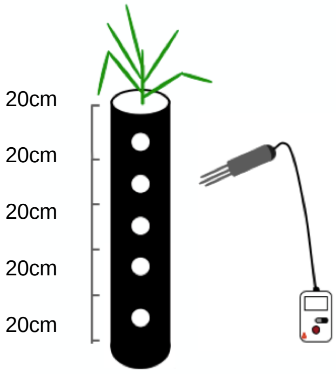


Figure 5.1. Columns constructed to grow plant material in glasshouse experiment II showing 20 cm increments where soil capacitance measurements were taken.

5.1.2 Irrigated glasshouse experiment (2013)

Glasshouse experiment III included a subset of the genotypes previously grown in the field in order to perform measurements of traits at the leaf level. In 2013, a glasshouse experiment was established to measure a range of parameters on a sub-set of those ancient wheat species that displayed favourable characteristics in previous investigations, however, this time, measurements were taken where irrigation was not limited. Seeds of the listed genotypes were sown into 2.54cm compartments within 14 x 20 module trays using John Innes No. 3 high fertility potting compost mix, on 7 December 2012. Plants were vernalised in a cool glasshouse for 62 days. Seedlings were transplanted into individual pots on 13 February 2013 and were checked and irrigated when necessary. Conditions were uniform within the glasshouse, after transplanting, plants were watered without limitation using a drip irrigation system. The experiment was established as a randomised complete block design with three blocks. Each block contained three pots per genotype.

5.2 Glasshouse measurements (GH 2012 and 2013)

5.2.1 Gas exchange response measurements (CO_2 and light responses)

In both 2012 and 2013 glasshouse experiments, gas exchange measurements were performed *in situ* using an Open Licor 6400 infra-red gas analyser portable photosynthesis system (Licor Lincoln Nebraska, NE, USA) between the hours of 10am and 3pm to limit the variation imposed by stomatal behaviour. All gas exchange measurements were made at ambient humidity (50–60%) on the mid-end section of the main stem flag leaf. Block temperature and humidity levels were maintained at 20°C and 50–60%, respectively in order to increase uniformity of results.

5.2.1.1 CO_2 response curves (irrigated GH experiment 2013 only)

In 2013, carbon dioxide response curves were obtained by monitoring gas exchange responses of the photosynthetic activity in leaves to intercellular CO_2 mole fraction (C_i) and were performed *in situ*. Measurements took place between 29 June and 14 July 2011 using an Open Licor 6400 portable photosynthesis system (Licor Lincoln Nebraska, NE, USA) infra-red gas analyser. Carbon dioxide response curves (four per genotype) were obtained by monitoring gas exchange responses of the photosynthetic activity in leaves to intercellular CO_2 mole fraction (C_i). This was performed using the programming functions of the Licor 6400–40 during which the section of leaf encapsulated in the chamber was exposed to 1000 µmol m^{-2} s^{-1} as standard. Leaf temperature averaged 23°C. The leaf was exposed to a series of ambient CO_2 concentrations: 385, 250, 100, 80, 60, 40, 400, 600, 800 and 1200.

5.2.1.2 Light response curves (GH experiment 2012 only)

In 2012, flag leaf response to differing levels of quantum flux (2000, 1500, 1000, 750, 500, 250, 100, 50 and 0 µmol m^{-2} s^{-1}) ranging from high to low so as to reduce the period the leaf spent acclimating and the effects of high light intensities on stomata. Due to the time consuming nature of these measurements however, these measurements were performed in glasshouse experiment II only. Carbon dioxide levels were maintained at 400 µmol m^{-1} throughout. Average leaf temperature was 24°C. A large amount of data had to be discarded due to machinery malfunction, and as a result light curve data was analysed using an unbalanced ANOVA. To ascertain quantum yield, photosynthetic response of leaves exposed to quanta below 200 µmol m^{-2} s^{-1} and under were plotted using linear regression in GraphPad Prism 6 (GraphPad software Inc. CA, USA).

5.3 Water Use Efficiency

5.3.1 Flag leaf ITE measurements (2012 and 2013 GH experiments)

Flag leaf measurements were performed in both glasshouse experiments, where appropriate variations in methodology between the two are described. ITE (mol mol^{-1}) was derived using the measured rate of photosynthesis and transpiration measured at a given point in time giving a ratio indicating the accumulation of carbon to water lost through stomata. Leaves were exposed to a constant photon flux rate of 2000 µmol m^{-2} s^{-1} and 385 µmol m^{-1} CO_2. In the 2012 glasshouse experiment (under drought), leaf photosynthesis, stomatal conductance and transpiration rates were measured simultaneously for all genotypes at two points (growth stage 39 as well as 61 + ~10 days) for pre- and post-anthesis characterisation of genotypes, whereas ITE measurements in the 2013 glasshouse experiment were performed at bi-weekly intervals beginning before anthesis to characterise ITE throughout development of the flag leaf.

5.3.2 Carbon isotope discrimination (GH 2013 only)

In the 2013 glasshouse experiment only, carbon isotope discrimination was used as another means to assess post-anthesis WUE. Prior to plant growth and development analysis, all grain was harvested. Grain samples were analysed for carbon isotope composition ($\delta^{13}C$) using mass spectrometry at the NERC Isotope Geosciences Laboratory, UK. This was converted to carbon istope discrimination ($\Delta^{13}C$) using the following equation:

$$\Delta (‰) = [(\delta_a - \delta_p)/(1+ \delta_p)] \times 1000 \qquad \text{Equation 5.1}$$

where δ_p is the carbon isotope composition ($\delta^{13}C$) of the plant material and δ_a, the $\delta^{13}C$ of atmospheric CO_2, taken as -8‰ (Farquhar *et al*., 1989).

5.3.3 Measuring soil capacitance for water uptake (2013 GH experiment only)

Using fresh and dry weights of known soil volumes coupled with capacitance measurements (using the Theta Probe Soil Moisture Sensor ML2 with data logger, Delta-T Devices, Cambridge) at each soil level (20, 40, 60, 80 and 100cm), it was possible to determine the volumetric soil moisture content of the entire column. This was used as an indicator of the 'water holding capacity' for the purpose of this experiment. The average soil moisture content of soil at 100% WHC was 0.655 $m^3\ m^{-3}$. The first column of each strip (5 columns per genotype) was used to measure soil moisture content, after which each column within that strip was restored to 80% WHC pre-anthesis, and 50% WHC post-anthesis. Soil moisture measurements were then made approximately every four days at which time the columns were irrigated accordingly. This data was coupled with biomass data to produce biomass to water uptake ratios providing the WUE throughout the growing season.

5.4 Plant growth and development analyses

5.4.1 Biomass and yield

In the water limited glasshouse experiment (2012), destructive sampling methods were used where biomass was assessed at key growth stages, sampling one column per growth stage, per genotype. Plants were cut at soil level where all above ground biomass was collected and transported to the laboratory after which all components of the canopy were separated, oven dried at 80°C until a constant was reached, then subsequently weighed. The final two columns remaining at full senescence were used as harvest samples. In the fully irrigated glasshouse experiment (2013) however, destructive analyses were carried out only once plants had reached full maturity and had therefore undergone full canopy senescence.

5.4.2 Biomass and yield

In 2013, plants were cut at soil level, all material collected and subsequently components of the canopy were separated (grain and total biomass is specified in the results section), dried at 80°C until they reached a constant weight. This was performed once the canopy had fully matured, post-harvest only.

5.5 Specific leaf area (2013 GH experiment only)

In the 2013 irrigated glasshouse experiment, several mature, fully expanded flag leaves without any obvious symptoms of pathogen attack were collected per genotype. The flag leaf areas were measured and recorded after which they were oven dried at 80°C until a constant weight was achieved. The dry weight per unit area was then calculated allowing assessment of leaf density.

5.6 ‘Stay green’ assessments

In both glasshouse experiments (2012 and 2013), ‘stay green’ persistence of the canopy was measured using a Minolta Chlorophyll Meter SPAD-502 (Minolta Camera Company Limited). SPAD measurements were performed on each plant at 3 points on the flag leaf, and the average of these was recorded. Measurements began as soon as the flag leaf emerged for each genotype and continued approximately every five days until senescence of the flag leaf was complete.

5.7 Data analyses

All data was stored using Microsoft Excel 2010 for Mac or GraphPad Prism 6 for Windows. Analyses of variance were performed to determine the significance of differences for parameters between genotypes using GenStat (16th Edition, VSN International Ltd.) for Windows (XP).

6. Glasshouse Experiment Results

6.1 Carbon dioxide response curves

6.1.1 Biochemical aspects of photosynthesis (2013 GH experiment only)

Exposure of a leaf to varying atmospheric CO_2 concentrations (C_a) will affect photosynthetic rates and the intercellular CO_2 mole fraction (C_i) within a leaf. The relationship between these parameters is not linear and therefore provides a means by which the biochemical and stomatal limitations on photosynthesis may be assessed quantitatively (Long & Bernacchi, 2003). The following section outlines various biochemical capacities (primarily for V_{cmax}, J_{max} and TPU) investigated in order to observe whether there are fundamental differences for carbon assimilation between genotypes. Figure 6.1 shows a typical response when the leaf was exposed to varying CO_2 concentrations, generated by a curve fitting tool (Sharkey *et al*., 2007) from which the values in Figure 6.2 and 6.3 derive. In the irrigated glasshouse experiment (2013), AC_i curves were performed pre- and post-anthesis to see whether there was any variation due to developmental stage.

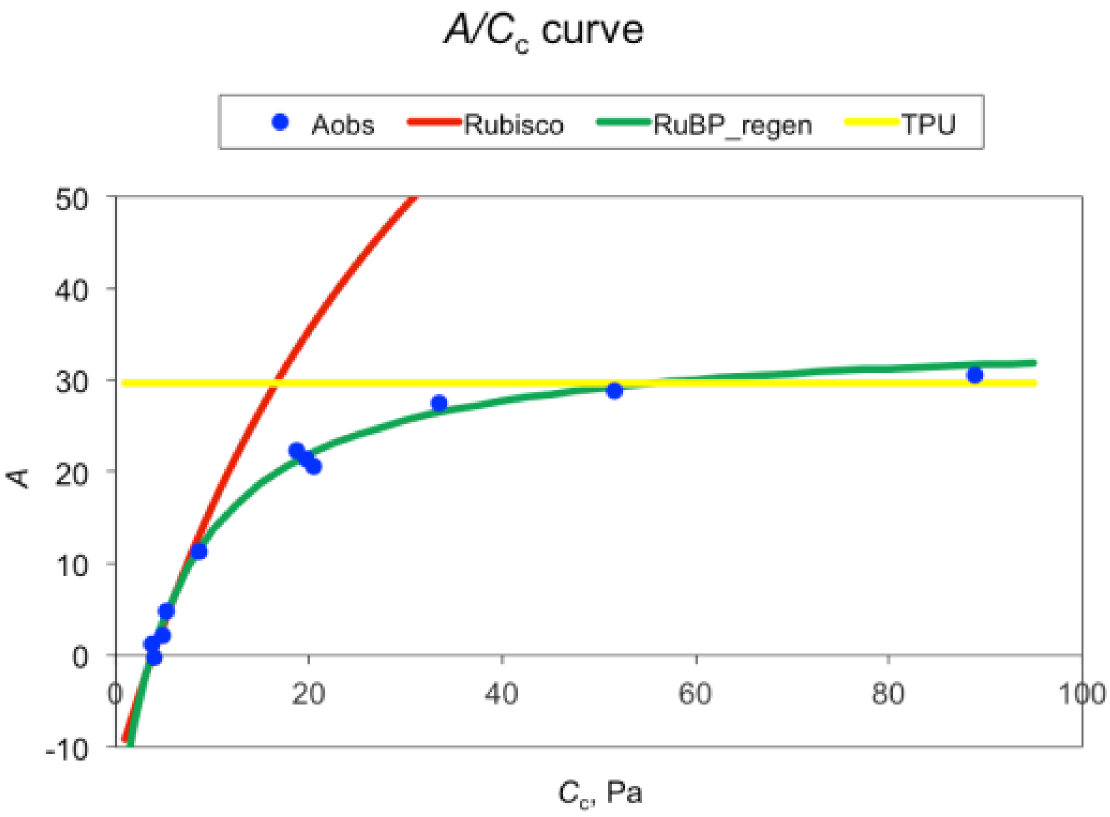
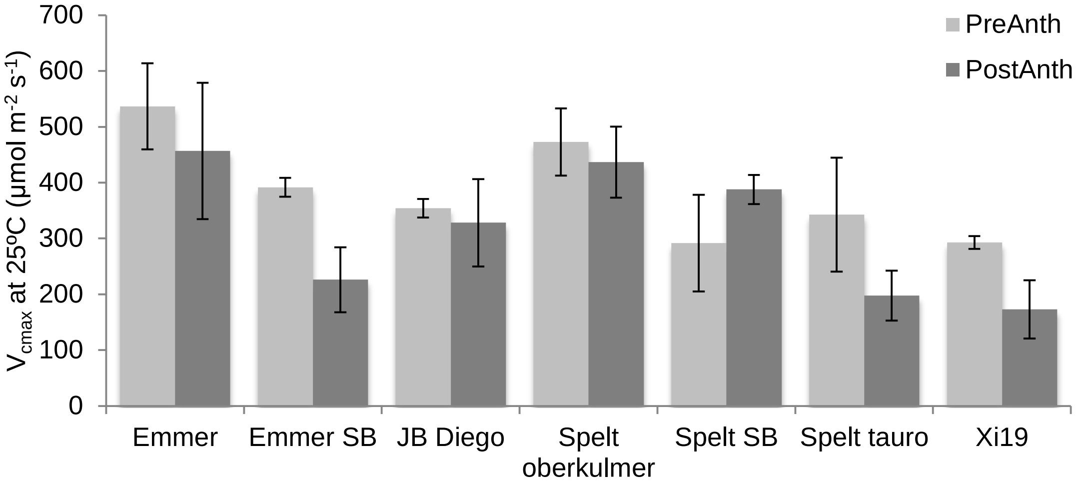


Figure 6.1. Example genotypic responses of exposure to varying carbon dioxide concentrations within the leaf chamber. Using the model constructed by Sharkey *et al*. (2007), C_i was converted into C_c (chloroplast CO_2) plotted against the net photosynthesis for the 8 genotypes; the figure shown demonstrates this for MBW, JB Diego. Blue circles (A_{obs}) denote the observed photosynthetic rate at known C_c. The red line represents the part of the photosynthetic CO_2 response that is limited by Rubisco. The green line corresponds to the RuBP-regeneration state and the yellow boundary symbolises the TPU state.

Figure 6.2 (A and B) shows that cultivars investigated displayed a range of different responses to CO_2 atmospheric concentrations, however differences were not significant except for triose phosphate limitation ($P = 0.021$) and respiration ($P = 0.011$). TPU ranged from 4.32 to 10.26 µmol m^{-2} s^{-1} (spelt SB and emmer, respectively) pre-anthesis and from 4.11 to 25.63 µmol m^{-2} s^{-1} post-anthesis (Xi19 and spelt Oberkulmer). TPU generally tended to be higher in ancient wheat cultivars compared to modern bread wheat and was highest in emmer, emmer SB, spelt Tauro and JB Diego, pre-anthesis. This indicates that these genotypes had a lesser limitation to increasing atmospheric CO_2 compared to other genotypes. This was also the case for spelt Oberkulmer and emmer post-anthesis, which indicates that TPU is not necessarily consistent through the life of the flag leaf. R_d was also significantly different between genotypes and across growth stages ($P = 0.011$). Emmer had the greatest R_d pre-anthesis, whereas spelt Oberkulmer had the greatest post-anthesis (14.14 and 35.26 µmol m^{-2} s^{-1}, respectively). Xi19 had the lowest R_d both pre- and post-anthesis (6.95 and 5.18 µmol m^{-2} s^{-1}, respectively).

The maximum catalytic rate of Rubisco ranged from 291.5 to 536.6 μmol m^{-2} s^{-1} (pre-anthesis) and 173.1 to 456.8 μmol m^{-2} s^{-1} post-anthesis. Differences were not significant although the general trend implies that V_{cmax} reduces with flag leaf age in all cultivars investigated, except for spelt SB. Rate of electron transport (J) did not show any statistical significance, which parallels that observed in the preliminary set of carbon response data (data not shown).

A



B

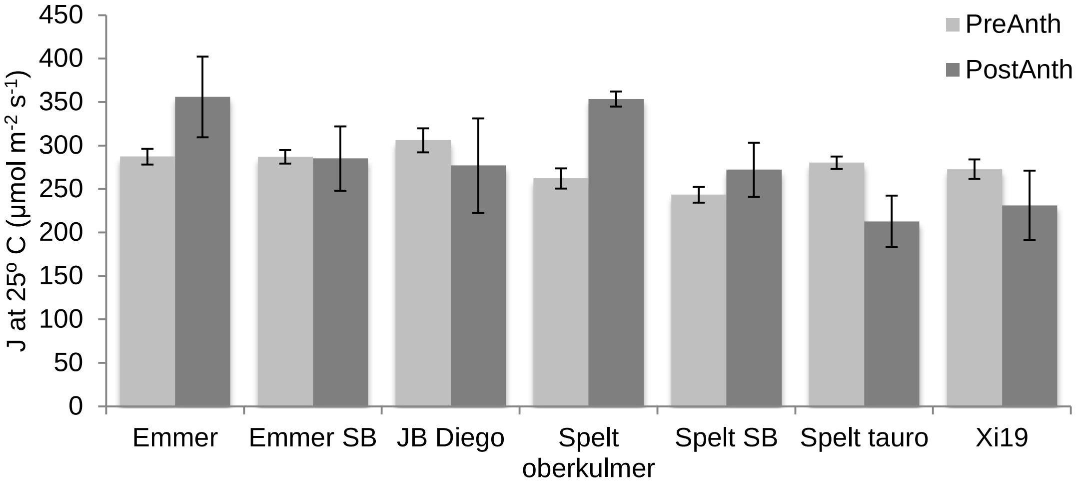
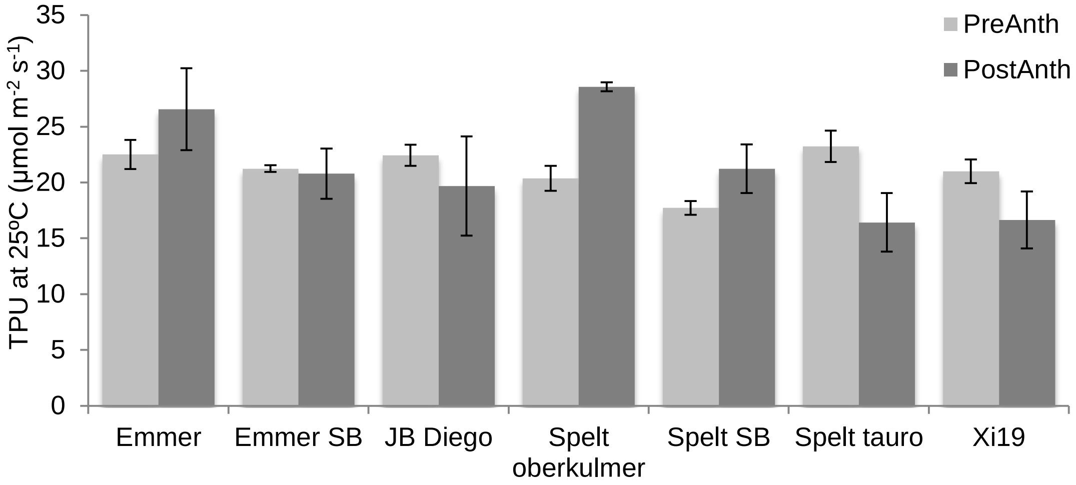


Figure 6.2 A. Values determined from fitted A/C_i (A/C_c) curves, with a normalised leaf temperature (25°C). Figure A shows the maximum rate of carboxylation (V_{cmax}) and B the rate of electron transport (J). The bars represent the standard error of the mean (where n = 4). B. Values determined from fitted A/C_i (A/C_c) curves, with a normalised leaf temperature (25°C).

C



D

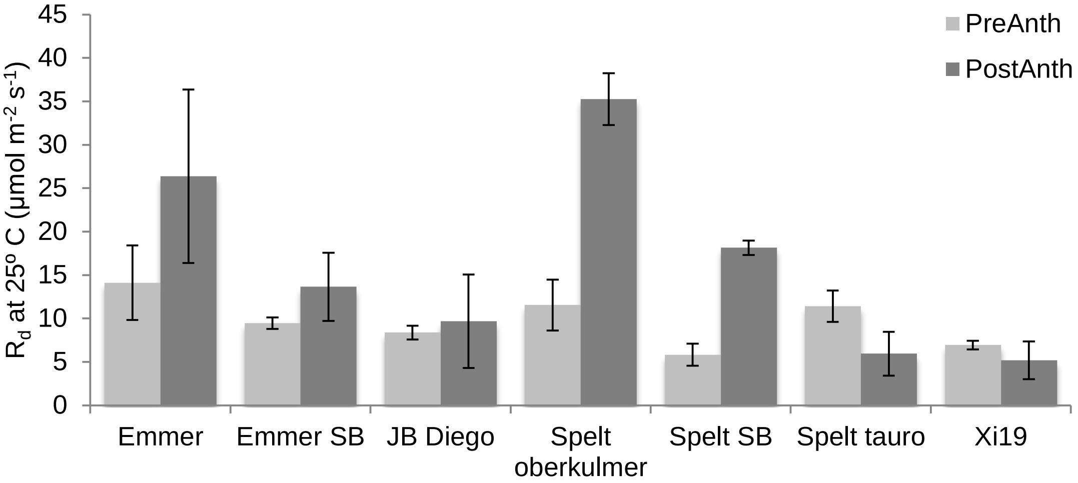


Figure 6.2 C shows the triose phosphate utilisation (TPU) and D the determined respiration rates (R_d) of each of the investigated genotypes. The bars represent the standard error of the mean (where n = 4).

There were no significant differences when data were analysed by species and results were adjusted to account for any variability (25ºC) (Figure 6.3). Once again however, TPU was highest for emmer, both pre- and post-anthesis (as also observed in preliminary data collected, although this data has not been shown).

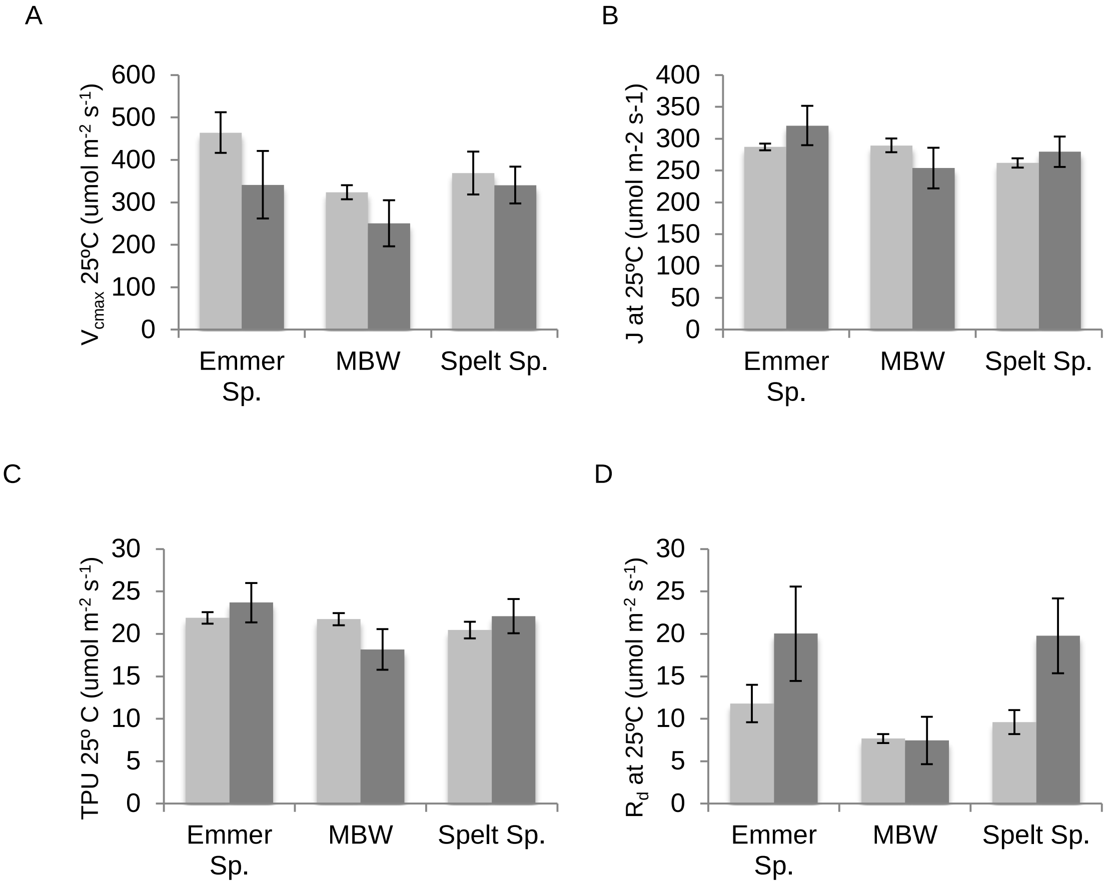


Figure 6.3. Values determined from fitted A/C_i (A/C_c) curves, with a normalised leaf temperature of 25ºC. A shows the maximum rate of carboxylation (V_{cmax}), B the rate of electron transport (J), C the triose phosphate utilisation (TPU) and D the determined dark respiration rates (R_d) for each of the investigated species. The bars represent the standard error of the mean.

6.2 Photosynthetic light response curves (2012 GH experiment only)

Genotypes responded to changing light intensity displaying a logarithmic response curve in which initial photosynthetic rates were light limited, producing a linear slope from which maximum quantum yields were obtained. Other parameters were also derived from the various phases of the response such as A_{sat}, indicating the point at which the photosynthetic reaction was limited by Rubisco or CO_2. This was the point at which no significant increase in photosynthetic activity occurred as the leaf became light saturated. All genotypes displayed this trend however there was some variation for these parameters (shown in Table 6.1). Table 6.2 also shows species differences between these assessed traits.

6.2.1 A_{sat}

Due to the asymptotic nature of the leaf light response, A_{sat}, was considered to be the greatest reading of *A* at the end of the non-rectangular hyperbola response curve. There was significant variation among genotypes ($P < 0.001$), however differences between pre- and post-anthesis were not significant and neither was the combination between factors (Table 6.1). Generally, A_{sat} decreased from pre-anthesis (21.44 μmol m^{-2} s^{-1}) to post-anthesis (18.96 μmol m^{-2} s^{-1}). At growth stage 39, einkorn SB, emmer and Xi19 showed higher than average flag leaf A_{sat}, interestingly, the former two also displayed higher RUE compared to other genotypes in both field trial years. All other genotypes were observed to have quite similar A_{sat} values, these ranged from 16.59 to 21.40 μmol m^{-2} s^{-1}. Using A_{sat} as a means to assess overall photosynthetic capability, spelt Tauro performed poorly compared to other genotypes (16.54 μmol m^{-2} s^{-1}). These results indicate that photosynthetic capability generally reduced with flag leaf age, suggesting that the flag leaf may contribute to carbon accumulation to a greater extent pre-anthesis. However Xi19, spelt Tauro and spelt SB had greater A_{sat} post-anthesis. Superior A_{sat} was observed in emmer, emmer SB and einkorn SB pre-anthesis, indicating that these genotypes had an enhanced ability to assimilate carbon at saturating irradiances. An increased ability to assimilate carbon, perhaps due to reduced Rubisco limitation, or alternatively increased Rubisco concentration and activity, has been previously reported to correlate with increased A_{sat} (Evans, 1996; Terashima *et al.*, 2011). Although einkorn SB and emmer had high pre-anthesis A_{sat}, these species 'lost' the largest proportion of pre-anthesis photosynthetic capability over the life of the flag leaf compared to other genotypes (there was a 37 and 21% reduction in photosynthetic capability, respectively). The photosynthetic capability of spelt SB and spelt Tauro increased, although this was only marginal (altering maximum photosynthetic rates by -1 and -2%, respectively). Despite being low, the A_{sat} for spelt genotypes were fairly consistent from pre- to post-anthesis and actually increased with leaf age for spelt SB and Tauro. Results indicate that although overall, spelt species may have low photosynthetic capabilities; these capabilities are much more conserved throughout the life of the flag leaf. This stability for carbon accumulation could indicate that the 'stay green' trait suggested by SPAD measurements and visual scoring (detailed in the previous chapter) was 'functional', i.e. was still photosynthesising and therefore contributing to carbon assimilation. Thus, although other genotypes may well have an enhanced ability to assimilate carbon at limited and saturating light intensities, spelt Tauro and SB may maintain A_{sat}, providing a basis for the high RUE previously reported.

Table 6.1: Radiation use characteristics of seven ancient wheat varieties and modern bread wheat, Xi19 for comparison. Parameters reported include the maximum rate of photosynthesis in saturating light (A_{sat}), the saturation at which leaves became saturated with light, quantum yields and respiration (R_d) for pre and post anthesis periods.

Genotypes	A_{sat} (μmol m^{-2} s^{-1})		Quantum yield (μmol m^{-2} s^{-1})		Light saturation (μmol quanta m^{-2} s^{-1})		Respiration (μmol m^{-2} s^{-1})	
	Pre-anth.	Post-anth.	Pre-anth.	Post-anth.	Pre-anth.	Post-anth.	Pre-anth.	Post-anth.
Einkorn	19.88	17.91	0.0423	0.0404	411.40	*	-0.35	*
Einkorn SB	28.22	17.65	0.0542	0.0373	445.00	473.30	-0.50	-0.84
Emmer	27.12	21.42	0.0489	0.0509	551.80	391.20	-0.84	-0.89
Emmer SB	21.40	19.18	0.0413	0.0493	499.60	364.50	-0.79	-0.95
Spelt oberk.	16.59	16.53	0.0373	0.0388	472.50	393.00	-0.78	-1.41
Spelt SB	17.50	17.73	0.0355	0.0436	443.00	354.30	-1.17	-1.29
Spelt tauro	16.54	18.68	0.0384	0.0405	416.90	470.20	-1.68	-2.04
Xi19	25.01	26.55	0.0505	0.0527	475.20	435.50	-0.37	-1.02
Mean	21.44	18.96	0.0436	0.0442	463.43	411.71	-0.81	-1.21
P Genotype		< 0.001		0.017		0.728		0.01
P GS		0.485		0.215		0.095		0.148
P Interaction		0.539		0.559		0.599		0.986
SED (DF)		2.838 (49)		0.00672 (47)		91.31 (47)		0.6597 (42)

* denotes missing results

Table 6.2: Radiation use characteristics of four wheat species. Parameters reported include the maximum rate of photosynthesis in saturating light (A_{sat}), the light intensity that saturates leaf photosynthesis, quantum yields and respiration (R_d) for pre and post anthesis periods.

Genotypes	A_{sat} (µmol m^{-2} s^{-1})		Quantum yield (µmol m^{-2} s^{-1})		Light saturation (µmol quanta m^{-2} s^{-1})		Respiration (µmol m^{-2} s^{-1})	
	Pre-anth.	Post-anth.	Pre-anth.	Post-anth.	Pre-anth.	Post-anth.	Pre-anth.	Post-anth.
Einkorn	21.35	17.75	0.0443	0.0385	417.00	473.30	-0.38	-0.84
Emmer	23.31	20.67	0.0439	0.0504	517.00	382.30	-0.81	-0.91
MBW	25.01	26.55	0.0505	0.0527	475.20	435.50	-0.37	-1.02
Spelt	16.78	17.47	0.0375	0.0404	432.70	409.10	-1.42	-1.60
Mean	21.44	18.96	0.0400	0.0500	460.48	425.05	-0.74	-1.09
P Genotype		< 0.001		0.002		0.402		0.001
P GS		0.650		0.149		0.134		0.215
P Interaction		0.640		0.269		0.256		0.821
SED (DF)		1.933 (57)		0.00472 (55)		62.59 (54)		0.4716 (49)

* denotes missing results

Assessment of A_{sat} by species showed significant species differences ($P < 0.001$) (Table 6.2). Emmer and MBW species had the greatest A_{sat} observed (23.31 and 25.01 µmol m^{-2} s^{-1}) compared to spelt species which displayed the lowest photosynthetic capacity (16.87 µmol m^{-2} s^{-1}) pre-anthesis post-anthesis, although differences were not significant between growth stages and species. Photosynthetic capacity for MBW and spelt increased with flag leaf age indicating these two species to make a greater contribution to carbon assimilation post-anthesis, potentially useful in the UK winter wheat growing season when incident radiation is greatest. Results suggest that there are genotypic and species differences for photosynthetic capability. These may be indicative of chloroplast function linked to the photochemical processes of Rubisco (Beadle & Long, 1985) or alternatively the amount of *in vivo* Rubisco which can be rate limiting in carbon assimilation (Murchie *et al.*, 2009). Alternatively, differences may derive from biochemical or mechanistic variances between species or alternatively may be due to the differences in developmental rates observed. Theoretically, it may be possible to increase light photosynthetic rates by increasing the photosynthetic machinery leaf per unit area (although there are limitations on accumulated protein per chloroplast and numbers of chloroplasts per mesophyll cell) (Murchie *et al.*, 2009). It is therefore possible that the variance observed for A_{sat} in the current experiment is due to differences in leaf architecture between genotypes.

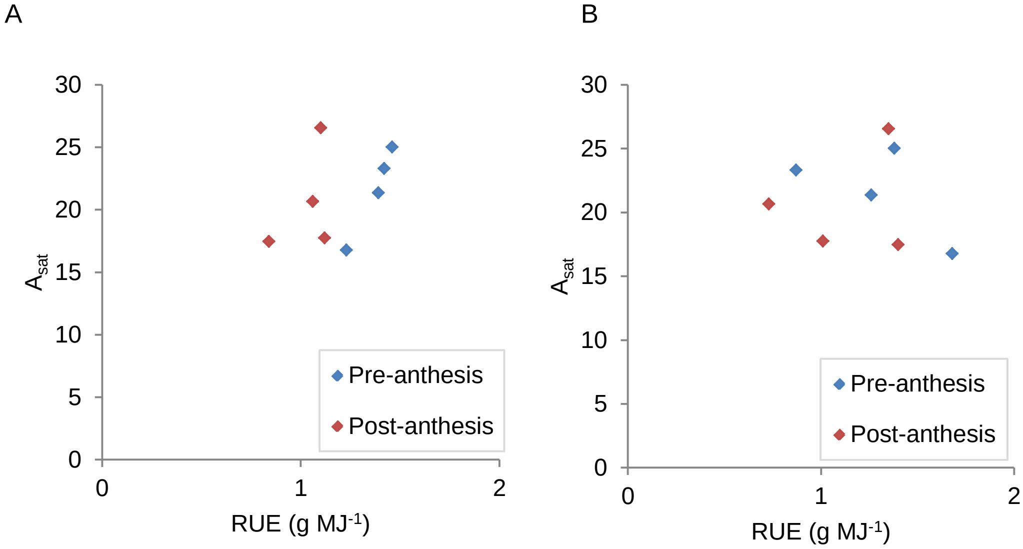


Figure 6.4. RUE and A_{sat} correlations. A. 2010 species pre- and post-anthesis RUE against species pre- and post-anthesis GH II A_{sat} ($r = 0.0232$). B. 2011 species pre- and post-anthesis RUE against species pre- and post-anthesis GH II A_{sat} ($r = -0.0822$).

Results indicated that einkorn SB and emmer have high A_{sat} rates although this was not reflected in RUE from the 2010 and 2011 field trials. It would be expected that increased maximum photosynthetic ability would denote highly efficient use of radiation (therefore high RUE), although it is also pertinent that carbon assimilation involves a large array of integrated reactions and processes. Additionally, 2010 and 2011 RUE values were plotted for those genotypes where A_{sat} was obtained in the drought glasshouse experiment. RUE and A_{sat} for pre- and post-anthesis were plotted separately however no correlations were significant except for pre-anthesis A_{sat} against pre-anthesis RUE in 2010 ($P = 0.0116$). A positive relationship between pre- and post-anthesis A_{sat} and RUE however, was observed in both years (Figure 6.4). Snaydon (1991) stated that increased A_{sat} does not necessarily denote an enhanced ability to produce biological yield, particularly under typical environmental conditions, which are often less than optimal. As a result, many studies have shown that the A_{sat} is rarely achieved (Murchie *et al*., 2009). This makes it particularly difficult to describe carbon assimilation in any way that is meaningful for agricultural purposes although Dempster (2013) and Ngyuen (2014) did find a relationship between glasshouse measured A_{sat} and field measured RUE.

6.2.2 Light saturation point

The light intensity at which leaves were saturated was defined as the point at which increments in light intensity no longer impacted photosynthetic rates. At this stage, ATP and NADPH was being produced in surplus where light independent reactions fix CO_2, and therefore ambient CO_2 concentrations become limiting. Results showed that emmer, emmer SB and Xi19 had the highest pre-anthesis light saturation point (Table 6.1). The light saturation points of einkorn SB and spelt Tauro increased from growth stage 39 to 61, despite the consistent A_{sat} observed at these growth stages in spelt Tauro and the much reduced A_{sat} from growth stage 39 to 61 in einkorn SB. Generally, flag leaf light saturation reduced from growth stage 39 to 61 in all genotypes, except for einkorn SB and spelt Tauro, potentially an adaptation that increases flag leaf productivity during periods when irradiance is typically higher (in the UK winter wheat growing season), however, genotypic differences and growth stage differences, as well as the interaction between these factors, were insignificant. Assessed by species also, there were no significant differences (Table 6.2).

6.2.3 Quantum yield

Quantum yield is a measure of photosynthetic efficiency expressed in moles of photons absorbed per mole of CO_2 gained. Quantum yield (Φ) is commonly reported to be higher within C_4 plant species due to the modified carbon accumulation mechanisms which reduces CO_2 limitations and increases internal resistances (Aggarwal & Sinha, 1983). However, variation for Φ in C_3 genotypes may also indicate differential C_i or increased internal resistances enhancing photosynthetic efficiency at low light levels. Φ observed in the drought glasshouse experiment ranged from 0.036

to 0.053, which are consistent with the findings of Ehleringer & Pearcy (1983) for the average Φ of a C_3 grass species (0.053 µmol m^{-2} s^{-1}). The quantum yield of the genotypes assessed showed significant genotypic variation (P = 0.017) (Table 6.1). Pre-anthesis, average quantum yield was 0.0436 µmol m^{-2} s^{-1}. Einkorn SB, emmer and Xi19 had high quantum yield (0.05417, 0.0489, 0.05052 µmol m^{-2} s^{-1}), which was also the case for emmer and Xi19 post-anthesis (0.05094 and 0.05267 µmol m^{-2} s^{-1}, respectively) although generally, Φ was relatively consistent across the two time periods. Large quantum yields indicate that initial photosynthetic rates, at low light intensities, are high, implying that initial increments in light intensity were better utilised. This may be a result of lower limitation on Rubisco by CO_2 concentrations (C_i). The general trend showed that quantum yield increased from growth stage 39 to 61 in all genotypes except for both einkorn species, however differences between the two growth stages were not significant. When quantum yield was assessed by species, there were significant differences (P = 0.002) (Table 6.2); MBW had the highest quantum yield both pre- and post-anthesis followed by emmer and einkorn. Spelt had the lowest quantum yield at both stages. Additionally, positive associations were observed between RUE and Φ in 2010 and 2011 (data was not shown).

6.2.4 CO_2 loss through respiration

Research has shown respiration processes to have protective and metabolic roles in plant growth and development, particularly whilst under environmental stresses. However, the process also has significantly deleterious effects. Respiration processes whilst plants are in lit conditions refer to CO_2 production from mitochondria and accounts for significant proportions of carbon lost by plants (Miao *et al*., 2009). This has been highlighted when doubling the CO_2 concentrations, biological yield production increases for a range of crops (Murchie *et al.,* 2009). Furthermore, there are theoretical models that suggest that under favourable conditions, removal of respiration could enhance yields considerably (Murchie *et al*., 2009). There was significant variation for respiration rates among genotypes (P = 0.01) (Table 6.1). The average respiration rate was -0.81. Einkorn, einkorn SB and Xi19 had the greatest R_d, implying that einkorn, einkorn SB and Xi19 may possess increased photosynthetic efficiency, simply through minimal respiration processes within the leaf. The largest deviation from the mean was observed in spelt SB and spelt Tauro (-1.17 and -1.68, respectively). Post-anthesis, the average respiration rate reduced to -1.20. Einkorn SB and emmer had the highest R_d (-0.84 and -0.89, respectively). Spelt genotypes all had comparably lower R_d than other genotypes (-2.04, -1.41 and -1.21 for spelt Tauro, spelt Oberkulmer and SB, respectively). During the life of a crop, the relative contributions of respiration to growth and maintenance alters with age and weight of the crop (Azam-Ali & Squire, 2003) and although respiration was observed to reduce between pre- and post-anthesis, indicating that more assimilates were lost due to respiration, no differences were observed between growth stage or growth stage and genotype combined. Respiration rates were not significantly different by species or growth stage (Table 6.2).

6.3 Water Use Efficiency

6.3.1 Water limited glasshouse experiment WUE (2012)

6.3.1.1 ITE measurements under drought

Carbon gain and water loss in plants are intricately related, the ratio between net photosynthetic rate and transpiration rate are coupled through stomatal regulation (Jianlin *et al*., 2008; Haefele *et al*., 2008). As discussed in the introduction, water use efficiency, defined in the current study as instantaneous transpiration use efficiency (ITE) in simplified terms can be enhanced by 1) increasing photosynthetic rates or by 2) reducing transpiration losses (Peleg *et al*. 2005). The ratio between the two is highly dependent on stomatal conductance (Hirasawa & Hsiao, 1999). Emmer, emmer SB and spelt SB had high ITE (8.39, 8.05 and 7.11 mol mol^{-1}, respectively) (Table 6.3). MBW (Xi19) and einkorn (7.02 and 7.30 mol mol^{-1}) performed better than einkorn SB, spelt Oberkulmer and spelt Tauro (6.67, 6.65 and 6.28 mol mol^{-1} respectively) for ITE although differences were not significant by genotype. ITE was measured across two dates, assessing pre- and post-anthesis ITE. ITE in emmer and all spelt cultivars were relatively conserved from pre- to post-anthesis, although did reduce over time from 7.40 mol mol^{-1} at growth stage 39 to 6.50 mol mol^{-1}, at growth stage 61 (P = 0.001), indicating that younger leaves were generally more efficient in their water use for all genotypes except einkorn and spelt Tauro.

Table 6.3. The instantaneous transpiration use efficiency, photosynthetic and transpiration rates of seven ancient wheat varieties and modern bread wheat, Xi19, for comparison pre- and post-anthesis (2013 glasshouse experiment).

	ITE (mmol mol^{-1})		Photosynthetic rate (μmol m^{-2} s^{-1})		Transpiration rate (mmol m^{-2} s^{-1})	
	Pre-anth.	Post-anth.	Pre-anth.	Post-anth.	Pre-anth.	Post-anth.
Einkorn	7.30	7.18	22.81	16.10	3.40	2.49
Einkorn SB	6.67	5.90	20.94	13.82	3.46	2.24
Emmer	8.39	6.83	24.19	20.15	3.27	3.45
Emmer SB	8.05	3.87	18.54	15.04	2.41	4.18
Spelt Oberk.	6.65	7.52	13.34	14.30	2.07	2.08
Spelt SB	7.11	6.66	17.53	13.08	2.69	1.96
Spelt Tauro	6.28	6.64	15.98	13.97	2.62	2.52
Xi19	7.02	6.03	23.46	17.06	3.38	3.38
Mean	7.40	6.50	19.44	14.82	2.82	2.58
P Genotype		0.150		<0.001		<0.001
P GS		0.001		<0.001		0.649
P Interaction		<0.001		0.079		<0.001
SED (DF)		0.8388 (291)		2.052 (291)		0.4652 (291)

Also noteworthy was einkorn which, despite having poor emergence in previous field trials, in addition to high RUE, did show high ITE which was also relatively conserved between pre- and post-anthesis. Although for the most part data analysis was directed towards emmer and spelt cultivars in particular, high RUE (observed in field trials) and the seemingly consistent ITE, does show that this genotype may warrant further investigation.

Table 6.4. Instantaneous transpiration use efficiency, photosynthetic and transpiration rates of four wheat species both pre- and post-anthesis (2013 glasshouse experiment).

	ITE ($mmol\ mol^{-1}$)		Photosynthetic rate ($\mu mol\ m^{-2}\ s^{-1}$)		Transpiration rate ($mmol\ m^{-2}\ s^{-1}$)	
	Pre-anth.	Post-anth.	Pre-anth.	Post-anth.	Pre-anth.	Post-anth.
Einkorn sp.	7.15	6.44	22.37	14.79	3.42	2.35
Emmer sp.	8.22	5.57	21.37	17.98	2.38	3.76
MBW	7.02	6.03	23.46	17.06	3.38	3.38
Spelt sp.	6.68	6.82	15.71	13.68	2.48	2.21
Mean	7.40	6.50	20.77	15.88	2.82	2.58
P Genotype		0.499		<0.001		<0.001
P GS		<0.001		<0.001		0.725
P Interaction		<0.001		0.029		<0.001
SED (DF)		0.6767 (299)		1.69 (299)		0.3742 (299)

When assessed by species, emmer had the highest water use efficiency pre-anthesis followed by einkorn, modern bread wheat and spelt (P <0.001) (Table 6.4). Furthermore, spelt displayed increased ITE from growth stage 39 to 61 whereas all other species declined (as observed when these results were analysed by genotype) however, this is primarily owing to spelt Oberkulmer and spelt Tauro (spelt SB did not show a percentage increase in ITE over time). This indicates that under drought conditions, as were imposed in the 2012 glasshouse experiment, spelt genotypes maintained water use efficiency in the later stages of the growing season whereas the other wheat species lost water use efficiency over time. It has been reported that high WUE at anthesis could be a useful trait, particularly where temperatures and radiation are high and water availability is low.

6.3.1.2 Photosynthetic rates under drought

Average photosynthetic rate, pre-anthesis was 19.04 mmol m^{-2} s^{-1}. Emmer, Xi19, einkorn and einkorn SB had higher than average rates of photosynthesis whereas spelt Oberkulmer and spelt Tauro had the lowest rates overall (Table 6.3). Genotypic differences were highly significant (P <0.001) and so too were the differences between growth stage (P <0.001). Photosynthetic rates reduced over time to 14.82 mmol m^{-2} s^{-1}. The lowest percentage decline in photosynthetic activity over time occurred for spelt Tauro, emmer and emmer SB (12.58, 16.70 and 18.88 %), interesting as these genotypes have been previously been observed to have superior radiation use efficiencies in field trials. Einkorn, einkorn SB and Xi19 reduced by the largest degree (29.41 and 34.00 and 27.28%). This reduction in photosynthetic rate from growth flag leaf emergence to after anthesis was characteristic of all genotypes except for spelt Oberkulmer which showed a pre- to post-anthesis increase of 7.2%. Emmer species displayed high ITE pre-anthesis, which may have derived from high pre-anthesis photosynthetic rates.

Photosynthetic rate was significantly different by species (P = 0.029) (Table 6.4). Spelt had the lowest photosynthetic rate (15.71 mmol m^{-2} s^{-1}) pre-anthesis whereas einkorn, modern bread wheat and emmer had comparably high photosynthetic rates (23.46, 22.37 and 21.37mmol m^{-2} s^{-1}). Post-anthesis, emmer and modern bread wheat maintained their high photosynthetic rate whereas einkorn and spelt photosynthetic rates decline substantially.

6.3.1.3 Transpiration rates under drought

Transpiration rates ranged from 2.07 - 3.40 mmol m^{-2} s^{-1} pre-anthesis and from 1.96-4.18 mmol $m^{-2}s^{-1}$ post-anthesis (Table 6.3). Average transpiration remained constant from pre- to post-anthesis and there was no clear trend; differences between growth stages were not significant. Spelt species had comparably low transpiration rates pre- and post-anthesis whereas the highest transpiration rates were displayed by einkorn species and Xi19 pre-anthesis, and emmer SB, emmer SB and Xi19 post-anthesis (4.18, 3.45 and 3.38mmol m^{-2} s^{-1}, respectively). Differences between genotypes were significant (P <0.001), as was the genotypic interaction with growth stage (P <0.001). Assessed by species, transpiration rates were highly significant (P <0.001) (Table 6.4). Emmer and spelt species had low pre-anthesis transpiration rates, compared to einkorn and modern bread wheat. Post-anthesis, emmer and MBW had the greatest transpiration rates and conversely spelt had very low transpiration rates.

The results gained here are indicative of differing mechanisms through which ITE is maintained; whereas emmer had the greatest photosynthetic rates on both dates, in contrast, spelt had considerably lower photosynthetic rates, although this was offset by lower than average transpiration rates. Therefore emmer maintained ITE through high photosynthetic rates, whereas spelt cultivars (particularly spelt Oberkulmer which actually increased ITE from pre- to post-anthesis), had amongst the lowest transpiration rates observed indicating that the mechanism by which ITE is controlled may well differ. Furthermore, although emmer and emmer SB both had high ITE pre-anthesis, increased photosynthetic capacity of the leaf was only observed in emmer. Emmer SB did not show a conserved ITE across sampling dates indicating that this trait may be characteristic of emmer rather than of the emmer species in general. The differences between the mechanisms for ITE between spelt and emmer corroborate preliminary studies (Isaaks, 2009). The 2012 glasshouse experiment maintained under drought imposed post-anthesis, shows that emmer and spelt in particular may have leaf level characteristics that confer adaptations making them more conservative of water and therefore suited to water limited environments. Additionally, whereas the emmer genotype and spelt cultivars displayed either high photosynthetic rates or low transpiration rates, einkorn had a high photosynthetic rate pre-anthesis for high ITE and low transpiration post-anthesis and therefore seemingly uses both mechanisms to conserve ITE.

6.3.1.4 Internal stomatal CO_2 concentrations under drought

Table 6.5 shows results for the genotypic assessments, whereas Table 6.5 shows averages across species. ITE has been determined using measurements of instantaneous photosynthetic rate and transpiration rate however, the dynamic nature of these measurements mean that the observed values can be analysed further. This analysis relates to stomatal behaviour of which conductance and internal stomatal versus atmospheric carbon dioxide concentration can be informative of the way in which plants utilise water and CO_2 because photosynthesis and water loss through transpiration are interdependent processes. The influx and outflow of these gases underlies the plants' capacity to acquire CO_2 and the efficiency with which it does this.

Table 6.5. Stomatal responses during ITE measurements of seven ancient wheat varieties as well as modern bread wheat, Xi19, for comparison both pre- and post-anthesis. The table includes internal stomatal CO_2 concentrations, the ratio between internal stomatal CO_2 concentrations and atmospheric CO_2 concentrations, stomatal conductance as well as intrinsic water use efficiency.

	Internal stomatal CO_2 concentrations (C_i) (μ mol mol^{-1})		Internal stomatal/atmospheric [CO_2] (C_i/C_a) (μ mol mol^{-1})		Conductance (G_s) (μ mol mol m^{-2} s^{-1})	
Genotype	Pre-anth.	Post-anth.	Pre-anth.	Post-anth.	Pre-anth.	Post-anth.
Einkorn	201.6	200.8	0.5127	0.5084	0.2216	0.1746
Einkorn SB	191.8	225.3	0.4882	0.5691	0.1887	0.1391
Emmer	229.9	285.2	0.5896	0.7280	0.3493	0.4963
Emmer SB	273.8	301.5	0.6971	0.7663	0.3048	0.4540
Spelt Oberk.	134.1	153.8	0.3403	0.3900	0.0964	0.1064
Spelt SB	182.9	172.5	0.4651	0.4365	0.1488	0.1005
Spelt Tauro	196.6	233.8	0.5171	0.5304	0.1520	0.1334
Xi19	265.1	332.7	0.6783	0.8458	0.3977	0.5150
Mean	214.40	222.80	0.5491	0.5499	0.2409	0.2106
P Genotype		<0.001		<0.001		<0.001
P GS		0.07		0.019		0.168
P Inter.		0.607		0.062		0.010
SED (DF)		32.95 (291)		0.05225 (291)		0.06188 (291)

The current experiment assessed the variability for the internal stomatal CO_2 concentration for each of the genotypes (Table 6.5). C_i averaged 214.40 pre-anthesis and 222.80 µmol mol^{-1} post-anthesis, there was no significant difference in C_i between growth stages. C_i was highest in emmer, emmer SB and MBW, Xi19 (229.9, 273.8 and 265.1 µmol mol^{-1}) and lowest for spelt Oberkulmer and SB (134.1 and 182.9 µmol mol^{-1}) pre-anthesis, although einkorn species also had lower than average internal stomatal CO_2 concentrations. Genotypic differences were highly significant ($P <0.001$) although the interaction was not. Genotypic differences were also mirrored post-anthesis with emmer, emmer SB and Xi19 having the greatest internal concentrations of CO_2. As would be expected, where photosynthetic rates were reported to be high in Table 6.5, C_i was also high for the same genotypes, indicating that the internal concentrations of CO_2 could have a bearing on photosynthetic activity. Species differences were not statistically significant (Table 6.6).

Assessment of the ratio between internal and atmospheric CO_2 concentration showed emmer, emmer SB and Xi19 to have a high ratio between internal and external CO_2 concentrations where these genotypes had greater C_i/C_a than the pre-anthesis average (0.5491 µmol mol^{-1}). This was also the case post-anthesis where emmer, emmer SB and Xi19 had the greatest ratio of internal stomatal to atmospheric CO_2 concentrations (0.7280, 0.7663 and 0.8458 µmol mol^{-1}) expected as measurements were carried out in a fixed atmospheric CO_2 concentration. As was the case of C_i assessment, C_i/C_a was significantly affected by growth stage ($P = 0.019$) and by genotype ($P <0.001$) however the interaction between the two was not ($P = 0.062$). Collating pre- and post-anthesis C_i/C_a and ITE data showed no significant correlations.

Table 6.6. Parameters indicating stomatal behaviour during ITE measurements both pre- and post-anthesis. The table includes internal stomatal CO_2 concentrations, the ratio between internal stomatal concentrations and atmospheric CO_2 concentrations, stomatal conductance as well as intrinsic water use efficiency.

	Internal stomatal CO_2 concentrations (C_i) (μ mol mol^{-1})		Internal stomatal/atmospheric [CO_2] (C_i/C_a) (μ mol mol $^{-1}$)		Conductance (G_s) (μ mol mol $^{-1}$)	
Genotype	Pre-anth.	Post-anth.	Pre-anth.	Post-anth.	Pre-anth.	Post-anth.
Einkorn sp.	199.3	215	0.5069	0.5434	0.2139	0.1541
Emmer sp.	251.8	292.1	0.6433	0.7443	0.327	0.4784
MBW	265.1	332.7	0.6783	0.8458	0.3977	0.515
Spelt sp.	172.7	193.3	0.4449	0.4648	0.1338	0.1148
Mean	222.23	258.28	0.56835	0.649575	0.2681	0.315575
P Genotype		<0.001		<0.001		<0.001
P GS		0.018		0.003		0.162
P Inter.		0.668		0.035		<0.001
SED (DF)		27.13 (291)		0.04382 (299)		0.04897 (299)

When assessed by species, C_i/C_a was significant (P = 0.035) (Table 6.6). Results mirrored the genotypic assessment of data where emmer and MBW had comparably high internal stomatal CO_2 to atmospheric CO_2 concentrations, both pre- and post-anthesis when compared to einkorn and spelt species.

6.3.1.5 Stomatal conductance for CO_2

Conductance was higher in genotypes with higher photosynthetic rates (Table 6.5). Stomatal conductance was highest in emmer, emmer SB and Xi19, whereas spelt species had the lowest conductance observed pre-anthesis. This genotypic ranking was also mirrored post-anthesis. Genotypic differences were significantly different (P <0.001) as was the interaction between genotype and growth stage (P = 0.010). Differences among species were also highly significant and reflected that observed when this trait was analysed by species; emmer and MBW had much greater conductance compared to einkorn and spelt species (Table 6.6).

High transpiration efficiency in wheat is usually associated with lower stomatal conductance and photosynthetic rates (Fischer, 2011) which occurs when CO_2 diffusion into the leaves and diffusion of water vapour out is largely shared, therefore when stomatal control reduces the amount of water lost, photosynthesis also reduces. All spelt cultivars displayed this trend with low stomatal conductance (G_s) ranging from 0.096 – 0.149 μmol m^{-2} s^{-1} (pre- and post-anthesis) and therefore also displayed low photosynthetic rates to increase ITE. The converse was true for emmer however, which had among the highest conductance rates observed thus enabling higher photosynthetic rates to be achieved. This would likely make the crop more 'conservative' of water, which could increase overall WUE of these plants. On the other hand, stomatal conductance for einkorn does also seem to be intermediate of those of the emmer cultivars and spelt cultivars with lower photosynthetic and higher transpiration rates compared to emmer and spelt, respectively.

6.3.1.6 Soil water extraction

Additionally, during the 2012 glasshouse experiment, the use of columns allowed soil capacitance measurements to be made using a theta probe. This made it possible to calculate the quantities of water extracted per column soil layer as well that extracted by the plants per column (data not shown). Cumulative water uptake data was used to quantify overall WUE (derived from the quantity of biomass relative to the accumulated water extracted from the soil profile). Table 6.7 shows the average WUE for genotypes where emmer and spelt Oberkulmer had the greatest WUE observed (3.93 and 4.51) (P <0.001). Spelt Tauro and einkorn also had comparably high WUE. Spelt SB and MBW, Xi19 were the least efficient in terms of biomass produced to water utilised. These results corroborated those previously observed ITE results indicating that spelt Oberkulmer, Tauro and emmer had high WUE. These results corroborated that observed through ITE measurements.

Table 6.7. WUE (ratio of biomass to cumulative water extracted) (g l^{-1}) for genotypes in the 2012 glasshouse experiment.

Genotypes	WUE (g l^{-1})
Einkorn	3.45
Einkorn SB	2.73
Emmer	3.93
Emmer SB	2.97
Spelt Oberkulmer	4.51
Spelt SB	1.87
Spelt Tauro	3.23
Xi19	2.82
Genotype	0.22
GS	<0.001
Interaction	<0.001
SED (DF)	1.209 (46)

6.3.2 Irrigated glasshouse experiment (2013)

Following on from the results obtained in 2012, it was apparent that there were traits that had the potential to increase water use efficiency under drought, the aim of the 2013 glasshouse experiment therefore was to assess whether the differences in mechanism for ITE was apparent under full irrigation. Instantaneous transpiration measurements were performed over the experimental period, from emergence of the flag leaf until flag leaf senescence. In this experiment the flag leaf was used as a representative to gauge the photosynthetic activity of each of the genotypes.

6.3.2.1 ITE measurements within an irrigated environment

Condon *et al*., (2004) states increments in both carbon gained and water transpired to be instrumental for the enhancement of WUE. Assessment of ITE in the 2013 GH experiment focused on a subset of genotypes to further investigate the mechanism observed in the 2012 GH experiment, from which it was hypothesised that emmer had high photosynthetic rates corresponding with high conductance, and spelt had reduced transpiration rates corresponding with low conductance. Average ITE at the outset of sampling was 7.58 mol mol^{-1} (Figure 6.4). Emmer SB and spelt Tauro had significantly greater ITE compared to other genotypes. Spelt Oberkulmer, spelt SB and both modern bread cultivars however, were also shown to be efficient in terms of leaf water use. Emmer had the lowest transpiration efficiency (6.83 mol mol^{-1}) on 169 DAS but did not decline to the same extent as JB Diego or Xi19 (2.88 and 3.17 mol mol^{-1}), consistent with findings from the glasshouse experiment in 2012. ITE decreased over time from 7.59 (169 DAS) to 4.23 mol mol^{-1} as would be expected of aging flag leaves but was maintained to a greater extent towards the end of the season in spelt compared to MBW genotypes.

Figure 6.5 shows that generally, ancient wheat species (emmer and to a lesser degree, spelt species) remain quite consistent for ITE throughout the life of the flag leaf however, modern bread wheat genotypes tended to decline quite rapidly from 196 to 208 DAS. Differences in ITE were highly significant between genotypes ($P < 0.001$) and sampling dates ($P < 0.001$). The interaction between the two was also highly significant ($P < 0.006$). The current experiment aimed to assess genotypes without resource constraint however all previous work had grown genotypes in water-limited conditions. Modern bread wheat declined more rapidly after 174 DAS whereas emmer and spelt had the greatest ITE, indicating that these were the most efficient throughout the sampling period ($P < 0.001$). Towards the end of sampling, species were ranked similarly compared to 2012 glasshouse experiment, where spelt had the greatest ITE. Emmer species flag leaves had fully senesced by the final sampling date therefore MBW had the lowest recorded ITE. Results do indicate the greater water use efficiency (at the leaf level) of ancient species overall, particularly after anthesis (Figure 6.6). However the mechanisms previously observed were not seen to the same extent, therefore it is possible that these genotypes employ these mechanisms only when under severe drought stress (as imposed in the 2013 GH experiment).

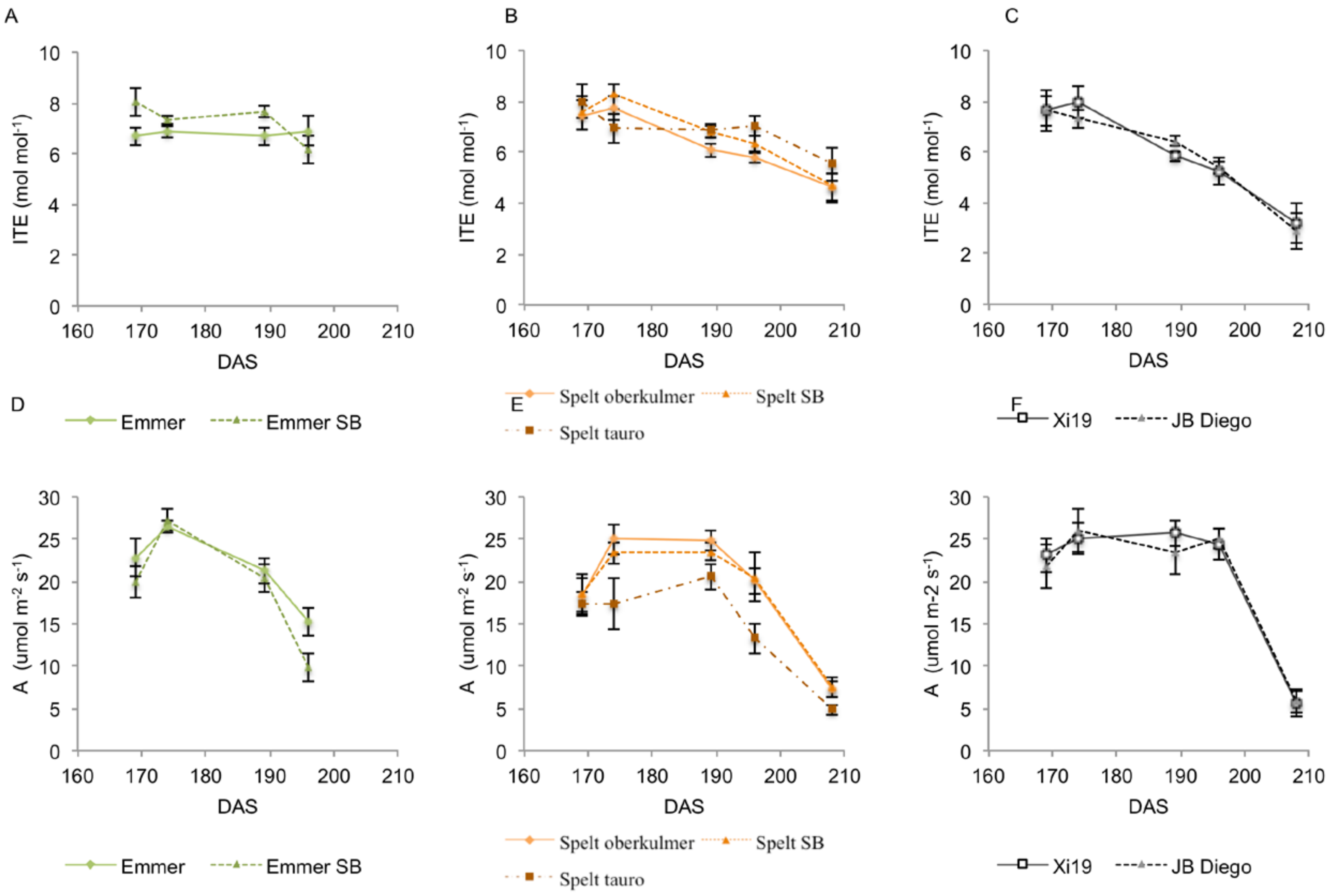


Figure 6.5 i: Instantaneous transpiration use efficiency (A, B and C), photosynthetic rate (D, E and F) and transpiration rate (G, H and I) of a subset of the original ancient wheat species including all emmer (A, D and G) and spelt species (B, F and H) as well as modern bread wheat (C, F and I) for comparison from growth stage 31 onwards (error bars denote SEM).

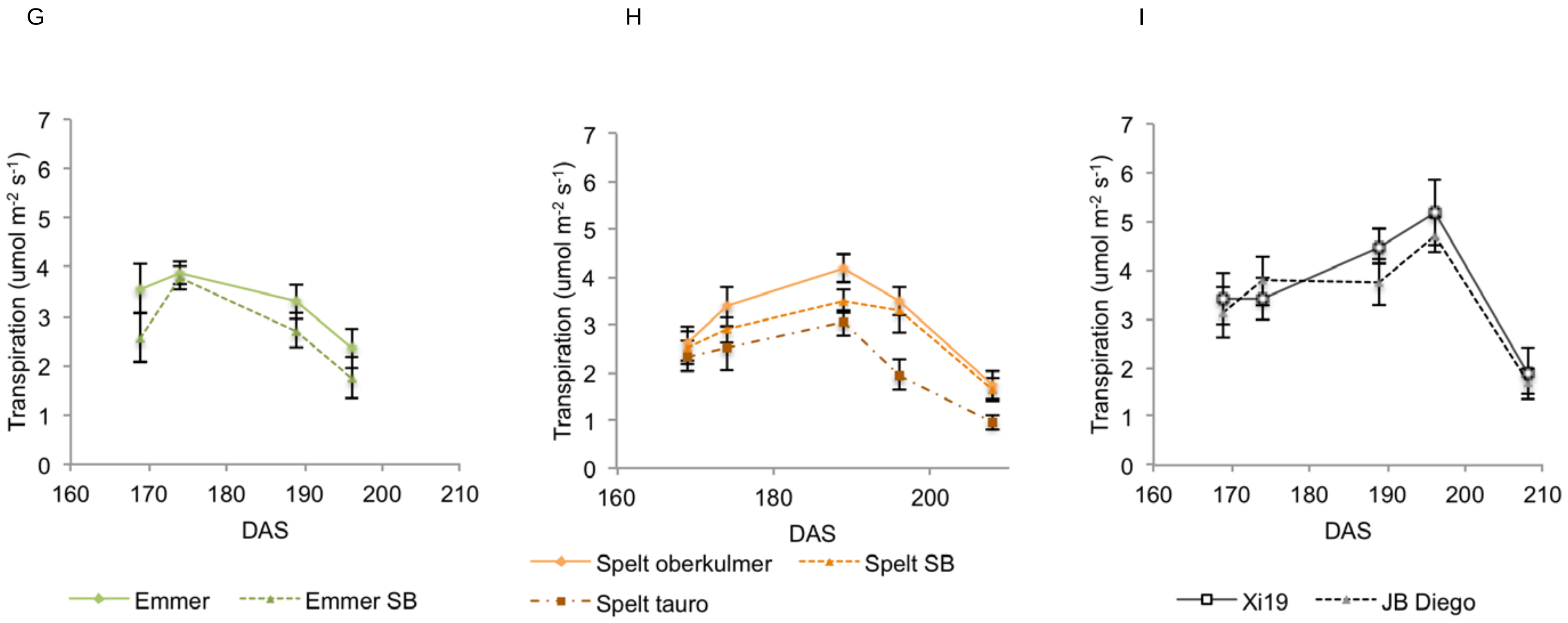


Figure 6.5 ii: Instantaneous transpiration use efficiency (A, B and C), photosynthetic rate (D, E and F) and transpiration rate (G, H and I) of a subset of the original ancient wheat species including al emmer (A, D and G) and spelt species (B, F and H) as well as modern bread wheat (C, F and I) for comparison from growth stage 31 onwards (error bars denote SEM).

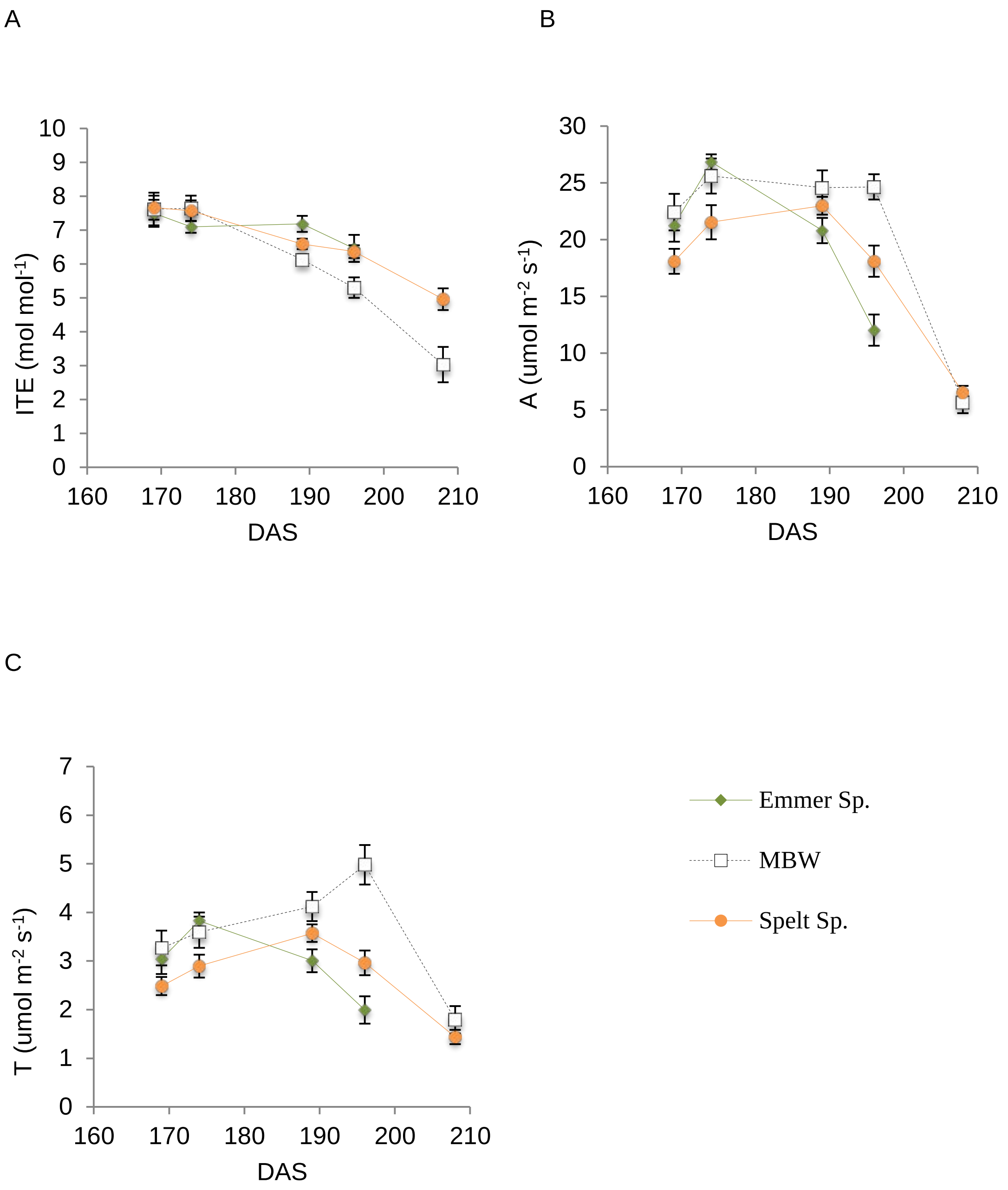


Figure 6.6. A, Instantaneous transpiration efficiency, B, photosynthetic rate and C, transpiration rate of a subset of the original ancient wheat collated by species including all emmer and spelt species as well as modern bread wheat for comparison from growth stage 39 onwards.

6.3.2.2 Photosynthetic rates in an irrigated environment (2013 GH experiment)

Photosynthetic rates averaged 20.27 µmol m^{-2} s^{-1} at the outset of sampling to 5.77 µmol m^{-2} s^{-1} on the final sampling date, indicating that the general trend was that photosynthetic capability reduced with flag leaf age (Figure 6.5 A and B). Emmer showed greater than average photosynthetic rates (22.83 µmol m^{-2} s^{-1}) while spelt genotypes had significantly lower photosynthetic rates ranging from 18.27 to 17.4 µmol m^{-2} s^{-1} at 169 DAS. All genotypes peaked for photosynthetic activity on 174 DAS, particularly emmer and emmer SB (26.48 and 17.41 µmol m^{-2} s^{-1}), indicating that emmer can maintain consistent (emmer) or high ITE (emmer SB) within an irrigated environment as a result of high photosynthetic rates (and comparatively lower transpiration rates) as was also observed in the 2012 GH experiment under drought. However, photosynthetic rates declined after 174 DAS for emmer and leaves senesced before the final measurement date; on these sampling days modern bread wheat cultivars had the highest photosynthetic rates observed. Differences were significant between by genotype (P <0.001), sampling date (P <0.001), as well as the interaction between the two (P <0.001).

6.3.2.3 Transpiration rates within an irrigated environment

The potential mechanism highlighted from 2012 GH experiment, indicated that spelt genotypes tended to maintain ITE due to lower transpiration whereas this derived from increased photosynthetic rates in emmer species under drought. As observed previously, transpiration was lower than the overall average for all spelt cultivars on all sampling dates. Emmer, Xi19 and JB Diego had comparatively high transpiration rates throughout the course of the experiment. Differences between genotype and sampling date were highly significant (P <0.001). Transpiration rates were also significantly different between species over the sampling period (P <0.001), with modern wheat having the highest transpiration rates throughout sampling, indicating the greatest water loss via the leaf. Results therefore indicate whether plants are being maintained under drought (as in the 2012 GH experiment) or within a fully irrigated environment (as in the 2013 glasshouse), the comparatively low transpiration for spelt in particular makes the leaf conservative in terms of water and therefore could offer an avenue through which WUE may be enhanced.

6.3.2.4 Stomatal CO_2 concentrations within an irrigated environment

C_i is a measure of CO_2 concentrations within the stomatal cavity, and therefore provides an indication of the quantity of substrate that is present for photosynthesis. Measurement of C_i allows assumptions to be made regarding the limitations on photosynthesis relating to biochemical and diffusion factors (Farquhar & Sharkey, 1982). Differences between C_i were highly significant between genotypes and sampling date (where P <0.001) however, were not significant for the interaction (P = 0.06) (Figure 6.7). As with preliminary results (data not shown), internal carbon dioxide concentrations were highest in those genotypes that displayed greater photosynthetic rates, such as emmer and emmer SB, particularly on 169 and 174 DAS. When analysed by species, modern bread wheat had a comparatively high C_i, followed by spelt and emmer (P <0.001), which reflects the higher photosynthetic rates of modern bread wheat (Figure 6.7). This trend was also reflected when data was analysed by species (Figure 6.8).

6.3.2.5 Stomatal conductance

As well as C_i, conductance plays a major role in photosynthesis as it provides information about the diffusivity of gases into and out of the leaf. Spelt genotypes had the lowest conductance throughout sampling. Spelt Tauro and spelt SB had low stomatal conductance (g_s) (Figure 6.7). Modern bread wheat had high mesophyll conductance, which consequently increased C_i, therefore increasing photosynthetic rates. All cultivars except spelt genotypes had greater than average g_s. Differences between genotype and sampling date were significant ($P < 0.001$). Results show that conductance consistently declined from the first sampling date to 208 DAS (0.178 to 0.0952) for all genotypes. Generally conductance was highest in those genotypes that displayed greater photosynthetic rates due to increased C_i, such as emmer and emmer SB ($P < 0.001$) (Figure 6.7) on 196 and 174 DAS which may indicate differences in leaf physiology. These could be caused by increased stomatal aperture density, which are also likely to increase the amount of the primary substrate for photosynthesis (CO_2) that diffuses into the leaf. These results were mirrored in the species analysis where generally spelt species had lower C_i and g_s when compared to modern bread wheat in particular (Figure 6.8).

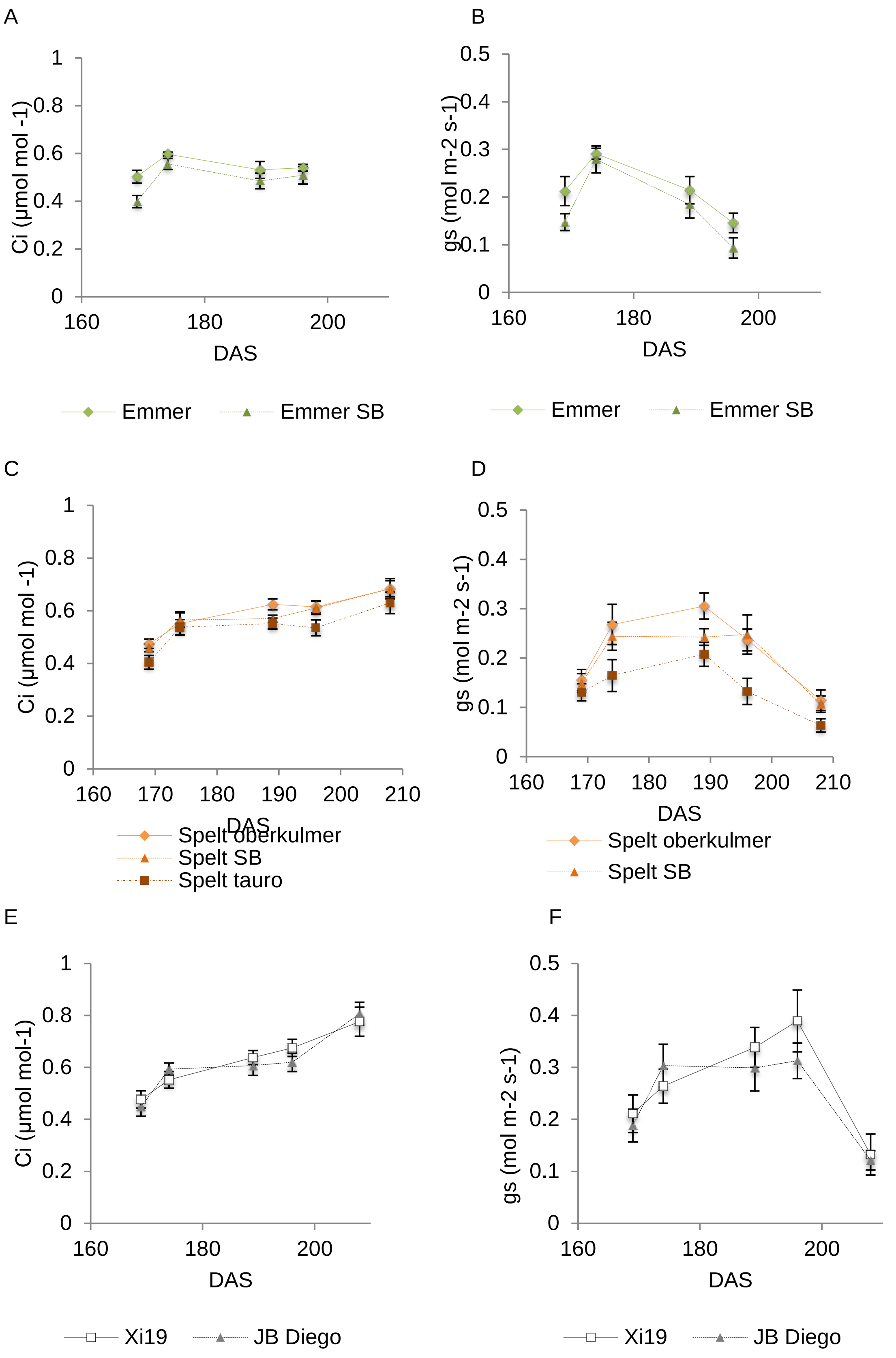


Figure 6.7. Stomatal responses simultaneously measured during ITE measurements of 5 ancient wheat and 2 modern bread wheat cultivars both pre- and post-anthesis. Figure includes sub-stomatal CO_2 concentrations and stomatal conductance (g_s) against DAS.

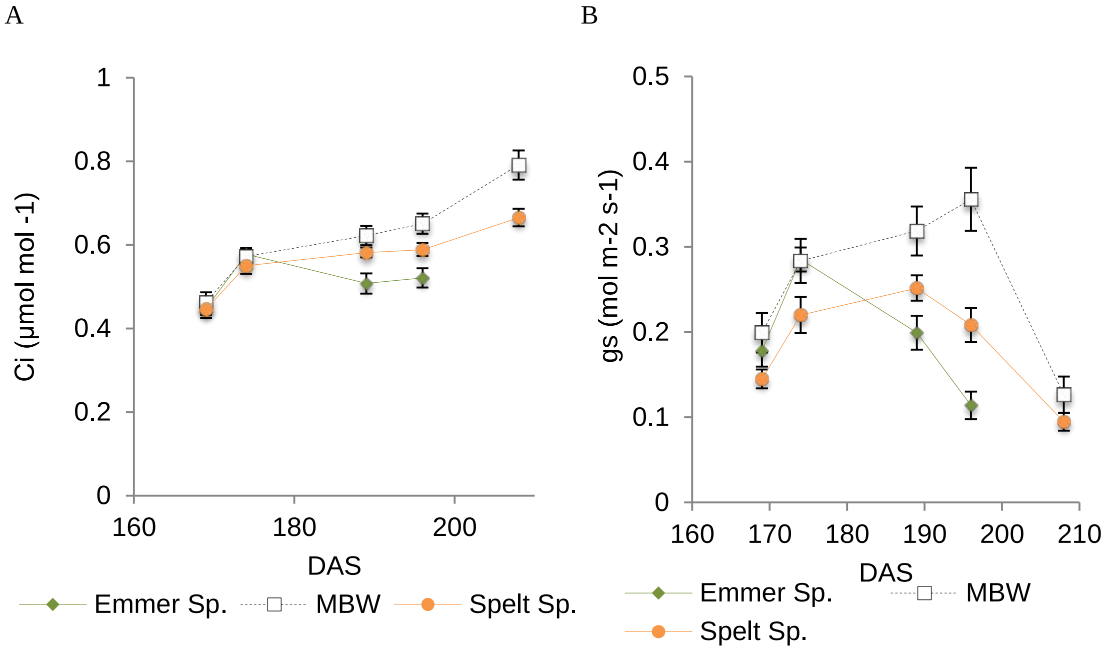


Figure 6.8. Stomatal responses simultaneously measured during ITE measurements of three species both pre- and post-anthesis. A shows sub-stomatal CO_2 concentrations and B shows stomatal conductance (g) against DAS.

6.3.2.6 Carbon isotope discrimination of grain

The grain harvested from the 2013 GH experiment was analysed using carbon isotope discrimination as a means to assess the WUE of investigated genotypes throughout the grain filling period. Carbon isotope discrimination has been widely used as a means to assess WUE and generally the lower the carbon isotope discrimination, the higher the water use efficiency. The grain content is therefore used as a measure of WUE throughout the grain filling period compared to ITE and water to biomass ratio measurements which assess WUE at the leaf level and throughout the life of the plant, respectively (Figure 6.9).

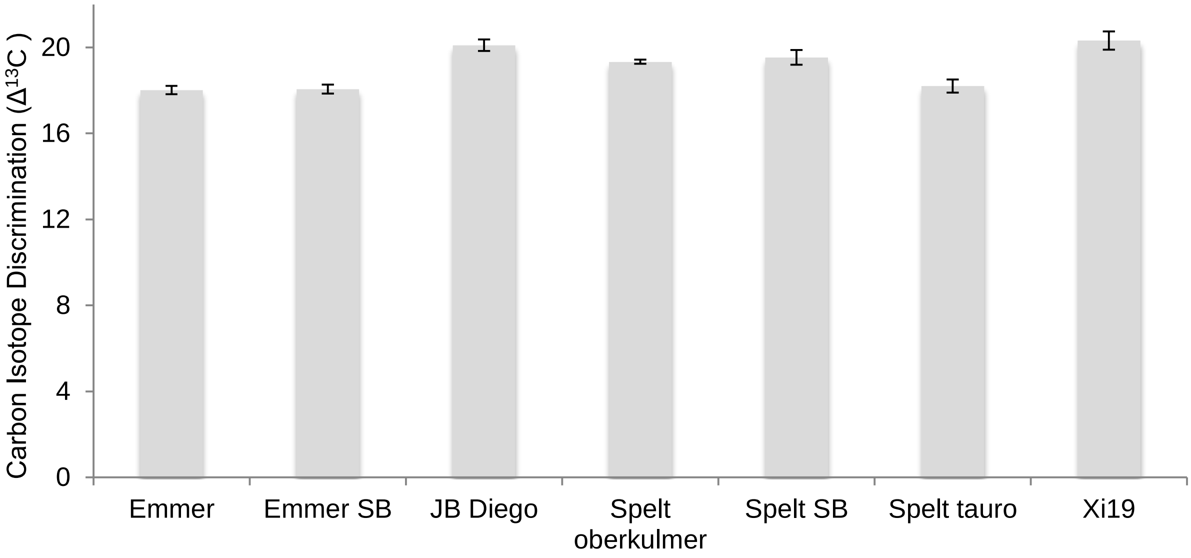


Figure 6.9. Carbon isotope discrimination for five ancient wheat cultivars and two modern bread wheat cultivars measured in the 2013 irrigated glasshouse experiment maintained (error bars represent SEM).

Emmer, emmer SB and spelt Tauro, previously observed to have high WUE through ITE measurements, had significantly lower Δ^{13}C (P <0.001) (Figure 6.9). Although this was not necessarily the case on individual measurement dates when the data was observed over the course of sampling, findings here are consistent with ITE results from the irrigated glasshouse experiment and also corroborate the drought glasshouse experiment where cultivar emmer, and spelt genotypes, showed an enhanced ITE. JB Diego and Xi19 displayed the highest (20.1 and 20.32, respectively). This was consistent with ITE results of the flag leaf over time. Also indicated was that modern bread wheat had consistently lower water use efficiency throughout the entire life of the leaf in ITE results, which is also indicated in Figure 6.5. Similar rankings of ITE and Δ^{13}C indicate that WUE may well differ between genotypes, and species analysis showed the same trend where emmer and spelt species had greater Δ^{13}C than modern bread wheat which supports previous findings that wild and cultivated emmer genotypes have the potential for superior WUE (Peleg *et al.*, 2005; Al-Hakimi, 1998). These consistencies also indicate that higher ITE observed for both ancient wheat species may have had a bearing on the Δ^{13}C grain content.

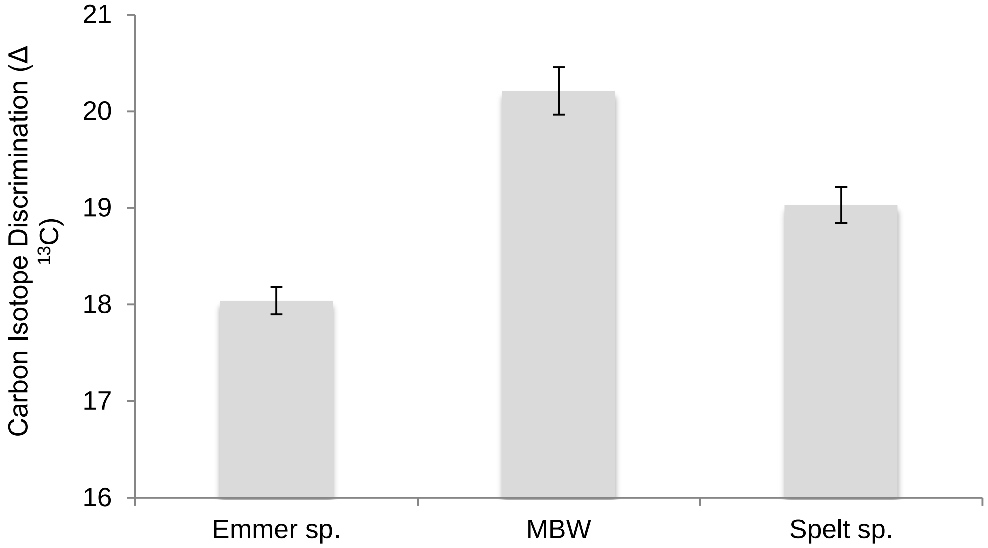


Figure 6.10. Carbon isotope discrimination for three wheat species measured from grain in the 2013 glasshouse experiment, maintained without water limitation (error bars show SEM).

When analysed by species, $\Delta^{13}C$ was significantly different (P <0.001). Similar rankings of ITE and $\Delta^{13}C$ indicate that WUE may well differ between genotypes, and species analysis showed the same trend where emmer and spelt species had lower $\Delta^{13}C$ than modern bread wheat (20.21) which supports previous findings (Figure 6.10) indicating that emmer and spelt had the greatest WUE throughout the grain filling period. Water use efficiency, particularly post-anthesis has been widely associated with yield advantage.

6.4 Biological yield

6.4.1 Accumulated biomass (under drought in 2012)

In the 2012 drought glasshouse experiment, component analyses were performed to determine how much carbon was being assimilated and where it was being accumulated (data not shown). Additionally, harvestable yield, the total amounts of biomass accumulated (provided in m^{-2}) as well as Harvest Index were also calculated (Table 6.8). Spelt SB produced the least grain m^{-2} however this was thought to be a result of poor development, as many spelt SB plants produced prostrate tillers which did not develop to produce grain or had few shoots develop to grain filling. Unsurprisingly, modern bread wheat produced the greatest grain yield per m^2 (775g m^2). Differences between genotypes were highly significant (P <0.001). Modern bread wheat, Xi19, had a HI similar to theoretical upper limit (Araus *et al*., 2002) whereas ancient species had significantly lower HI. Spelt SB had the lowest HI of all genotypes observed. Harvest results highlight that the largest difference between these ancient wheat genotypes and modern bread wheat cultivars, at least from an agricultural perspective, seems to be the partitioning of resources (and potentially irregular development potentially due to inadequate vernalisation). Spelt cultivars and to a lesser degree emmer and einkorn cultivars, do have the capacity to assimilate and accumulate carbon however, it is the partitioning of this biomass that means that production is not viable for grain production. Since the theoretical limit for HI in MBW is nearing (0.62) (Austin *et al*., 1980), genotypes like spelt Oberkulmer may provide genetic variation for increased biomass to boost yields.

Spelt Oberkulmer and spelt SB produced among the largest amounts of dry biomass (2978 and 2989 g m^{-2}, respectively) although this was also thought to be an artefact of unusual development mentioned previously. Plants produced lots of tillers with few shoots actually setting grain, which may have been a result of incomplete vernalisation. It is interesting to note, that although Xi19 produced among the least total biomass, grain yield was significantly better, a trait common in modern wheat through breeding since HI was a large focus of many breeding initiatives.

Table 6.8. Grain harvested (m^{-2}), total above ground biomass and harvest index of seven ancient and one modern bread wheat from glasshouse experiment II where plants were grown in 1m columns.

Genotype	Grain yield (m^{-2})	Total biomass (g m^{-2})	Harvest Index
Einkorn	469.8	1222	0.38
Einkorn SB	447.6	1413	0.32
Emmer	493.5	1634	0.30
Emmer SB	448.7	1578	0.30
Spelt Oberkulmer	202.8	2978	0.13
Spelt SB	41.5	1989	0.07
Spelt Tauro	183.5	1875	0.17
Xi19	775.0	1269	0.61
P	<0.001	<0.084	<0.001
SED (37 DF)	98	291	0.04

Biomass and harvestable yield in the drought glasshouse experiment (2012) however, did not appear to mirror IRGA results. By removing the complexities of the dynamics between ITE and biomass production, it would be expected that those genotypes where photosynthetic rates were highest, may have had the greatest biomass production or grain yield. For genotypes such as Xi19, einkorn and emmer, this was not the case. However, this might be an oversimplification as spelt Oberkulmer, shown in Table 6.8 to produce the most biomass, was observed to have the most consistent photosynthetic rates from pre- to post-anthesis, inferring that although photosynthetic rates were comparably lower, compared to other genotypes, the consistency throughout the season may have compensated for this and therefore paid dividends in terms of biological yield production (as observed in Table 6.8)

6.4.2 Accumulated biomass (2013 irrigated GH experiment)

In the 2013 irrigated GH experiment, the total amount of biomass produced m^{-2} was calculated (Table 6.9), as well as the grain yield and harvest indices of each genotype. Surprisingly, einkorn SB produced the greatest amount of biomass (g m^{-2}) and grain yields (m^{2}) whereas Xi19 and JB Deigo produced the least total biomass (P <0.001). As observed in the drought glasshouse experiment (2012), although ancient wheat species, particularly emmer, emmer SB and spelt SB produced a large biological yield, this was not necessarily reflected in in the harvestable product. Einkorn SB produced the greatest harvest, however, much of this could be chaff as it was difficult to separate from grain. The high biological and harvestable yield observed for einkorn SB may also be instructive of high RUE observed in previous field trials, the high A_{sat} during light response measurements performed in the 2012 glasshouse experiment and are consistent with biomass 2012 GH biomass data. Spelt SB, emmer SB and JB Diego also produced a comparably large amount of grain per m^{2} (853.2, 707.5 and 1076.1g m^{2}) whereas emmer and emmer SB produced lower amounts of biomass and harvestable yields compared to genotypes such as spelt SB, einkorn and einkorn SB, however also had comparably low transpiration and photosynthetic rates. Furthermore, photosynthetic rates were lower for spelt Tauro which incidentally produced the least total biomass and yield. Conversely, Modern bread wheat, Xi19 and JB Diego produced the greatest yields and also showed the greatest C_i and stomatal conductance. Differences among species were highly significant (P <0.001), so too were differences between HI (P <0.001) (data not shown). Once again, results indicate that ancient wheat genotypes, particularly einkorn species, do have an ability to produce biomass. Generally results have indicated that ancient species do not partition assimilate to economic yield.

Table 6.9. Grain yield, biomass and the deduced harvest index from the 2012 GH experiment.

Genotype	Grain (m^{-2})	Biomass (g m^{-2})	Harvest Index
Einkorn	970.6	2350	0.42
Einkorn Hungarian	918.1	2304	0.39
Einkorn SB	1544.9	3484	0.44
Emmer	761.0	2440	0.31
Emmer SB	707.5	2160	0.33
JB Diego	1076.1	2126	0.50
Spelt Oberkulmer	777.7	2003	0.39
Spelt SB	853.2	2247	0.38
Spelt Tauro	651.9	2001	0.32
Xi19	729.8	1882	0.40
P	<0.001	<0.001	<0.001
SED (76 DF)	152.9	269.9	0.04

6.5 'Stay green'

6.5.1 Green area persistence (2012 GH experiment)

Christopher *et al*. (2008) states that increased flag leaf duration may afford plant the propensity to resist drought in certain environmental conditions. Green area persistence was observed in the 2012 GH experiment. Genotypic differences were significantly different (P <0.001) (Figure 6.11). As observed in previous experiments, plants generally followed the same trend, by maintaining flag leaf 'greenness' until approximately 160 DAS (genotypic data not shown). However, each of the spelt genotypes maintained green area with high SPAD values observed even beyond sampling indicating the presence of 'stay green' within drought conditions. This was observed to a greater extent in the current results compared to previous field trials, and was thought to have been emphasised in this experiment as a result of the drought imposed (80% pre-anthesis and 50% post-anthesis) potentially causing the expression of this tolerance trait in response to drought stress. Lawson *et al*. (2012) states that prolonged survival of green area may be a result of an enhanced ability to react to reactive oxygen species (ROS), which could be expressed within stressful environments or alternatively may be caused by an adaption in the chlorophyll catabolic pathway (Thomas *et al*., 2001). Alternatively, green persistence may be observed due to the abnormal development witnessed for some spelt plants. Figure 6.10 shows the analysis by species where differences were highly significant. Whereas MBW, emmer and einkorn species all had a high rate of chlorophyll reduction shown by the decline in SPAD to 0 (SPAD value) at ~180 DAS (P <0.001), spelt species conserved green area so although these genotypes had comparatively low SPAD values at the start of the season, they were able to maintain it for a longer duration. It could therefore be purported that spelt species, particularly spelt Oberkulmer, was able to maintain a high WUE (indicated through ITE measurements and biomass/water uptake ratios) as a result of the 'stay green' trait, particularly under drought. Further analysis should assess whether this persistent green area is photosynthetically active and whether it therefore contributes to drought tolerance.

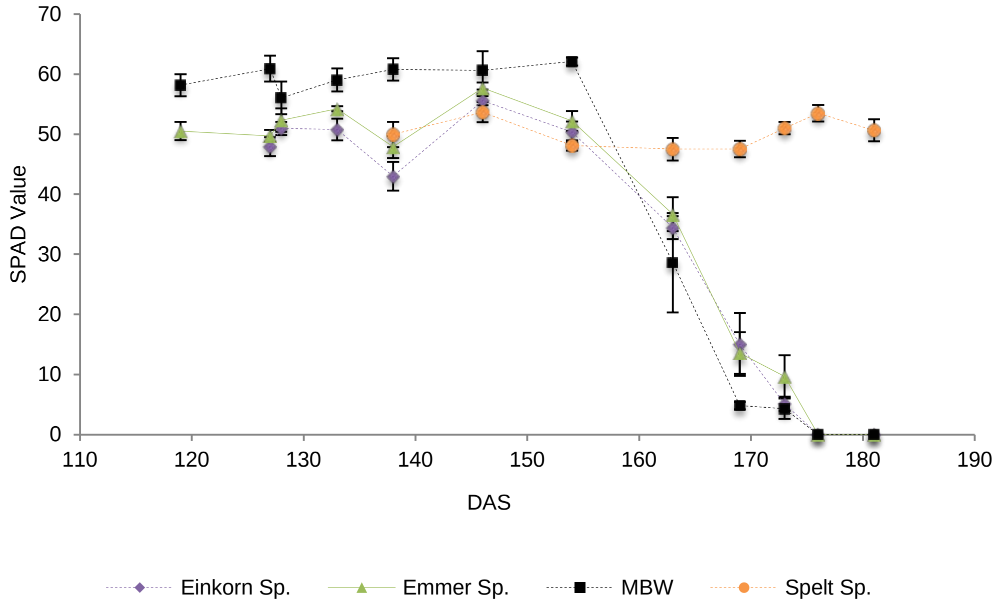


Figure 6.11. SPAD values throughout glasshouse experiment II (DAS) for all four investigated species (error bars denote SEM).

These data show the potential presence of green area persistence in spelt species, however interpretation of these results must also consider the disparity between species, particularly for developmental rates.

6.5.2 Green area persistence (2013 GH experiment)

Green area persistence was also monitored in 2013 where plants were fully irrigated. All genotypes followed the same general trend where flag leaf chlorophyll content, indicated by SPAD readings, was initially high at the beginning of sampling, which corresponded to flag leaf emergence. SPAD readings were significantly different by genotypes and sampling day (P <0.001) (genotypic data not shown) as were species differences and sampling day (P <0.001) (Figure 6.12). As observed in previous chapters, MBW generally had the greatest SPAD at the start of season. After approximately 191 DAS, all genotypes declined in terms of leaf ‘greenness’. Although results showed that SPAD readings significantly differed between genotypes and species, the irrigated glasshouse experiment (2013) did not indicate the presence of a ‘stay green’ trait, contradicting that already observed within previous experiments in the current project. It is possible that this was due to non-limiting irrigation which may have not been the case in the field trials of 2010 and 2011 where the trait was first observed since these were both rain-fed. This absence of ‘stay green’ in 2013 was unfortunate; since the ‘stay green’ trait could not be confirmed, it was not possible assess whether green area was photosynthetically active, although field experiments do indicate that spelt genotypes continue to accumulate biomass between anthesis and harvest. Modern bread wheat tended to have greater SPAD values early in the season and these were maintained throughout sampling. Einkorn species had the lowest leaf ‘greenness’ from 154 DAS to 191 (where values ranged from 37.88 to 46.66) however from 191 to 208 DAS emmer species showed the lowest SPAD values and therefore declined in terms of chlorophyll content of the flag leaf at a faster rate when compared to other genotypes. These results did not show the presence of green area persistence trait for spelt species, although spelt did perform similarly to modern bread wheat.

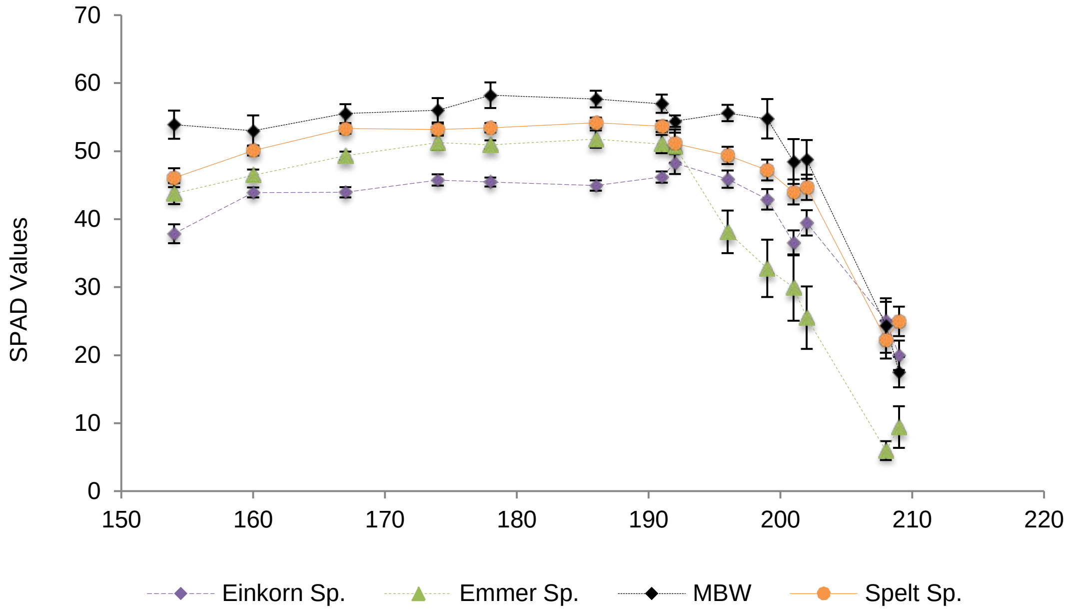


Figure 6.12. SPAD values throughout the 2013 GH experiment (DAS) for all four investigated species (error bars denote SEM).

6.6 Specific flag leaf area (2013 GH experiment only)

In 2013, as a means to extend the investigation of leaf level traits, the irrigated glasshouse experiment also included analyses of specific flag leaf area (SLA) relating to the leaf mass per unit area meaning that greater SLA equates to thinner leaves (Westoby *et al*., 2002) (Figure 6.13). Cornelissen *et al*. (2003) states that lower SLA values tend to correspond with high investments in leaf ‘defences’, particularly structural defences, associated with increased leaf lifespan (Cornelisson *et al*., 2003), potentially indicative of the ‘stay green’ trait observed in spelt cultivars in field experiments. Modern bread wheat had comparatively low SLA compared to several ancient species, indicating thicker leaves. SLA was significantly different between genotypes (P = 0.025). It would be expected that lower SLA would imply a greater *in situ* rate of photosynthesis, as observed by Poorter & Evans *et al.* (1998); SLA was greater for emmer and emmer SB, observed to have comparable high photosynthetic rates on 169 and 174 DAS and increased photosynthetic rates in previous experiments. It was thought that increased leaf thickness would be associated with high photosynthetic rates where greater leaf machinery per unit area may originate from extra layers or longer palisade cells, leading to increased chloroplast numbers and photosynthetic enzymes per unit leaf thus increasing photosynthetic capacity (Evans & Poorter, 2001). Results, however, showed that genotypes observed to have greater photosynthetic rates, in fact had higher SLA, inferring that they also had thinner leaves. Spelt Oberkulmer, Tauro and SB also had high

SLA, indicating thinner leaves. Although the converse was expected, these results may derive from lower leaf nitrogen content in the leaves of highly photosynthesising leaves, therefore leading to differences in the structural arrangement of photosynthetic components, which allowed greater access for CO_2 to catalytic sites within the leaf. Reich *et al*. (1998) state that for Rubisco to operate efficiently, the photosynthetic machinery needs to be spread thinly across the surface of the cell wall. Emmer and emmer SB were also observed to have high A_{sat} in the drought gashouse experiment (2012), which also corroborates these findings. Further investigation would require leaf physiological analyses, beyond the scope of the current project.

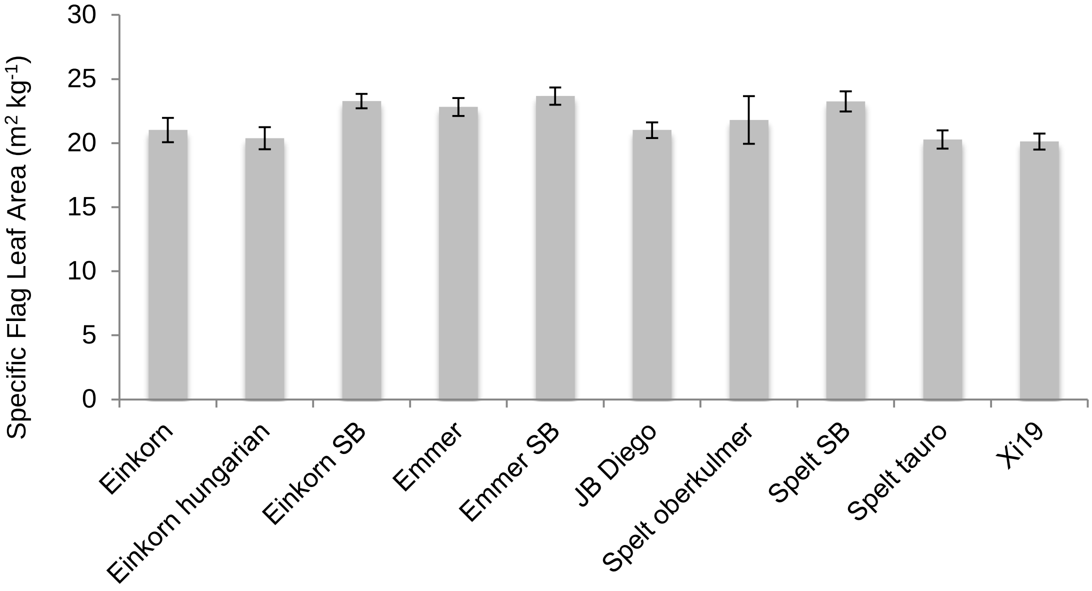


Figure 6.13. Specific flag leaf area for 5 ancient wheat cultivars and 2 modern bread wheat cultivars measured in gashouse experiment III (error bars denote SEM).

SLA by species was also significantly different (P = 0.043) (Figure 6.14) where emmer had the greatest SLA, followed by spelt then modern bread wheat (23.24, 21.92 and 20.56 m^2 kg^{-1}, respectively) indicating that, overall, ancient species had thinner leaves. This may be the underlying trait that confers consistently high photosynthetic rates and increased ITE observed in previous experiments (although this was not necessarily the case in the current glasshouse experiment since emmer and emmer SB only had increased photosynthetic rates on the first two sampling dates). Reich *et al*. (1998) state that thinner leaves, which presumably have smaller quantities of nitrogen per unit, would allow the substrate, CO^2, better access to catalytic sites thus increasing the activity of Rubisco.

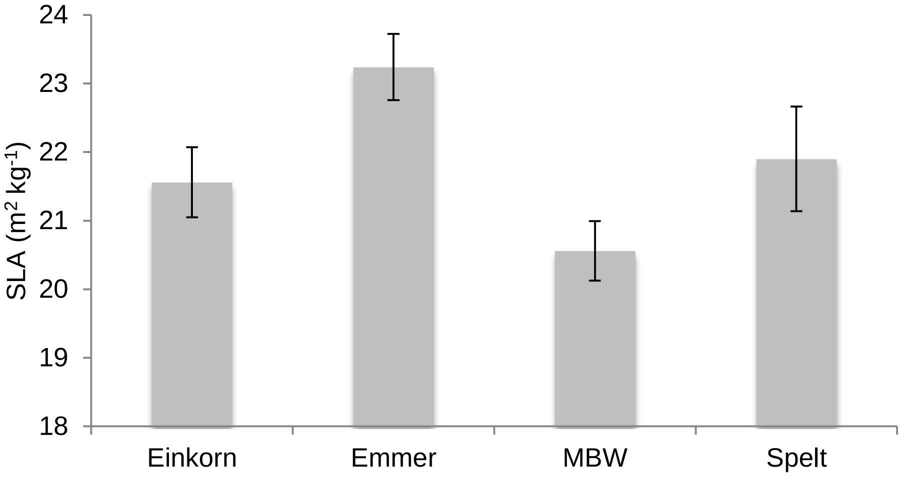


Figure 6.14. Specific flag leaf area (m^2 kg^{-1}) for five ancient wheat cultivars and two modern bread wheat cultivars measured in gashouse experiment III. Error bars denote SED.

6.7 Glasshouse experiment conclusions

This component of the experiment was a means to explore the selected genotypes with and without water limitation. Data gained here did show some consistencies with preliminary work (although this preliminary analyses was not shown), for instance investigation of biochemical properties of the leaf showed very little variation for carboxylation rates or electron transport, agreeing with Wullschleger (1993) who stated that these traits are highly conserved within crop species. Many of the ancient species, notably einkorn SB and emmer, displayed comparable photosynthetic rates when compared to modern bread wheat. Results also indicated that those genotypes that had previously displayed high RUE, also displayed greater A_{sat}, implying that the increased RUE observed, may have derived from this high photosynthetic capability. Glasshouse experiments also highlighted that several ancient wheat species, thought to originally derive from dry rain-fed environments, possessed increased ITE (namely emmer and spelt SB) where the mechanisms whereby ITE was maintained, were different between the emmer and spelt species (either through high photosynthetic rates or low transpiration rates, respectively). In addition, the 2012 GH experiment highlighted that biomass/water uptake ratios were significantly different indicating that there was variation for WUE throughout the entire plant cycle.

Biomass data corroborated that several ancient wheat species had comparable proficiency to produce yield when compared to modern bread wheat, inferring that spelt genotypes in particular, made a trade-off between moderate photosynthetic rates and comparably lower transpiration rates which were observed to benefit biological yield production. Additionally, increased canopy duration, shown to be particularly distinct during post-anthesis drought imposed in the 2012, was also thought to contribute to biomass production, although, as previously observed, this did not translate to harvestable yield. Glasshouse investigations highlighted that the value of these ancient wheat species may not derive from yield production but from a proficiency to produce biological yield, potentially a result of increased growth duration through delayed senescence and more conservative use of water resource, both of which are traits that could be useful in producing novel plant types for the production of more efficient.

7. General Discussion

Physiological and yield potential data for ancient wheat species is relatively sparse although spelt and emmer have been reported to performe well in low input conditions (Troccoli & Codianni, 2005). Other research indicates that old cereal cultivars have higher radiation use efficiency than their modern counterparts (Muurinen & Peltonen-Sainio, 2005). This project aimed to characterise the traits of ancient wheat species and compare them with the traits of modern bread wheat. In the first phase of the experiment, detailed analysis of the above ground partitioning, yield and morphology were studied for the eight ancient wheat species, six were found to be of interest for resource use efficiency or had been indicated to have favourable characteristics in preliminary studies such as WUE (Reported by Isaaks, 2009 and Sparkes, 2010). Further analyses assessed the biochemical properties of genotypes, after which a sub-set of five genotypes were selected for analyses to be scaled down to physiologically characterise leaves. The current chapter aims to integrate the main findings of the project, regarding the initial hypothesis and relevant findings previously reported in the literature.

Biological yield may be enhanced through several avenues; 1) the duration of the crop may be increased, 2) the fraction of solar radiation intercepted may be enhanced, or alternatively 3) the efficiencies at which individual plant metabolisms can convert resources into plant dry matter may be enhanced (Murchie *et al*., 2008). These factors culminate to determine the proficiency at which a species will translate resources into biological yield. There are a number of variables thought to influence RUE; the variation for some of these has been discussed in the current work.

7.1 Plant growth, development and canopy architecture

Morphological character traits, ultimately affect the amounts of photosynthetically active radiation (PAR) intercepted by a crop canopy, and therefore have a large bearing on biological yields. A vast number of elements will affect morphology including developmental rates, canopy architecture and biomass partitioning (Azam-Ali & Squire, 2002). Muurinen & Peltonen-Sainio (2006) state that canopy height influences radiation spread within the canopy; height influenced the fraction of light intercepted, radiation use efficiencies (RUE) as well as the biomass accumulation, in turn impacting GAI. When assessing extinction of light down the canopy, it was found that modern bread wheat intercepted the largest fraction of light per unit height, therefore intercepting more light within the uppermost layers of the canopy, allowing flag leaves, which are the main contributors to carbon assimilation, the greatest proportions of incident light. This was thought to be a more ‘economical’ use of radiation within environments such as those within the UK, where incident irradiance is often the limiting factor. Thus it can be stated that the altered morphology through the introgression of *Rht* in particular offers an advantage to modern bread wheat when compared to their much taller ancient progenitors.

Tiller development and economy was also investigated in the 2010 and 2011 field experiments. Tiller production was variable across years with ancient species, especially einkorn, producing more tillers than modern bread wheat cultivars in 2010, as was hypothesised at the outset of the project. The increased tiller production for ancient species in 2010, agrees with Critchley (2001), who stated that increased vegetative phase (due to slow development) increased the time frame where tiller production could occur. Furthermore, Sparkes *et al*., (2006) state that plant population affects tiller initiation therefore, this tiller production was probably influenced by large differences in population densities (due to the variable emergence). As a result therefore, the extent of the differences for tiller production were not conclusive since ancient wheat species had greater tiller production compared to MBW in 2010, whereas the converse was true in 2011. Tiller formation and economy was also thought to have influenced GAI; GAI developed until anthesis after which it declined, as did tiller production. This was particularly apparent for einkorn and einkorn SB whereby GAI increased from flag leaf emergence to anthesis, thought to be a result of increased shoot production at these points. Reynolds and Trethowan (2007) state that high tiller production is a mechanism whereby ground cover can be increased. The conclusions made from this component of the experiment are subject to the variation imposed by the differential emergence rates, which naturally, had an impact on plant population, tiller production and survival and therefore, also influenced light interception. It is therefore not possible to accept H_2, which stated that modern bread wheat would produce fewer tillers. Results did indicate however, that modern bread wheat did partition fewer assimilates towards tillers indicated by smaller proportions of biomass being directed to leaf and straw material, when compared to ancient wheat species.

7.2 Biomass production and RUE

In field experiments, bread wheat produced more biological yield throughout sampling when compared to ancient wheat species. Despite the comparatively low emergence observed, spelt species generally produced more biomass (g m^{-2}) compared to einkorn and emmer. Furthermore, spelt displayed increments in biomass from anthesis through to pre-harvest, although this was observed to a lesser degree in 2011. These trends were consistent increasing GAI observed in spelt species. Therefore, despite low plant densities, spelt indicated proficiency for biomass production, which was similar to that of modern bread wheat across both seasons. This was further supported by high RUE, particularly in spelt Oberkulmer and Tauro, both pre- and post-anthesis in 2011 when compared to all modern bread wheat cultivars, as was initially hypothesised. High RUE however, was not consistent for these genotypes in 2010 therefore H_5, stating that spelt germplasm would have greater RUE, cannot be accepted. Einkorn and einkorn SB also displayed high RUE across both experimental years, which was thought to be due to profuse tiller production as a result of low plant density. Einkorn species were also observed to produce reasonable yields in 2011 where ear indices were comparable to those of MBW. However, in 2010, spelt species directed the largest proportion of biomass towards the ear of the ancient species. These findings imply that the shorter culm length of modern bread wheat did not confer greater RUE as Toyota *et al*. (2010) suggested.

Biomass was also assessed in the glasshouse experiments that were established in 2012 and 2013. In the 2012 GH experiment, spelt Oberkulmer produced the greatest biomass, although this did not translate through to yield, which could have been a reflection of poor development observed for spelt genotypes. Low yields may also be indicative of the lower photosynthetic rates observed for these cultivars. Therefore, it is thought that this advantage for biomass production was a function of increased green area duration as well as photosynthetic stability from pre- to post-anthesis, rather than photosynthetic capability, indicating a green area persistence trait. Einkorn cultivars were also observed to produce comparable amounts of biomass in field trials however, this was thought to be a result of poor establishment and consequently, profuse tillering. These results support the hypothesis (H_1) stating that ancient wheat biomass production, particularly that of einkorn and spelt, would be comparable to modern bread wheat.

Investigation of canopy structure combined with assimilate partitioning data from pre-harvest analyses, indicated that the shorter stature of modern bread wheat, and greater GAI, allowed for greater harvest indices and larger quantities of assimilates to be partitioned towards the ear. However, the taller ancient wheat species, partitioned greater quantities of biomass towards tiller production. Further assessment of biomass partitioned to the ear in 2010, show emmer species to direct the largest proportion of biomass toward ear components, although this was not necessarily instructive of greater yield since JB Diego achieved the highest harvest index (HI). This supports H_4, suggesting that modern bread wheat partitioned more carbon towards the ear when compared with ancient species, and therefore had greater harvest indices. This implies that the structure (corroborated by high GAI and F) of JB Diego and modern bread wheat in general, to be preferable to that of emmer and ancient species. It is important to note that, although the economic yield of modern bread wheat was greater, a small proportion of this derives from the ability to produce biomass, but instead, originates from HI which has been selectively bred into germplasm (Shearman *et al*., 2005). This can be illustrated by einkorn, which had the lowest tiller survival rate, however despite this, still produced reasonable yields compared to other genotypes. It is therefore the ability to produce greater biological yields, rather than yield production in ancient wheat species that could provide the necessary genetic gains (Shearman *et al*., 2005). The investigations performed in 2010 and 2011 could not identify a clear trend in dry biomass partitioning by genotypes or by species, however Takahasi & Nakaseno (1990) suggested that biomass partitioning typically influences the fraction of irradiance that the canopy is able to intercept and thus will indirectly impact the amounts of biomass accumulated.

7.3 Photosynthetic radiation capture and conversion

It could be the case that enhanced RUE may stem from enhancements to the photosynthetic machinery or processes. Section 6.2.1 shows that there were positive associations between maximum rates of photosynthesis and RUE, indicating the importance of leaf level traits to overall productivity. Section 6, detailing the glasshouse experiment results, followed on from this by empirically determining and describing some of the carbon assimilation mechanisms and the limitations that may affect productivity at the leaf level.

7.3.1 Biochemical aspects of leaf physiology

An increase in carboxylation rates could be fundamental to superior RUE. It has been reported that variation in A_{sat} (2012 water-limited glasshouse experiment), should also reflect similar variation in related parameters such as maximum rates of carboxylation (V_{cmax}) (Reich *et al*., 1998). Einkorn and spelt Tauro, which both had high RUE in field trials (where only pre-anthesis RUE differed in 2011), had relatively high V_{cmax} also. Differences between genotypes for V_{cmax} however, were not significant; in fact little variation was observed for biochemical parameters, which was also the case in the preliminary assessment (although this data was not included in the current paper). This corroborates Wullschleger (1993), who found no differences in the biochemical reactions that regulated gas exchange between crop species.

In the irrigated glasshouse experiment (2013) (as well as preliminary glasshouse assessments) however, there were significant differences found for R_d and TPU. The former represents the CO_2 lost through respiration, resulting in assimilate losses. Respiration is an essential process which is affected by the efficiency of photosynthesis as well as the irradiance capture (Azam-Ali & Squire, 2004). The latter therefore, infers that respiration will be influenced by canopy architecture. Emmer and spelt Oberkulmer were observed to have high R_d in the irrigated glasshouse experiment. Consistently however, MBW showed reduced R_d when compared to ancient species (both pre- and post-anthesis), indicating that MBW had consistently lower respiration rates. This is pertinent since respiration can account for up to 30% of the rate of photosynthesis (Rawsthorne, 1992). The lower respiration rates of modern bread wheat therefore imply an increased efficiency. Furthermore, MBW maintained R_d from pre- to post-anthesis in the 2013 GH experiment, whereas emmer and spelt showed large increases after anthesis, indicating that R_d increased with leaf age. TPU in spelt Tauro and einkorn SB, genotypes that also displayed high RUE in previous field trials, were high, indicating lower limitation imposed by the products of photosynthesis (such as triose phosphate).

7.3.1 Photosynthetic capacity

At the outset of the project, it was hypothesised that ancient wheat genotypes and MBW cultivars would have comparable photosynthetic rates. Results suggested that this was not the case where, in the drought glasshouse experiment (2012), emmer and einkorn SB had greater photosynthetic rates, pre-anthesis. In the same experiment, A_{sat}, defined as the maximal rate of photosynthesis under light saturated conditions, was generally seen to reduce with time (from pre- to post-anthesis), indicating that the photosynthetic capabilities of the flag leaf reduced with age in all genotypes except for spelt SB, spelt Tauro and Xi19. Spelt Oberkulmer also displayed conserved A_{sat} photosynthetic rates from pre- to post-anthesis. Emmer, displayed high pre- and post-anthesis photosynthetic rates during ITE measurements (2012 GH). These genotypes therefore had an enhanced ability to assimilate carbon, which may derive from increased Rubisco concentrations per unit leaf area (Terashima *et al.*, 2011). Reich *et al*. (1998) state that variation for photosynthetic assimilation (A_{sat}) is primarily due to differences in biochemistry rather than differences in CO_2 supply. It was also these genotypes that had a greater ability to assimilate carbon in light limited conditions, indicated from high quantum yields. Therefore photosynthetic rates of emmer and einkorn SB are comparable to those of the MBW cultivar investigated. This supports H_6, which stated that the photosynthetic rates of modern bread wheat and ancient wheat species would not differ. This hypothesis can be accepted for einkorn SB (pre-anthesis only) and emmer, however must be rejected when considering all other investigated genotypes. A_{sat} results also suggest that spelt genotypes may contribute to carbon accumulation to a greater degree, post-anthesis, which could also be linked to the ‘stay green’ leaf traits observed in all spelt genotypes across all experiments. Furthermore, this carbon accumulation may also be related to the generally lower SPAD values observed for spelt genotypes pre-anthesis, which were seemingly ‘conserved’ throughout the life of the flag leaf.

It is possible that this variation derives from the intrinsic physiology of the leaf, which may be caused by 1) differential Rubisco limitations 2) Rubisco concentrations or 3) physiological variation in leaf structure. Although 1) and 2) were not investigated in the current work, investigation of SLA (in the irrigated glasshouse experiment), thought to represent the variation due to environmental adaptation (Reich *et al*., 1997), indicated that some variation may exist for leaf structure between these genotypes. SLA relates to the leaf area per unit mass invested, and therefore indicates efficient light capture and photosynthetic capacity (Reich *et al*., 1997). MBW had significantly thicker leaves, compared to ancient species, which Reich *et al*. (1998) infers reduced A_{sat} per unit nitrogen (N). Emmer had the thinnest leaves as well as high A_{sat} in the drought glasshouse experiment, which also supports this hypothesis.

Leaves are highly complex structures containing photosynthetic machinery however, the packaging and orientation of the various components is highly dynamic among species (Morison & Lawson, 2007). Photosynthesising cells and chloroplasts are arranged some distance from stomatal openings, thus in different species, the diffusion pathway for CO_2 differs. The relevance of this may be considered since it describes the by CO_2 diffusion pathway (Morison & Lawson, 2007). It is therefore likely that the structural differences between the two epidermal layers are indicative of the variation; leaf thickness (SLA), may be used to indirectly infer structural differences between genotypes. Results indicate that emmer had reduced leaf thickness compared to MBW, implying that Rubsico was spread more thinly across the cell wall, allowing CO_2 to diffuse to catabolic sites with greater ease (Reich *et al*., 1997). The thicker, more dense leaves within MBW may have caused leaf shading which could have limited light reaching the chloroplast, consequently reducing CO_2 access to active sites (Reich *et al*., 1997). Further assumptions can also be made from SLA; Reich *et al*. (1997) state that plants with high SLA, high A_{sat} and short leaf life spans (as observed for modern bread wheat species) commonly have high relative growth rates.

The increased A_{sat} of einkorn SB may also be linked with high RUE observed in both field trials. Despite being low, the A_{sat} for spelt genotypes were fairly consistent from pre- to post-anthesis and actually increased with leaf age for spelt SB and Tauro. This stability for carbon accumulation could indicate that the 'stay green' trait suggested by SPAD measurements and visual scoring were 'functional', i.e. was still photosynthetically active, and therefore contributing to carbon assimilation. Thus, although other genotypes may well have an enhanced ability to assimilate carbon at limited and saturating light intensities, spelt Tauro and SB may maintain A_{sat} for a longer duration, providing a basis for the high RUE previously reported. As well as spelt SB and Tauro, Xi19 showed increased A_{sat} from pre- to post-anthesis. Overall, the data combined is not clear cut, however there are inferences of greater carbon assimilation for some of the genotypes, one in particular being that of the 'stay green' trait in spelt. The exact causes of the variation for photosynthetic ability are not known, however results indicate that increased life-span and instantaneous productivity, indicated by A_{sat}, confer some advantage for RUE and biomass production. Further work could be carried out investigating the content and re-allocation of nitrogen to various photosynthetic pools, which may help to undercover the basis of the observed variation.

7.4 Water Use Efficiency

There is a requirement to identify plants which harbour underlying mechanisms for improved WUE (McAusland *et al*., 2013). As discussed in Chapter One, water use efficiency can be defined through 1) instantaneous transpiration use efficiency (A/T), 2) ratios of biomass to water uptake or 3) carbon isotope discrimination ($\Delta^{13}C$). It was hypothesised that since the ancient varieties included in the current study would have originated from the dry, rain-fed Fertile Crescent, they would possess traits that confer an adaption to limited water supply and therefore, be more efficient in water use. Similar rankings using all three methods indicate that WUE differs between genotypes and species where emmer and spelt species had greater $\Delta^{13}C$ than modern bread wheat. All glasshouse experiments indicate enhanced WUE in ancient wheat species, except for the 2013 GH experiment, where enhanced ITE was not observed to the same extent, potentially because drought conditions were not imposed. Even so, although ITE was not consistently high after the first sampling date, a trend was observed where ancient wheat species maintained ITE over time. Results support previous findings that wild and cultivated emmer genotypes have the potential for superior WUE (Peleg *et al*., 2005; Al-Hakimi, 1998).

High ITE in wheat is usually associated with lower stomatal conductance and photosynthetic rates (Fischer, 2011). All spelt cultivars displayed low stomatal conductance (pre- and post-anthesis), which may be the origin of the low photosynthetic rates observed in spelt genotypes, therefore increasing ITE. Furthermore, spelt genotypes, particularly spelt Oberkulmer, consistently displayed low transpiration rates coupled with moderate photosynthetic rates. The large quantities of biomass produced however, suggest that the trade-off for lower photosynthetic rate and transpiration rate, leading to high ITE did not allow them to produce large economic yields however, paid dividends in terms of biological yield production. In contrast, emmer genotypes indicated that ITE was enhanced by increased photosynthetic rates. Furthermore, einkorn had high photosynthetic rates pre-anthesis for high ITE and low transpiration post-anthesis and therefore seemingly used both mechanisms to conserve ITE. Stomatal conductance for einkorn was also intermediate of those of emmer and spelt cultivars. This suggests that these ancient wheat genotypes, originating from the dry rain-fed Fertile Crescent, were more conservative in their use of water, supporting H_7. Additionally, results indicated that the WUE observed in ancient species derived from low transpiration and conductance in spelt species only. The contrasting mechanisms observed for emmer and spelt genotypes in the drought glasshouse experiment however, were not observed to the same extent in the irrigated glasshouse experiment (2013), thought to be a result of the difference in water regimes used. Whereas the irrigated glasshouse experiment aimed to assess genotypes without resource constraint, the previous work had grown genotypes in water-limited conditions. Therefore, it is possible that these genotypes employ these mechanisms when under drought stress. It might, therefore, be the case that this trait is a protection mechanism. Lawson *et al*. (2012) states that prolonged survival of green area may be a result of an enhanced ability to react to reactive oxygen species (ROS), which could be expressed within stressful environments or alternatively may be caused by an adaption in the chlorophyll catabolic pathway (Thomas *et al*., 2001). There is some debate as to the environmental conditions under which this trait is useful. Christopher *et al*. (2008) reported that, in wheat ‘stay green’ was only useful when water was available post-anthesis, while Borrell & Hammer (2000) found that ‘stay green’ in sorghum is associated with post-flowering drought resistance. Results corroborate the observation that spelt has greater canopy persistence (Sparkes, 2010). Larbi & Mekliche (2004) state that genotypes reported to have functional green area persistence during grain filling may be the key to securing wheat yields in semi-arid regions.

7.5 Leaf physiology: Green area persistence and SLA

The final aspect of the project related to the ‘stay green’ trait. It was thought that spelt cultivars would display green area persistence particularly post-anthesis when compared to all other genotypes. Christopher *et al*. (2008) states that increased flag leaf duration may afford plant the propensity to resist drought in certain environmental conditions. It could therefore be purported that spelt species, particularly spelt Oberkulmer, were able to maintain a high WUE (indicated through ITE measurements and biomass/water uptake ratios) as a result of the ‘stay green’ trait, particularly under drought. The extent to which spelt species stayed ‘green’ in the drought glasshouse experiment surpassed that observed in any of the previous experiments, and is hypothesised to be a result of the drought conditions imposed which may have led to the expression of these tolerance traits (Lawson *et al*., 2012).

The results of the irrigated glasshouse experiment demonstrated that ancient wheat species, particularly emmer species, had significantly greater specific leaf area, indicating reduced leaf thickness. Reich *et al*. (1998) report that increased nitrogen per unit area leaf follows from increased leaf thickness, associated with increased chloroplasts width, reducing the activity of Rubisco by reducing the diffusion of CO_2 to catalytic sites (Reich *et al*., 1998). Conellison *et al*. (2003) also state that low SLA tends to correspond with structural investments in leaf ‘defenses’. Spelt species had a high SLA and was also observed to ‘stay green’ post-anthesis. MBW however, had thicker leaves, which generally senesced earlier; Reich *et al*. (1997) suggest that this may be due to the overall cost where A_{sat} and respiration are dependent on the abundance of photosynthetic enzymes, pigments and other compounds. The high provision of these may provide high net CO_2 exchange, but ultimately, this will limit the longevity of the leaf (Reich *et al.*, 2007). Additionally, environmental conditions also influence SLA, where species grown within resource rich environments tend to display higher SLA overall (Cornellison *et al*., 2003); thus the non-limiting conditions in which plants were maintained may be a confounding factor which meant that emmer, which did not display green area persistence, had higher SLA than with spelt species.

Results from all experiments (except the 2013 GH experiment) indicate that spelt species have increased green area longevity compared to the other species assessed, supporting H_3. Further analyses should assess this ‘stay green’ trait under non-limited water and drought conditions, and investigate the extent to which photosynthetic area is maintained and functional in the two environments. Increasing photosynthesis is a promising avenue through which productivity may be enhanced. Clearly, the maximum leaf photosynthetic rate is the main theoretical variable that influences RUE (Sinclair & Muchow, 1999) however, the current work also suggests that duration could enhance photosynthetic assimilation.

7.6 Further work

This project, for the most part, explored selected genotypes without water limitation. Data gained here did show some consistencies, however it might be useful to characterise these genotypes under severe drought, as this seems to be the environment in which the ‘stay green’ trait and WUE (through the ITE mechanism), has been most apparent. These may allow RUE and WUE to be related as Caviglia & Sadras (2001) state they are. The project aimed to be as comprehensive as possible however, with the benefit of hind-sight, all glasshouse experiments should be established using soil columns and include additional investigation of root biomass. It was clear that plant emergence continued to be a constraining factor, particularly in field experiments, which limited identification of interception traits. Avenues to circumvent this would be to treat seed in order to increase viability or alternatively increase seed rates, and then thin plants to the equivalent population densities. Canopy traits were measured as comprehensively as possible however, the analyses could further be enhanced by placing sensors in the field and recording continuous data even between growth stages.

The scope for developing the current project is expansive. With the prospect of techniques that allow greater throughput as well as a higher resolution of traits, WUE in these species could be explored to provide much firmer conclusions. The techniques used all have specific drawbacks, for instance $\Delta^{13}C$ are destructive measurements that are based on cumulative seasonal conditions, and therefore may mask specific phenotypic traits (McAusland *et al*., 2013); the use of a range of methods allows WUE to be investigated at different scales which can indicate consistencies in the data; these have proved to be useful in the current work. However, Lawson & Morison (2006) and McAusland *et al*. (2013) report techniques whereby leaf diffusion can be visualised in order to screen genotypes for gas exchange. This would allow spatial patterns of CO_2 concentrations, which factor in the dynamics between photosynthetic rates and stomatal conductance and allow these to be characterised, allowing a more comprehensive understanding of WUE (McAusland *et al*., 2013). Such results could elucidate the variation for diffusion of gases into and out of the leaf. Structural analyses, such as microtome sectioning would also be valuable. This type of study would be useful for emmer and spelt genotypes, which have shown high photosynthetic ability and tolerance to water deficit.

Additionally, given the apparent differences for water uptake and use between genotypes, WUE in relation to root traits should be investigated. An example of which observed synthetic wheat derivatives which had genes from the diploid wild species *T. tauschii* (DD genome), showed that plants had similar dry weight to control cultivars however, roots were distributed more deeply into the soil profile (Reynolds *et al*., 2007). Additionally, root screening techniques, such as WinRhizo (Regent instruments Inc., Quebec, Canada) could be utilised as these are less time consuming and provide a more informative analyses than biomass growth analyses (McAusland *et al*., 2013).

7.7 Results synopsis

The current work investigated several morphological and physiological parameters of ancient and modern bread wheat cultivars, some of which appear to influence the efficiency and assimilation of carbon and water. The current work shows sufficient variation to suggest that survival and duration may be the priority in ancient wheat rather than productivity, with some genotypes indicating increased WUE and leaf longevity. In field trials high RUE was observed for several of the ancient wheat species. Although during the outlined experiments there were confounding factors which made the origin of this efficiency unclear, further research did highlight potential factors that may have contributed to these findings. The most prominent were 1) green area longevity and 2) enhanced WUE, both of which contributed to a proficiency to produce biological yields comparable to those of MBW.

Spelt species consistently displayed green area longevity, essentially lengthening the growing season and therefore leading to greater biomass accumulation; such a trait could be essential to increase wheat productivity allowing cultivars to take advantage of the high incident radiation at the close of the UK winter wheat growing season when modern wheat tends to senesce. The green area longevity observed in spelt appeared to be conserved throughout the species, presenting in all genotypes investigated, however it emerged that there may be some plasticity for this trait which was indicated by the lack of ‘stay green’ where plants were watered without limitation. These findings do however highlight a successful adaptation within drought prone environments, which could prove to be of value to future modern wheat production through breeding.

Additionally, investigation of leaf-level water use highlighted variation between species for the mechanism by which ITE was maintained, traits which could be utilised in order to produce novel wheat varieties with adaptations to specific environmental conditions. An example of which is the production of the Australian varieties, Drysdale and Rees, where backcrossing techniques allowed ITE to be enhanced whilst maintaining traits that afforded the varieties protection against biotic stresses (Condon *et al.*, 2004). Enhanced WUE and green area longevity traits could provide promising adaptations to plants within resource-limited environments. Therefore they highlight avenues through which the biological yield per unit resource may be boosted in the production of modern wheat cultivars. Such traits may prove to be useful to supplement the variation for resource use in modern bread wheat.

7.8 Conclusions

Despite a broad analysis of these genotypes, further clarification of the mechanisms responsible for these adaptive traits is required. Therefore, this research provides a foundation for the development of quantitative trait investigations that should be performed within controlled environments. The current work, however, does suggest that stability, survival and duration may be prioritised over productivity in the ancient wheat growth cycle, with some genotypes indicating increased RUE, WUE and leaf longevity. The origin of the variance for RUE was not clear, although further research did highlight the potential factors that may have contributed to these findings, namely green area longevity, intrinsic photosynthetic capability of the leaf and enhanced WUE; all of which contributed to a proficiency to produce biological yields that were comparable to those of MBW. Furthermore, leaf level IRGA also highlighted variation for the mechanism whereby species maintained ITE, which could be harnessed to produce novel wheat varieties with adaptations to specific environmental conditions. Close collaboration of molecular physiology and cell biology would also be an advantage with the potential to use genetic microsatellites with a range of metabolic and structural capabilities that may be altered through gene expression. The array of genomic data available, and the availability of genetic markers, could allow genetic trait mapping, which further increases the feasibility of marker assisted breeding (Lawson *et al*., 2012). This collection of traits, therefore, does highlight potential successful adaptations conferring resource use efficiency, which could be highly valuable to supplement the allelic variance within the MBW gene pool for increased yield stability and productivity in increasingly limited environments and has widely associated with increased crop productivity.

8. References

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