The Physiological Processes Determining Grain Yield Potential in Winter Wheat

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Abstract

Winter wheat (*Triticum aestivum* L.) is the most important crop grown extensively in the UK. There is a gap between yield production and world demand for wheat. So, there is a need to fill this gap. The overall objective of the present study is to investigate the physiological determinants of grain yield potential in winter wheat grown in UK conditions using doubled-haploid lines derived from a cross between winter wheat varieties Rialto and Spark. These varieties are known from previous work to contrast for source and sink type traits and for the presence/absence of the 1BL/1RS translocation and the *Rht-D1b* semi-dwarf allele.

Three field experiments (at Sutton Bonington, University of Nottingham 2003/4, 2004/5 and 2005/6) were conducted examining 25 DH lines of the Rialto x Spark population and the two parents. Two post-anthesis shading treatments (with and without shading) were applied to six genotypes (four DH lines and two parents) in 2003/4 and 2004/5. Two post-anthesis de-graining treatments (with and without de-graining) were applied to twelve genotypes (ten DH lines and two parents) in 2003/4 and 2004/5. Detailed measurements of grain growth were carried out on the two parents for five pre-determined grain positions within the ear. A range of physiological traits were measured, including developmental stages, light extinction coefficient, radiation interception, radiation-use efficiency (RUE), green area and biomass, stem water-soluble carbohydrate reserves, floret fertility, potential grain weight, grain filling rate and duration, final grain weight and combine grain yield.

A source-sink balance model which quantified the source: sink balance during grain growth indicated that the four DH lines and the two parents were sink-limited during grain growth. The sink manipulation treatment (de-graining) generally confirmed the sink limitation in this population although small positive responses of grain growth to de-graining for a few lines indicated they may have been close to source limitation. Radiation-use efficiency measured as the regression slope of dry matter

on accumulated PAR intercepted between onset of stem elongation and anthesis differed amongst genotypes in 2004, 2005 and 2006. Pre-anthesis RUE was positively correlated with each of grains m^{-2} and specific leaf weight (SLW). The 1BL/1RS translocation increased RUE significantly. The characterisation of the 25 DH lines in this study showed that the differences in grain yield were positively correlated with grains m^{-2} but not individual grain weight. There was positive correlation between stem WSC reserves and yield in one of the experimental seasons, 2006. The ten DH lines and the two parents differed in the length of the period between GS31 and GS61 by nine days. There was a positive correlation between the duration from GS31 to GS61 and radiation interception during this period which positively affecting grains m^{-2}

Rate and duration of grain growth and final grain weight were assessed for five grain positions (G1 to G5) for Rialto and Spark under 50% shading and a control treatment. Rialto had heavier grains associated with a longer grain filling duration than Spark. The five grain positions had similar durations of grain filling but differed in final grain weight and rate of grain growth. Grains in the central spikelet (G1, G2 and G3) were sink-limited as they did not respond to de-graining. However, grains in the basal (G4) and apical (G5) spikelets were marginally source-limited since their final weight was increased by de-graining. These results suggest that breeders should consider selecting for extra grains in proximal grain positions in basal spikelets (e.g. G4) rather than in distal grain positions in central spikelets, because these grains in this position were heavier, had faster filling rate and had the ability to respond to extra assimilates later in the season.

Harvest biomass was positively correlated with grain yield amongst the DH lines. So traits to improve biomass whilst maintaining harvest index may be important for future breeding progress. It is suggested that breeders might select for an extended duration between GS31 and GS61 and higher RUE (via high SLW) to improve grains m^{-2} and yield potential in future years. They also should select for higher stem carbohydrate reserves to increase source size alongside grain sink size.

GENERAL ABBREVIATIONS AND ACRONYMS

% = percentage

µg = microgram

1BL/1BR = chromosome translocationof the short-arm of the rye-chromosome 1R into wheat chromosome 1B

ABA = Abscisic acid

ADAS = Agricultural Development and Advisory Service

AFLP = Amplified fragment length polymorphism

AGDM = above-ground dry matter

ANOVA = analysis of variance

APSIM = Agricultural production systems sIMulator

C3 = carbon-3

C4 = carbon-4

CIMMYT = Centro International de Mejoramiento de Maiz y Trigo

cm = centimetre

Co_2 = carbon dioxide

D = days

Df = degrees of freedom

DH = doubled-haploid

g = gram

GAD = green area duration

GAI = green area index

GS = plant growth stage (Zadok's)

ha = hectare

HI = harvest index

k = extinction coefficient

Kg= kilogram

LTM = long term mean

m = metre

mg = milligram

MGW = mean grain weight

mm = millimetre

N = nitrogen

°C = degree Celsius

PAR = photosynthetically active radiation

PGW = potential grain weight

QTL = quantitative trait loci

Rht-D1b= semi-dwarfing allele

Rubisco = ribulose-1, 5-bisphosphate carboxylase-oxygenase

RUE = radiation-use efficiency

SLW = specific leaf weight

t = tonne

UK = United Kingdom

WSC = water soluble carbohydrate

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1 Introduction

Yield potential is the yield of a cultivar grown in an environment to which it is adapted, when nutrients and water are non-limiting, and when pests, diseases, weeds, lodging and other stresses are effectively controlled (Evans and Fischer, 1999). Genetic yield potential of wheat (*Triticum aestivum* L.) has been increasing at an annual rate of approximately 0.9% in high input regions like the UK over the last 30 years (Calderini, Reynolds and Slafer, 1999a). However, global demand is predicted to increase by 1.6% per year over the next 20 years; from the current production of 617 million tonnes (F.A.O, 2006). So there is a need to increase the rate of relative genetic gain in wheat yields to keep up with world demand for food. Genetic yield potential can be increased by direct selection of high-yielding lines. But the low heritability of grain yield may result in a slow response to selection (i.e. result in slow progress), as yield is also influenced by the environment. Alternatively, indirect selection by targeting physiological traits may be more effective than direct selection for grain yield itself (Reynolds *et al.*, 2000).

1.1 The physiological basis of genetic gains in yield potential in UK wheat

In the United Kingdom, yields of winter wheat have increased progressively during the period from 1950, mainly through increases in grains m^{-2} and harvest index. The progress has been found to be coupled with the introduction of the semi-dwarfing gene (*Rht-D1b*) in the late 1970s that allows greater partitioning to the ear hence greater grain number, and use of greater amounts of N fertilizer, associated with shorter more lodging-resistant cultivars. The reduced-height allele effect and other physiological changes have increased yield potential by 110 kg ha^{-1} per year, but yield potential of modern cultivars is still considered to be limited by grain sink strength (Austin *et al.*, 1980a; Austin, 1999). Shearman *et al.* (2005) found that in cultivars released in the UK between 1972-1995 yield increased due to continued increases in grains m^{-2} and harvest index. Additionally they found an increase in harvest biomass production in cultivars introduced since about the mid 1980s.

Wheat yield is determined by the number of grains per unit area (grain sink size) or the photosynthetic capacity of the crop to fill the grains (grain source). Yield is

ultimately limited by either the source or the sink, which ever is the lower. So increasing yields in future years will depend on: (i) identifying new genetic resources with enhanced grain number, and (ii) increasing photosynthetic source in tandem to ensure that the extra grain sites are being fully exploited.

Examining the physiological basis of sink limitation in UK germplasm depends on the development of the appropriate crosses between genotypes contrasting for ear fertility traits influencing grains m^{-2} (sink strength). One such cross is the doubled-haploid mapping population derived from a Rialto x Spark cross utilized in the present study.

1.2 Major genetic introductions in UK wheat

Some of the major steps in enhancing yield potential of wheat in the last decades have been achieved by introduction of new genes.

1.2.1 *Rht-D1b* semi-dwarfing allele (formerly *Rht2*)

The semi-dwarfing allele *Rht-D1b* was introduced in to UK cultivars in mid 1970s (Gale and Youseffian, 1985). Semi-dwarf cultivars generally have superior grain yield potential and enhanced lodging resistance (Hedden, 2003). This progress has facilitated the greater use of N fertilizer associated with the development of shorter and lodging-resistant cultivars. The *Rht-D1b* allele reduces sensitivity to gibberellic acid (GA), which is essential for stem elongation (Flintham *et al.*, 1997). In favourable environments, the reduced demand for assimilates by a shorter stem and stiffer straw results in improved assimilate partitioning to the developing ears, leading to higher spikelet fertility and more grains per ear.

Semi-dwarf wheats have smaller cell dimensions (Butler *et al.*, 2005), smaller leaves, but compensate with increased photosynthetic rates resulting in a biomass similar to that of tall cultivars (Morgan, Lecain and Wells, 1990; Flintham *et al.*, 1997). The introduction of the *Rht-D1b* allele has therefore potentially caused a modification in the source-sink balance, as the sink size has been increased (through increased floret survival), with no apparent increases in the source size.

1.2.2 1BL/1RS chromosome translocation

The 1BL/1RS wheat– rye (*Secale cereale*) translocation, involving the short arm of rye chromosome 1R, has been extensively used in wheat breeding programmes across the world in recent decades (Rajaram, Villareal and Mujeeb-Kazi, 1990). Genes located on the fragment of rye chromosome have been reported to determine wide adaptation , high yield potential and race-specific disease resistances to leaf rust (*Puccinia recondita*), stem rust (*Puccinia graminis*), yellow rust (*Puccinia striformis*) and powdery mildew (*Blumeria graminis*) (McIntosh, 1983; Heun and Fishbeck, 1987; Rajaram *et al.*, 1990; Villareal *et al.*, 1998). Winter wheat cultivars incorporating 1BL/1RS were first released in the UK in the late 1980s, e.g. Hornet and Haven (introduced 1987). Most of the race-specific disease resistances associated with 1BL/1RS in UK cultivars have been overcome in recent years. It is possible, however, that there is a residual benefit of the translocation for grain yield productivity, but the extent of this has yet to be quantified in the UK's temperate environment.

Rajaram *et al.* (1983), examining a range of CIMMYT winter wheat cultivars worldwide, found 1BL/1RS to confer greater grain yield in hard red winter wheat. Carver and Rayburn (1994) showed 1BL/1RS to increase grain yield (9-10%) associated with greater harvest AGDM. On the other hand, there was a neutral effect of 1BL/1RS on both grain yield and AGDM comparing F_9-derived sister lines in two genetic backgrounds of soft red winter wheat in Montana (McKendry, Tegue and Miskin, 1996) and no consistent differences were reported between 1BL/1RS and 1B lines by Moreno-Sevilla *et al.* (1995).

In the UK, there have been few previous reports of effects of 1BL/1RS on grain yield productivity and associated physiological traits. A field study by Foulkes *et al.* (1998) examining 17 UK-grown winter wheat cultivars suggested 1BL.1RS to be associated with greater grain yield and AGDM, however this investigation did not utilize precise genetic stocks.

2 Literature Review

This literature review is organized into three main parts. The first part deals with resource capture and its effect on yield-determining plant processes. The aim here is to describe the effects of solar-driven growth processes, in particular radiation capture and radiation-use efficiency and the associated variables. The second part deals with the physiological aspects of yield formation by relating various plant and canopy processes to the determination of the numerical components of grain yield. The last part deals with the evidence for sink limitation in wheat with regard to source-sink manipulation experiments. The main aim in the literature review is to assess the evidence from previous studies for traits associated with components of grain sink size that can be used as selection criteria in breeding programmes.

2.1 Source and sink limitation to yield potential

The physiology of crop development is a major domain of any crop research, as most effects of certain environmental factors on crop growth and yield differ depending upon the developmental stages (Thorne and Wood, 1987; Savin and Slafer, 1991; Slafer, Satorre and Andrade, 1994; Slafer and Satorre, 1999). Many researchers have shown that grain yield is more sensitive to environmental factors during some developmental phases than others (Landes and Porter, 1989; Slafer, Calderini and Miralles, 1996). In wheat, development is a continuity of three major phases: the vegetative phase, when the leaves are initiated; the reproductive phase, when spikelets are initiated and floret development occurs until the number of fertile florets (i.e. the number of grains) is determined; and the grain-filling phase, when the grain first develops the endosperm cells and then grows to determine the final grain weight (Miralles and Slafer, 1999). Before anthesis, the number of grains is determined and after anthesis the grains are actually filled and the individual grain weight is achieved. Thus, the number of 'ears per unit area' and grains per ear' (or grains per unit land area) and the mean 'individual grain weight' are the major yield components (Miralles and Slafer, 1999; Peltonen-Sainio *et al.*, 2006). In general, the period between terminal spikelet initiation and anthesis is of greatest importance

(Siddique *et al.*, 1989b; Slafer, Andrade, and Satorre, 1990; Savin and Slafer, 1991; Reynolds *et al.*, 2000; Richards, 2000, Reynolds, Pellegrineschi and Skovmand, 2005). This is because the genetic progress in grain yield has been shown to be better related to the number of grains per unit land area than to individual grain weight (Thorne and Wood, 1987; Slafer and Andrade, 1989; Slafer and Savin, 1994; Reynolds *et al.*, 2005; Shearman *et al.*, 2005). The duration of these phenol-phases is controlled by both vernalization and photoperiod genes (Vrn and Ppd, respectively). The UK winter wheat has a vernalization requirement and is photoperiod sensitive.

2.2 Yield component model

The fraction of above-ground dry matter produced by a crop, which is harvested as yield, is often referred to as the harvest index (HI) (Donald, 1968). Grain yield is the product of ear number per unit ground area (N_e), grain number (N_g) and mean grain weight $(\hat{W}_g)$ at harvest. This is expressed in the following equation as:

$$Y = N_e \cdot N_g \cdot \hat{W}_g \quad \text{Equation (2.1)}$$

For analytical purposes it is convenient to consider yield as the product of the number of grains per unit area and the mean grain weight at harvest. The former is normally determined by the time of anthesis, whereas the latter is determined after anthesis. Thus the most important yield component determined by post-anthesis assimilation is grain size (Calderini *et al.*, 1999a; Donmez *et al.*, 2001; Acreche and Slafer, 2006; Bingham *et al.*, 2006a). So, yield is potentially limited by both the sink (the number of grains m^{-2}) and the source, depending on the season and time of day (Evans and Wardlaw, 1996). If assimilate supply is insufficient to meet sink demand, potential sink size cannot be fully expressed. When maximum expression of potential sink size is achieved, no further increases in assimilate supply can affect grain growth. In this way, either source or sink may limit grain growth. Thus, the grain yield of wheat depends on the formation, translocation, partitioning and accumulation of photosynthates during grain-filling. So, photosynthetic activity of leaves (source), and storage ability of grains after anthesis (sink) are the potential

limiting factors for the grain yield of wheat (Evans, Wardlaw, and Fischer, 1975, Ma, MacKown, and Van Sanford, 1990; Wang *et al.*, 1994).

In general, a convenient way to analyze a trait is to divide it into simpler components. Grain yield can be divided into either physiological (Biomass x HI) or numerical (Ears m^{-2} x grains ear^{-1} x grain weight) (yield) components models. The physiological model gives a wider and comprehensive description to the factors underpinning yield formation and the interrelationship between these factors than the numerical model. This means that the physiological model may be more useful in identifying the traits and processes limiting yield potential than the yield components model.

2.3 Resource capture and the crop growth processes

The physiological principles governing the response of crops to environmental and weather factors requires a critical consideration as they have direct effects on dry matter production and yield in three main ways as detailed by Gallagher and Biscoe (1978) and Black and Ong (2002). These are: (a) the interception of radiation by the green canopy, (b) the efficiency with which intercepted radiation is converted to dry matter, and (c) the partitioning of dry matter between economic yield and the rest of the crop. Similarly, Miralles and Slafer (1999) and Reynolds *et al.* (2005) showed the yield potential (YP) as a function of the above mentioned traits and expressed it as

$$YP = LI \times RUE \times HI \quad \text{.....Equation 2.2}$$

Where LI is the light interception; RUE is the radiation-use efficiency and HI is the harvest index.

This model will used in this section as a framework for describing the physiological processes underpinning genetic progress in yield potential.

2.3.1 Radiation interception

Most of the previous studies have shown that the above-ground biomass depends on the total amount of solar radiation intercepted during the growing season or that crop yield is strongly related to cumulative radiation interception (e.g. Gallagher and Biscoe, 1978; Black and Ong, 2002; Muurinen and Peltonen-Sainio, 2006). The fraction of the radiation intercepted by a crop canopy depends mainly on its green area index (GAI). For most cereal crops, when GAI reaches 4-5, more than 95% of the incident, photosynthetically-active radiation (PAR; 400-700 nm) will be intercepted by the green canopy (Sylvester-Bradley *et al.*, 1997; Reynolds *et al.*, 2005). So any factor that restricts the rate of expansion of leaf surface will directly limit the dry matter production of the crop until a GAI of 4-5 is achieved. Thus, the fraction of radiation intercepted by the crop is a function of the GAI, and the efficiency with which the green area intercepts solar radiation, described by the light extinction coefficient (K). The extinction coefficient, itself, is calculated from measurements of GAI and the fractional radiation interception. Saeki (1960) cited in Yunusa *et al.* (1993) reported that differences in K were related to variation in the distribution and orientation of leaves. These relationships were quantitatively expressed in a form of Beer's law by Monsi and Saeki (1953) and described by Monteith (1965):

$I = I_o e^{-kl}$ .....Equation 2.3

Where I_o and I are the incident solar radiation and the radiation intercepted by the canopy, respectively. L and K are GAI and extinction coefficient, respectively.

Light is extinguished in a homogenous medium according to Beer's law, so the Monsi-Saeki equation assumes a random leaf distribution. There is a diminishing increase in fractional interception (F) as the size of the canopy increases that is described by the negative exponential relationship (Hipps, Asrar, and Kanemasu, 1983). The fraction of radiation intercepted (F) can be calculated from L, if K is known:

$F = 1\text{-}e^{-kl}$.....Equation 2.4

In wheat, K_{PAR} values amongst genotypes have been reported to be in the range 0.37 to 0.82 (Younusa *et al.*, 1993; Calderini, Dreccer and Slafer, 1997; and O'Connell *et al.*, 2004). Moreover, Muurinen and Peltonen-Sainio (2006) reported genotypic differences in K among cereal species with 0.70 as an average value of K for wheat cultivars in Finland.

Leaf angle affects K. Most modern wheat cultivars have erect leaves. Leaf area production and leaf area persistence are the important components for effective capture of solar radiation and further conversion into crop dry matter. Thus photosynthesis depends on the persistence of existing green leaf area and all assimilate that is produced is being used to fill grains. So persistence of green leaf area has a direct implication for the economic yield of wheat. Similarly associated effects of leaf senescence at the end of maturity have a direct bearing on effective radiation capture (Gallagher and Biscoe, 1978; Sinclair and Muchow, 1999; Reynolds *et al.*, 2005). Under optimal conditions more than 90% of the light is generally intercepted by wheat from canopy closure until the onset of senescence. This means that the stay-green trait can result in increasing the amount of radiation interception (Jenner and Rathjen, 1975; Reynolds, Sayre and Rajaram, 1999; Reynolds, Pellegrineschi, and Skovmand, 2005). The stay-green trait is reported to have genetic variation under post-anthesis drought in wheat (Verma *et al.*, 2004; Reynolds *et al.*, 2005) and it is easy to select either visually or by using spectral reflectance techniques. However, there are no reports regarding the association between the stay-green trait and genetic improvement in yield under optimal conditions (Reynolds *et al.*, 2005).

2.3.2 Radiation-use efficiency

There have been two main approaches to predict crop growth and productivity which are either based on 'solar-driven' approach or on the 'water-driven' approach. The solar-driven approach uses solar radiation as the primary driving force for the photosynthetic process and thus predicting biomass, whereas, the 'water-driven'

growth engines uses the correlation between water evapo-transpired and the photosynthetic process to predict biomass.

In this thesis, the solar-driven approach has been used to quantify the basis of biomass production and its influence on grain sink size. The simple way to express the commercial yield of a crop (YP), from emergence to harvest is:

$$YP=HI \cdot RUE \cdot F \int I_o \, dt \quad \text{.....Equation 2.5}$$

Where, HI is the harvest index; RUE is the radiation-use efficiency; F is the fraction of I_o intercepted by the canopy; I_o is the incident solar radiation.

This modelling approach assumes that there is an approximately linear relationship between the total accumulated dry matter and the amount of intercepted solar radiation, over the same measurement time. 'RUE' represents the slope of the linear relationship and has been shown to be substantially constant during the growing season for given environments and cultivars (Monteith, 1977, Gosse *et al.*, 1986; Sinclair and Muchow, 1999; Araus *et al.*, 2002).

Almost all plant life on Earth can be divided into two categories based on the way they assimilate carbon dioxide into their systems.C3 plants include more than 95% of the plant species on earth including wheat. C4 plants include such crop plants as sugar cane and maize. They are the second most prevalent photosynthetic type. During the first steps in CO_2 assimilation, C3 plants form a pair of three carbon-atom molecules. C4 plants, on the other hand, initially form four carbon-atom molecules. In the absence of stress, RUE is often conservative, typically ranging between 1.0 and 1.5 g MJ^{-1} for C_3 species in temperate environments (Monteith and Elston, 1983), 1.5–1.7 g MJ^{-1} for tropical C_3 species (Kiniry *et al.*, 1989; Monteith, 1990) and up to 2.5 $gMJ^{-1}$for tropical C_4 cereals under favourable conditions (Squire, 1990).

Radiation-use efficiency can be calculated from the differences in biomass between two consecutive harvests divided by the corresponding amount of radiation intercepted. Since this method suffers from large errors associated with calculated differences, fitting a linear relationship between biomass and intercepted radiation has been widely used to find RUE as the slope of the fitted curve (Sinclair and Muchow, 1999).

Although RUE is evidenced to be constant during the growing season (Gosse *et al.*, 1986), decreases in RUE late in post-anthesis period have been observed in many studies (e.g. Green, 1987; Garcia *et al.*, 1988; Fischer, 1993). The possible reasons attributed to this are: decline in the canopy photosynthetic efficiency due to the end of leaf production and reduction in photosynthetic capacity of existing leaves as a consequence of progressive senescence (Gallagher and Biscoe, 1978) associated with losses in leaf nitrogen content (Sinclair and Horie, 1989), changes in temperature (Garcia *et al.*, 1988), changes in respiratory load during the grain filling stage (Gallagher and Biscoe, 1978; Gimenez, Connor, and Rueda, 1994) and variation in biomass partitioning between roots and shoots (Kiniry *et al.*, 1989). But such declines in RUE later in the post-anthesis period may have a small effect because senescence occurs from the bottom leaf layers canopy upward. Additionally, Calderini *et al.* (1997) reported that RUE was unchanged or faced a small reduction during the later stages of grain filling in the modern Argentinean wheat compared to their predecessors.

There are many experimental investigations into genetic and environmental variation in RUE of wheat. The range of locations and experimental conditions offer greatest genetic variation in growth conditions. Studies in Australia (Gregory, Tennant, and Belford, 1992; Gregory and Eastham, 1996) indicated that the range of RUE values may not reflect the potential RUE for wheat. Several studies have reported on the genetic variation in RUE in wheat. Comparing three spring wheat cultivars introduced successively with breeding Yunusa *et al.* (1993) indicated that the most recently released cultivar had the greatest RUE. On the other hand, Calderini *et al.* (1997) comparing seven spring wheat cultivars released over the period from 1920

to 1990 in Argentina found no change in RUE with year of release. In the UK, Shearman *et al.* (2005) reporting on eight representative winter wheat cultivars introduced from 1972 to 1995 found that significant genetic changes with grain yield were associated with changes in pre-anthesis RUE of about 0.012 g MJ^{-1} yr^{-1}. This was shown to be consistent with a tendency for an increase in AGDM at GS61 with breeding, which in turn appeared to be associated with greater specific leaf weight (SLW), implying that modern UK wheat cultivars may have a more photosynthetic apparatus per unit leaf area. In general, breeding had small effect on RUE (Siddique *et al.*, 1989a; Slafer *et al.*, 1990; Calderini *et al.*, 1997). Because HI approaching its theoretical upper limit of *ca.* 0.62 (Austin, 1980), breeders should try to focus on the constraints RUE as an avenue for improving yield, knowing that RUE theoretically can be enhanced at canopy, leaf and biochemical levels (Reynolds *et al.*, 2000; 2005)

2.3.2.1 Physiological avenues for potential increases in RUE

Leaf photosynthesis:

Several researchers have proposed that increases in leaf photosynthetic rate during grain filling should result in higher wheat grain growth rates (Simmons, Crookston and Kurle, 1982; Frederik and Camberato, 1994). However, selection for higher rate of leaf photosynthesis during grain filling has generally been found to result in little increase in grain yield (Austin, 1989). Within a genotype, the lack of increase in grain growth rate when leaf photosynthesis is increased indicates photosynthate supply may not be limiting grain growth rate, at least during the early stages of grain filling before rapid leaf senescence and loss of photosynthetic activity occur. Most studies conducted to understand the relationship between yield improvement and light saturated net carbon exchange rate of flag leaves. But there were factors that reduce the amount of net carbon fixed which is available for growth such as respiration of assimilates during the dark period (Amthor, 1989), loss of carbon from root exudates and tissue necrosis. Also most of the leaves in canopy are shaded.

Leaf angle:

In theory, the RUE and hence, yield potential might be improved especially in high radiation environments if the canopy has an erect leaf angle (Duncan, 1971; Reynolds *et al.*, 1999). This hypothesis was supported by a 4% increase in yield of wheat isolines carrying this trait in the UK (Innes and Blackwell, 1983).

Reducing photorespiration

Theoretically, RUE can be increased by reducing photorespiration through increasing the affinity of Ribulsoe-1,5-bisphosphate carboxylase/ oxygenase (Rubisco) for CO_2 and hence decreasing its oxygenase activity (Reynolds *et al.*, 2000; Araus *et al.*, 2002). Modest variation for CO_2 specificity has been found in land plants (Parry, Keys, and Gutteridge, 1989; Delgado *et al.*, 1995), with wheat having among the highest values for crop species. However, marine algae were reported to have much higher values (Read and Tabita, 1994; Uemura *et al.*, 1997). Transforming wheat Rubisco genetically via molecular techniques from its current specificity of 95 to potential values of 195, corresponding to thermophilic alga *Galderia partita* may be possible. However, Rubisco has a protective role in dissipating excess energy, with O_2 uptake in the light playing a significant role in preventing chronic photoinhibition under field conditions (Osmond and Grace, 1995). In such cases, it might be of counter-productive to alter Rubisco's oxygenase specificity, especially in crops subjected to abiotic (e.g. drought or temperature) stresses (Araus *et al.*, 2002), unless other means of using excess reducing power, e.g. through the Mehler-ascorbate peroxidase reaction (Osmond and Grace, 1995) and by cycling carotenoid pigments (Gilmore, 1997), can also be genetically modified to compensate.

Leaf N content and specific leaf weight:

Leaf N concentration may affect the photosynthetic capacity of the leaves (Dreccer *et al.*, 2000) and thus RUE (Sinclair and Muchow, 1999). In theory, photosynthetic rate could be enhanced in more illuminated upper leaves if there was an altered

distribution of the N within the canopy, with more N being partitioned to the upper leaf layers and less N remaining in the lower leaves (Dreccer *et al.*, 2000).

Specific leaf weight (SLW; g leaf DM m^{-2} leaf area) is a measure of leaf thickness. Leaves of high SLW usually have high chlorophyll content and high content of photosynthetic enzymes per unit leaf area (Morgan *et al.*, 1990; Miralles and Slafer, 1997). Shearman *et al.* (2005) showed a positive association between leaf thickness and yield potential in winter wheat. Leaf thickness is positively correlated with leaf photosynthetic rate (Gifford and Evans, 1981). SLW was reported to increase in response to the *Rht-D1b* allele (Miralles and Slafer, 1997). A leaf of high SLW does not tend to expand horizontally and therefore tends to be narrow and erect. Leaf size was reported to decrease as a direct effect of the semi-dwarfing genes (Calderini, Miralles and Sadras, 1996). However, this small reduction in leaf area is not considered a disadvantage due to a negative relationship between leaf area and photosynthetic rate (Austin, 1982). Pyke and Leech (1985) similarly reported reduction in leaf area by dwarfing genes did not reduce photosynthesis because the semi-dwarf allele increased the concentration of Rubisco in the leaves.

Methodologies for screening RUE:

One of the most important features for a selected trait is the ability to carry out precise, high-throughput measurements. Stomatal conductance (gs) and canopy temperature depression (CTD; canopy minus air temperature) are examples of high-throughput, remote-sensing traits (Amani, Fischer, and Reynolds, 1996; Ayeneh *et al.*, 2002) which are mechanistically linked to photosynthesis. CTD is measured within few seconds with an infrared thermometer and gs can be measured with either a viscous-flow porometer or an infra-red gas exchange analyzer (CIRAS) (Richards, Condon, and Rebetzke, 2001). Leaf cooling is the main function of transpiration. If photosynthesis is reduced, the stomata will be more closed and the leaf temperature will rise due to the lack of transpirational cooling. This means that canopy temperature is acting as a sensor of plant transpiration and photosynthesis (Araus *et al.*, 2002). This sensor has been widely used in agronomy and breeding with the development of the infrared thermometer which enables the breeder or researcher to

assess temperature remotely and quickly (Araus *et al.*, 2002; Ayeneh *et al.*, 2002). CTD is used as a tool for selection for yield potential. Under irrigated conditions, stomatal conductance was positively correlated with grain yield in wheat (Reynolds *et al.*, 1994; Amani *et al.*, 1996; Fischer *et al.*, 1998). Such correlations were strongest when CTD was measured in the middle of the day (between 12 and 4 pm) and were not affected by the stage of development (Amani *et al.*, 1996). Therefore, CTD can be used to select for high yield potential in wheat (Reynolds *et al.*, 1994, 1998; Amani *et al.*, 1996)

2.3.3 Partitioning of dry matter

The main cause for genetic gain in yield since the 1960s has been a consistent increase in the partitioning of biomass towards reproductive organs, without major changes in biomass production (e.g., Austin *et al.*, 1980a; Deckerd *et al.*, 1985; Cox *et al.*, 1988; Slafer and Andrade, 1989; Slafer *et al.*, 1994; Calderini *et al.*, 1997). Thus, most researchers attributed higher yields to enhanced harvest index (HI) from more favourable partitioning of assimilates from the vegetative stem tissues to the grain (Austin *et al.*, 1980a; Sayre *et al.*, 1997). Because of this increase in partitioning, modern cultivars in some countries have already reached harvest indices that are close to a theoretical upper limit of about 62% as estimated by Austin, (1980). It is clear, therefore, that one of the future aims of genetic improvement is to increase the biomass production while maintaining the present values of grain partitioning (Austin *et al.*, 1980a; Slafer and Andrade, 1991; Reynolds *et al.*, 2000). High HI was associated with more grains per spikelet and ear number per unit area (Slafer and Andrade, 1991; Sayre *et al.*, 1997; Brancourt-Hulmel *et al.*, 2003; Shearman *et al.*, 2005). Grain number is established at around the time of anthesis. It is suggested that the strong competition between the growth of the ear and vegetative sinks during 20–30 days before anthesis could affect floret survival and hence grain number in wheat (Miralles *et al.*, 1998a). Ear dry matter at anthesis reflects the quantity of assimilates invested in reproductive growth and several authors have found a linear relationship amongst genotypes between ear

biomass at anthesis and number of grains in wheat crops when water and nutrients were not limiting growth (e.g. Fischer, 1985; Savin and Slafer, 1991; Shearman *et al.*, 2005).

The increased number of grains per ear and, to a lesser extent, increased number of tillers, resulted in a close relationship between grain numbers per unit area and grain yield (Austin, Ford, and Morgan, 1989; Sayre *et al.*, 1997; Shearman *et al.*, 2005). Although, biomass has not genetically increased with yield potential in the last 15 years there are some reports of genetic progress in yield linked to biomass. During the 1990s, there have been several reports of biomass increases attributed to introduction of the 1BL/1RS translocation which was introduced into UK-bred wheat cultivars in the late 1980s. There have been few studies on the effects of breeding on harvest AGDM since this time (Donmez *et al.*, 2001; Shearman *et al.*, 2005).

2.3.4 The contribution of stem WSC reserves to genetic progress in yield

Increases in leaf photosynthesis during the early portion of grain fill are associated with increases in accumulated stem WSC reserves (Kiniry, 1993; Frederik and Camberato, 1995). Non-structural carbohydrate concentrations also increase during this time (Kiniry, 1993) and probably account for most of the vegetative dry weight increases. These carbohydrate reserves are found primarily in the stem and are utilised for maintenance respiration and grain fill (Austin *et al.*, 1977; Foulkes, Scott, and Bradley, 2002). Stem reserves may serve to maintain a linear rate of grain growth when photosynthate production declines during the latter portion of grain fill (Simmons, 1987; Crus-Augado *et al.*, 2000). There has been an increase in amounts of stem WSC with year of cultivar release in the UK of about 0.50 t ha^{-1} per decade (Foulkes *et al.*, 2002; Shearman *et al.*, 2005). Evidence for significant deposition of stem WSC reserves in grains in the absence of post-anthesis stress in wheat was presented by Gebbing, Schnyder, and Kuhbauch, (1999) and Shearman *et al.* (2005). Under normal growing conditions, the lag period at the end of grain fill may be caused by the depletion of carbohydrate reserves. Analysis of source-sink interactions should consider the role of alternative sources in the plant (Schnyder, 1993). In wheat, special attention is given to the stems, since competition exists

between the growing upper internodes and reproductive organs in the weeks before anthesis (Cruz-Aguado *et al.*, 1999). The outcome of the competition depends on both genotype and environment. Recently, there was evidence that the trait is of high heritability (Ruuska *et al.*, 2006). So, it should be possible to breed for high stem reserves.

2.4 Effects of initiation and appearance of vegetative and reproductive organs

2.4.1 Leaf and spikelet primordia initiation

Miralles and Slafer (1999) reviewed the events that take place in the various developmental phases in wheat. The mature grain contains the embryo and enough stored reserves, in the endosperm, for germination. The shoot apex of the embryo has three or four leaf-primordia and a tiller bud protected by the coleoptile (Hay and Kirby, 1991).

In wheat, the leaf primordia are initiated at a single rate on a thermal time basis. The reciprocal of this rate, the thermal time between the initiations of two consecutive leaf primordia, is called the plastochron (Wilhelm and McMaster, 1995). Although there is genetic variation for plastochron (Evans and Blundell, 1994), a common value of about 50°C d, above a base temperature of 0°C, has been reported in most literature (Kirby *et al.*, 1987; Delecolle *et al.*, 1989). Some time later than floral initiation the first double ridge is initiated, when leaf and spikelet primordia appear as double ridges around the shoot apex. The lower ridge is the leaf primordium and the upper ridge is spikelet primordium (Gardner, Hess and Tryone, 1985). The double-ridge stage has been frequently used as an indicator of the end of the vegetative phase, but it is recognized that the first spikelet primordium may be initiated before the first double ridge appears (e.g. Delecolle *et al.*, 1989; Kirby, 1990). The phase of the spikelet initiation that commenced at floral inititation continues until the initiation of the terminal spikelet in the apical meristem, when the maximum number of spikelets is fixed (Miralles and Slafer, 1997). The length of the phyllochron (thermal time between the appearances of two successive leaves) and the period between flag leaf appearance and anthesis ensures a minimum duration

for reproductive development. The longer the period from seedling emergence to floral initiation, the higher the number of leaf primordia that have to appear after floral initiation (Hay and Kirby, 1991), and consequently the longer the reproductive phase from floral initiation to anthesis. Temperature has the most significant influence on rate of leaf appearance and hence thermal time is frequently used in most studies to analyze the dynamics of leaf appearance (Gallagher, 1979; Baker, Gallager and Monteith 1980; Baker *et al.*, 1986; Kirby *et al.*, 1989; Slafer *et al.*, 1994; Calderini *et al.*, 1996; Slafer and Rawson, 1997).

The reproductive stages of development are the period between initiation of floret development to anthesis. The timing of the floral initiation switch is under control of Ppd and Vrn genes. UK winter wheats are photoperiod sensitive. The rapid ear and stem growth phase (terminal spikelet stage to anthesis) is crucial in determining yield potential since floret abortion occur during the latter stages of this period and the presence of more resources would reduce floret abortion hence increase fertile floret number (number of grains per ear). The duration of the rapid ear and stem growth phase is approximately 20 d in irrigated spring wheat (Fischer, 1985). During this period final grain number is determined, not only determining the partitioning of photoassimilates to yield and increased the grain sink strength (Reynolds *et al.*, 2005), but also influencing photosynthetic assimilation rate during grain filling (Bingham, 1969; Reynolds *et al.*, 1999; 2000). The duration of this phase shows genetic variability (Slafer and Rawson, 1994). Because of this, this phase is of great importance in establishing the yield components and hence in determining yield (Kirby, 1988). In the previous phase (spikelet initiation phase), 8-10 floret primordia per spikelet were formed, also tiller number was determined. However in the rapid ear growth phase, only 2-5 floret primordia will survive to produce grains. Brooking and Kirby (1981) proposed that floret per spikelet death might be due to competition for limited resources, while others (e.g. Cotterell, Dale and Jeffcoat, 1981) suggest it is due to the effect of hormones. Numerous studies have shown that hormones may be implicated in determining grain set in wheat (Rawson and Evans, 1970; Evans, Bingham, and Roskams, 1972; Waters, Martin, and Lee, 1984; Zeng, Morgan, and

King, 1985; Wang *et al.*, 2001). Wang *et al.* (2001) studied the regulating effect of exogenous hormones on floret development and reported that Zeatin promoted floret development, significantly increased the number of fertile florets as well as grain set and increased the sugar concentrations in ears at anthesis. In contrast, indole- 3-acetic acid (IAA), giberellic acid (GA-3) and abscisic acid (ABA) inhibited floret development, with different patterns for each of the hormones. For example, IAA inhibited the development of the whole ear and all florets in the spikelets such that grain loss occurred in all positions in the spikelets. GA-3 increased the number of fertile florets per ear, but decreased grain set of the third floret in each spikelet. ABA inhibited floret development, and decreased the number of fertile florets and grain set. The inhibitory effect of ABA was mainly on the first and third florets in each spikelet. While Whingwiri and Stern (1982) proposed that there is a critical stage of development and that any florets initiated after this stage do not develop into grain. In the final stages of the rapid ear growth phase, the carpels are fertilized and grain growth occurs. The growth of wheat grain is classically sigmoidal in form: it starts with a short lag phase which is followed by a longer and rapid linear period of growth, at the end of which the rate of dry matter accumulation slows, until reaching physiological maturity, after which, no further additions are made to grain mass (Stone and Savin, 1999). Recently, the *Gn1a*, which is a gene for cytokinin oxidase/dehydrogenase (OsCKX2), an enzyme that degrades the phytohormone cytokinin was identified in rice (Ashikari *et al.*, 2005). It reduced the expression of *OsCKX2,* which causes cytokinin accumulation in inflorescence meristems and increases the number of grains and hence grain yield.

2.5 Effects of photoperiod on the relative duration of the stem elongation phase

Many studies in wheat and barley point to the potential advantages of increasing the proportion of thermal time to anthesis by alter the duration stem-elongation period to favour ear partitioning and increase ear biomass at anthesis, hence raise grain number per unit area (Kernich, Halloran, and Flood, 1996; Slafer and Rawson, 1996; Gonzalez, Slafer, and Miralles, 2003; Slafer *et al.*, 2005; Reynolds *et al.*, 2005).

Extending the stem-elongation period has similarly been proposed as a strategy to increase yield potential in winter wheat grown in the UK (Sylvester-Bradley *et al.*, 2005). Delaying flowering in the UK could increase grain losses associated with late harvesting, so advancing GS31 while maintaining GS61 is the preferred pattern of development. An extended stem-elongation period should simultaneously favour greater ear biomass and stem WSC at anthesis. Indeed Foulkes *et al.* (1998) studied a set of 30 modern UK winter wheat cultivars and reported a positive linear relationship between the duration of stem-elongation period and each of stem WSC accumulation and yield. In general, there are some crude indicators that extending the stem-elongation period may offer an avenue for simultaneously increasing post-anthesis source and sink size hence yield potential in the UK environment. Since there has been no systematic change in phenology with recent UK breeding (Shearman *et al.*, 2005), this pattern of development would offer a novel strategy for raising yield potential. The length of developmental phases depends on their sensitivity to photoperiod and vernalization and on their intrinsic earliness (basic length of a phase). The genes required to manipulate the stem-elongation period independently of the whole period to anthesis period have not yet been identified. However, there is some prospect for doing so, since recent field investigations have confirmed that the thermal duration of the stem-elongation period is sensitive to changes in photoperiod after the onset of stem elongation (Whitechurch and Slafer, 2001; 2002). Craufurd and Cartwright (1989) observed that the maximum number of florets initiated in wheat spikelets was not affected by photoperiod and reductions in day length retarded floret development and delayed the time to reach the stage of fertile floret formation. So delaying the period during which floret primordia are competing for assimilates with other organs would allow for a higher proportion of total floret primordia at flowering and thereby increase the number of grains per ear (Gonzalez *et al.*, 2003).

Wheat crops develop many more tillers than survive to produce fertile ears (Duggan, Richards and Tsuyuzaki, 2002). Under optimal conditions, more than 1000 tiller m^{-2} may be produced by wheat at the beginning of stem elongation but less than half of

these survive (Stapper and Fischer, 1990; Berry *et al.*, 2003). The introduction of the tiller inhibition gene (*tin* gene) into wheat is beneficial in Australia since it reduced the incidence of unproductive tillers (Richards, 1988; Motzo, Giunta and Deidda, 2004). Although these unproductive tillers can re-translocate some of their assimilates, there is a cost in terms of soil water used for transpiration, structural material and respiratory losses (Richards *et al.*, 2002). Bread wheat lines in which tillering were genetically reduced was selected for the first time in Australia by Atsmon and Jacobs (1977). Plants containing the *tin* gene usually show '*gigas*' features, as they have thick culms, large leaves and ears and many grains per ear. The tiller inhibition gene is located on chromosome 1AS, at 10±3 map units from the locus for hairy glume. However, in the UK where attainable yields are much higher than in Australia, the benefit of tiller economy would probably not compensate for the predictions in fertile-shoots m^{-2} with the *tin* gene.

2.6 Potential grain weight and avenues for genetic improvement

Genetic progress in yield potential is strongly associated with increases in grain number, while individual grain weight has, if anything, decreased (for example, Sayre *et al.*, 1997). An important question is whether grain weight potential can be increased independently of increases in grain number. Simplistically, it can be argued that this inverse relationship is a necessary trade-off when more grains are competing for limited assimilates. Slafer *et al.* (1996) argued that there is limited evidence that assimilate limitation during grain-filling causes reduced grain weight and that a co-limitation (i.e. source-limited during endosperm cell division and expansion, then sink-limited during linear grain filling phase) of final grain weight is determined by both source and sink. This may occur, for instance, when grain weight potential is determined at an earlier stage during grain filling resulting in different potential sizes at different ear positions. The conclusion is that one avenue for increasing yield potential may be to exploit genes that increase grain weight potential, especially at ear positions that currently have low grain size. Richards (1996) suggests that while smaller grain size may be a pleiotropic effect of the

dwarfing genes, final grain size may be increased genetically by increasing the number of endosperm cells.

2.6.1 Determinant processes associated with potential grain weight

The beginning of grain development is characterised by rapid division of endosperm cells resulting in a dramatic increase in cell number, but little growth of individual cells (Brocklehurst, 1977). Assimilate levels at this time affect the rate of cell division (Brocklehurst, 1977). The potential grain size can be restricted by the number of endosperm cells, as each has a limited capacity for starch storage. Larger grains are therefore formed under conditions where source is not limiting during this phase (Dunstone and Evans, 1974; Brocklehurst, 1977; Singh and Jenner, 1982; 1984; Cochrane and Duffus, 1983), although potential endosperm cell number is also genetically determined (Dunstone and Evans, 1974; Brocklehurst, Moss, and Williams, 1978). Endosperm cell number therefore represents the potential of the grain to accumulate starch, and hence the potential final grain size (Brocklehurst, 1977; Singh and Jenner, 1984). The regulation of endosperm cell number by assimilate supply allows the source and sink to be maintained in a balance, with the potential grain weight geared to the likely subsequent assimilate supply. Many reports suggest that the total number of endosperm cells per grain is typically in the region of 100,000 (Briarty and Hughes, 1979; Jenner, Ugalde and Aspinall, 1991).

Cell division ceases between 14-20 d post-anthesis (Jennings and Morton, 1963; Briarty and Hughes, 1979; Nicolas, Gleadow and Dalling, 1985), at which time potential grain size is largely already determined. The genetic variation in duration of cell division is partly due to different temperature responses. Grains reach their maximum volume much earlier than their maximum dry weight, because endosperm cells are first filled mainly with water to establish maximum volume, and then water is replaced by assimilates (Martinez-Carrasco and Thorne, 1979; Pepler, Gooding and Ellis, 2006). It is this rapid increase in cell water content which determines cell and hence grain volume. Maximum grain volume may be physically restricted by glumes (Millet, 1986). Therefore potential grain size is ultimately a result of interactions between endosperm cell number and floret cavity volume. Termination

of rapid net water deposition in the grain occurs at about 14 d post-anthesis (Schnyder and Baum, 1992). This roughly coincides with the termination of cell division. Schnyder and Baum (1992) and Borras, Slafer, and Otegui, (2004) demonstrated a good relationship between maximum grain water content and mature grain dry weight. In further experiments, it has been demonstrated that mature grain weight is related to the grain water content at 28 d post anthesis (Macbeth, 1996).

2.6.2 Mechanisms influencing genetic variation in potential grain weight

Grain yield in wheat has been commonly found to be associated with grains m^{-2} (Fischer 1985; Magrin *et al.* 1993; Reynolds *et al.*, 2005; Shearman *et al.*, 2005) and much research has been carried out to explain the causes of differences in grains m^{-2} in relation to both environmental conditions (Fischer 1985; Savin and Slafer, 1991) and genetic improvement (Loss *et al.* 1989; Slafer *et al.* 1994; Shearman *et al.*, 2005). Although less variable than grains m^{-2}, individual grain weight is also an important source of variation in grain yield. However, there is a rather poor understanding of the determinants of grain weight in wheat. In general crop growth rate normally decreases during the period of grain growth and rate of grain growth is dependent on the stored assimilates as crop dry matter, i.e. the assimilate stored in the stem which is later translocated to the grain. In most situations under optimum growth conditions mean grain weight is a relatively conservative characteristic for most cereals (Gallagher, Biscoe, and Scott, 1975; Bingham *et al.*, 2006b), and yield variation is mostly varied by grain number per unit ground area and there has been little changes in grain weight with breeding. Thus if the genetic variation in grain size (or grain weight) could be identified it would be a great value in breeding programmes. There has been little change in grain weight with breeding. Many authors reported genotypic differences in grain weight (e.g. Macbeth, 1996; Reynolds *et al.*, 2005; Shearman *et al.*, 2005). Most differences in grain weight must be related to those in grain weight potential (i.e. the intrinsic capacity of grains to accumulate dry matter as defined by Bremner and Rawson (1978)). Temperature during grain-filling has been identified as a major environmental factor affecting grain weight and its physiological determinants (Sofield *et al.*, 1977). This effect has

also been found when relatively short periods of high temperature occur during grain-filling in wheat (Stone and Nicolas 1995) and barley (Savin and Nicolas 1996). These studies, based in controlled-environments, have clearly demonstrated that high temperatures during the grain-filling period reduce grain weight due to a reduction in the grain-filling duration (measured in calendar time), and this effect may not be reversed by increasing the availability of assimilates per grain (Slafer and Miralles 1992). The importance of the period immediately before anthesis for the determination of potential grain weight has been largely disregarded (Calderini *et al.*, 1999b). However, it is during this period that the structures of the ovary (grain pericarp after fertilization) are formed, and the importance of the pericarp tissues on early grain development was suggested many years ago (Rijven and Banbury 1960). The effect of environmental conditions immediately before anthesis on potential grain weight was investigated in wheat (Calderini *et al.*, 1999b). A linear and curvilinear relationship between potential grain weight and the carpel weight at anthesis was reported in barley (Scott *et al.* 1983) and wheat (Calderini *et al.*, 1999b), respectively. Caldereini *et al.* (1999b) suggested an existence of a carpel weight threshold of *c.* 1 mg at anthesis for achieving maximum grain weight and hypothesized that cultivars with grains of relatively low potential weight may be less stable in final grain weight than those with genetically heavy grains.

2.6.3 Grain position as a determinant of grain weight within the ear

Differences in grain size due to grain position within the spikelet are apparent shortly after anthesis, and are in ascending order of morphogenesis (Bremner and Rawson, 1978). However, by maturity, the differences change, so that the grain weight increases from the basal floret to the second floret, and thereafter decreases to the apical floret. Bremner and Rawson (1978) concluded that the basal grain of a spikelet has priority for the assimilate supply, but a limited growth potential.

The lower average grain weight frequently observed in modern cultivars (Slafer *et al.*, 1994; Calderini and Ortiz-Monasterio, 2003) could be an indirect effect of their improved grain set in the most distal positions within the ear. Miralles and Slafer

(1995b) reported that there are a higher proportion of grains from distal positions within the spikelets which in turn are of smaller weight potential and thereby reduce the average grain weight of semi-dwarf compared to the tall isogenic wheat lines. The weight of these distal grains was clearly less than that of the grains from florets more proximal to the rachis, and that difference was not reduced at all by doubling the source-sink ratio (Miralles and Slafer, 1995b). Calderini and Ortiz-Monasterio (2003) observed a difference in grain weight and nutrient concentration between grain positions within the ear that could lead to important implications for future wheat breeding strategies. For example, if plant breeders continue to strive for increased wheat yields by selecting for grain set in distal positions of the ear, the inherently smaller grain weight potential at these distal positions will likely limit advances in grain yield. Moreover, the added grains will have progressively lower mineral concentrations compared with proximal grains. Recently, wheat breeders at CIMMYT have developed synthetic wheats characterized by heavier grains than conventional hexaploid cultivars (Reynolds *et al.*, 1999; 2000). Synthetic wheats are a hybrid of tetraploid (*Triticum durum*) and diploid (*Triticum tauschii*) lines where the chromosome number has been doubled. In experiments where assimilate supply was increased by de-graining treatments within the spikelet during rapid ear-growth significant increases in grain weight potential of up to 12% were shown, while no effect on grain size was apparent when de-graining occurred a week after anthesis (Calderini and Reynolds, 2000). The authors therefore suggested that increasing the duration of the rapid ear growth phase may be a means to increasing assimilate availability for grain formation permitting larger grain weight potential.

2.7 Manipulation of source-sink relationships

Determining the physiological factors limiting yield is the first step to increase the yield potential of cultivated plants. For this reason, it is important to assess whether grain growth is limited by the availability of assimilates (source-limited) or by the capacity of the grain to store the available assimilate (sink-limited) (Patrick, 1988; Slafer *et al.*, 1996; Kruk, Calderini and Slafer, 1997; Richards, 2000). Attempts to identify physiological factors limiting yield must take account of source-sink

interactions (Patrick, 1988; Reynolds *et al.*, 2005; Bingham *et al.*, 2006a).The source-sink inter-relationship is influenced by both genotype and environmental factors contributing through variation in photosynthesis and assimilates partitioning (Wang *et al.*, 1994). These factors are described in the following section.

2.7.1 Evidence for sink limitation

A basic requirement for a trait to be a useful selection criterion is that it must be physiologically related to yield. Wheat grain growth is strongly limited by the size of the grain sink (Richards, 1996; Kruk *et al.*, 1997; Reynolds *et al.*, 2005; Shearman *et al.*, 2005). Grain number m^{-2} is the physiological trait most closely linked with genetic gains in grain yield (Fischer, 1985; Slafer and Savin, 1991; Savin and Slafer, 1994), and there is evidence for sink limitation in wheat (grains m^{-2}; Fischer and Stockman, 1986; Reynolds *et al.*, 2005; Shearman *et al.*, 2005). It seems, therefore, that genetic improvement of wheat grain yield may depend on achieving genetic gains in grain number m^{-2} (Waddington *et al.*, 1986; Slafer and Andrade, 1989). Grain number m^{-2} is related to ear dry-matter at anthesis (Fischer, 1985; Fischer and Stockman, 1986; Thorne and Wood, 1987; Shearman *et al.*, 2005). Thus, grain number is related to biomass production and biomass partitioning to the ear during the pre-anthesis period. Recently, there is evidence that anthesis biomass has increased with UK wheat breeding (Shearman *et al.*, 2005). Slafer and Andrade (1989) in Argentina assessed the effect of plant-breeding selection in six wheat cultivars released between 1912 and 1980 and reported that high number of grains m^{-2} in modern wheat cultivars was related to more photosynthetic assimilate being partitioned to the developing ears before anthesis. They also reported the positive association between grain yield and HI. Similar associations were also reported in previous studies (Austin *et al.*, 1980a; Brooking and Kirby, 1981; Corbellini and Borghi, 1985; Waddington, *et al.*, 1986; Shearman *et al.*, 2005).

Concurrent increase in ear number and decrease in vegetative mass indicates that new genotypes utilized assimilate more efficiently than early genotypes (Fischer, 1983; Shearman *et al.*, 2005). For example, the gain in grains per ear of wheat in the

Great Plains in USA is partly from an increase in spikelets per ear and mostly from an increase in grains per spikelet. Survival of florets to form grains is determined primarily by competition for assimilates for stem-elongation during the 3 week period before anthesis (Donmez *et al.*, 2001). The superiority of modern cultivars was mainly associated with more grains per unit area.

There is a rather poor understanding of the determinants of individual grain weight in wheat as discussed above, There are several reports that grain weight may be associated with changes in the availability of assimilates for grain growth (see Slafer and Savin 1994). These studies manipulated source: sink ratios at (or after) anthesis to test whether changes in grain weight are associated with assimilate availability. A few reports (Fischer and HilleRisLambers 1978; Simmons *et al.* 1982) concluded that final grain weight was to some extent limited by the source. In the wider analysis, however, Slafer and Savin (1994) showed that grain growth of modern cultivars is unlikely to be source-limited according to results of source-sink manipulation experiments. Similar results have been published for cultivars introduced in different years (Kruk *et al.* 1997) and for differences between Rht isogenic lines (Miralles and Slafer 1995a).

2.7.2 Genetic variation in the source/sink balance

Source-sink balance is the differences between grain sink size and grain source size in the same units (Shearman *et al.*, 2005). Earlier studies indicated that grain yield potential progress associated mainly with an increased partitioning of photosynthates to grains (Austin *et al.*, 1980a; Gifford, Thorne and Hitz, 1984). In the UK, Austin *et al.* (1989) showed that, for cultivars introduced between 1908 to 1986, HI was positively associated with yield progress with only a slight (non-significant) increase in AGDM at harvest. Subsequently, genetic gain in grain yield of UK winter wheat released from 1972 to 1995 was reported by Shearman *et al.* (2005). This progress was not only attributed to increases in HI but also to significantly increased AGDM at harvest. Those authors reported that the progress in AGDM was contributed mostly in cultivars released after 1983 (i.e. covering the period after the introduction of the semi-dwarfing genes). Thus, biomass progress explained most of the yield

progress within the cultivars introduced after 1983. Such progress in AGDM resulted from greater pre-flowering growth as indicated by the significant positive linear relationship between pre-anthesis RUE and anthesis AGDM.

Slafer and Savin (1994) analyzed data from 15 studies, where source and sink were manipulated at or shortly after anthesis. They concluded that although both source and sink may limit the yield potential, sink is generally more limiting during grain filling. An increase in AGDM and yield was reported to be associated with the introduction of the Lr19 translocation from A. *elongatum* (Singh *et al.*, 1998; Reynolds *et al.*, 2005). Moreover, Reynolds *et al.*, (2001) reported an increase in AGDM resulting from improved source-sink balance with Lr19 in six near isogenic pairs for Lr19. This was due to higher assimilate partitioning to the ears at anthesis in which resulted in more grains (stronger sink) which in turn increased the photosynthetic rate during grain filling in these lines.

Many authors have studied the relationship between source and sink during different eras. For example, Koshkin and Tararina (1989) and Calderini *et al.* (1997) analyzed the impact of source-sink manipulation on sets of cultivars and reported that the most modern cultivars were more responsive to source limitation than older cultivars. Furthermore, Shearman *et al.* (2005) reported that modern winter wheat genotypes were not source-limited because breeders have select for source indirectly while improving sink strength, but the progress in source may be slower than in sink which might result in source limitation in future years.

2.7.3 Evidence from source/sink manipulation experiments

Source/sink relationship varies in different ecological conditions (Wang and Conner, 1996). Many experiments have been reported in which source-sink balance is modified by shading, defoliation, thinning or de-graining (e.g. Slafer and Savin, 1991; Savin and Slafer, 1994; Ma, MacKown, and Van Sanford, 1995; Kruk *et al.*, 1997; Voltas, Romagosa, and Araus, 1998). Shading treatments have been widely used to study grain growth response to reduced assimilate supply (Brocklehurst *et*

al., 1978; Stockman, Fischer and Brittain, 1983; Singh and Jenner, 1984; McMaster, Morgan and Willis, 1987; Grabau, Van Sanford and Meng, 1990; Savin and Slafer, 1991; Shearman, 2001). Pre-anthesis shading reduces grain number while post-anthesis shading reduces grain size (Stockman *et al.*, 1983; Grabau *et al.*, 1990; Savin and Slafer, 1991; Shearman, 2001). The reduction in yield by post-anthesis shading is expected to be greater in more-source-limited cultivars. Fischer and Stockman (1980), Kuhbauch and Thome (1989) and Sherman (2001) reported a reduction in weight of grains from different positions within the ear in response to shading, with the largest reduction for the proximal grains within a spikelet (Singh and Jenner, 1984). RUE has been reported to increase as a result of shading (Sinclair and Muchow, 1999). A compensation effect might therefore be present, to limit the extent to which the crop becomes source-limited and it is possible that there may be genetic variation in this capacity for compensation.

De-graining has been widely applied to test the effect of extra assimilates on the remaining grains (Koshkin and Tararina, 1989; Ma *et al.*, 1990; 1995; Bindraban, 1996; Calderini and Reynolds, 2000; Calderini, Reynolds and Slafer, 2006). If the de-grained crop is source-limited, the grain growth rate and final grain weight will be increased. On the other hand, if the crop is sink-limited, the final grain weight will not increase because there is no room in the grain to accommodate extra assimilates (Ma *et al.*, 1990; 1995; Calderina and Reynolds, 2000; Borras *et al.*, 2004; Calderini *et al.*; 2006). This means that the grain reached it is maximum size. Slafer and Savin, (1994a), Kruk *et al.* (1997) and Calderini *et al.* (2006) reported no increase in grain weight as a result of de-graining performed ten days after anthesis. Thus, the cultivars were not source-limited. This was because de-graining was applied after the cessation of the endosperm cell division. However, Koshkin and Tararina, (1989) conclude that modern spring wheat varieties were source-limited because the final grain weight was increased as a result of 50% de-graining treatment performed at anthesis. However, this reported increase in final grain weight occured because de-graining was performed at anthesis shortly after assimilate availability is crucial for the already active endosperm cell division. So,

the differences between these reports were due to differences in the time of de-graining as indicated by Calderini and Reynolds (2000) who imposed a de-graining treatment on synthetic hexaploid wheat lines for different grain positions within the ear at heading and seven days after anthesis and reported that the de-graining treatment at heading significantly increased grain weight, especially in distal positions. On the contrary, the de-graining treatment carried out after anthesis caused no increase in final grain weight implying that yield of these lines were sink-limited

2.8 Grain growth and photosynthetic rate

Grain dry weight accumulation is linear during most of the grain filling period (Egli, 1994; Pepler *et al.*, 2006). The rate of increase in wheat grain weight appears to be under genetic control (Darroch and Baker, 1990; Hunt, Van der Poorten and Pararajasingham, 1991; Bingham *et al.*, 2006b) with endosperm cell number having a positive effect on grain growth rate (Brocklehurst, 1977). Individual grain's position in the ear differs in rate of growth (Simmons *et al.*, 1982; Shearman, 2001; Dimmock and Gooding, 2002). However, there is little evidence to suggest that the growth rate of grains at a specific location in the ear and the average grain growth rate of all grains in the ear differ in response to a given treatment. When comparing genotypes, only a weak association has been found between grain growth rate and yield (Frederick, 1997; Van Sanford, 1985).

Shearman (2001) studying historic UK winter wheat cultivars released between 1964 and 1995 found an inverse relationship between grain growth rate and the thermal duration of grain fill. Others have found a similar negative association between the two variables (May and Van Sanford, 1992; Van Sanford, 1985). Frederik and Hesketh (1993) proposed that the high demand for assimilate with high seed growth rate increases leaf senescence, shortens the duration of seed fill, and results in a smaller seed in soybean. Similar relationships may also occur in wheat, thus explaining the inverse relationship between the rate and duration of grain growth.

However, grain filling rate may also be partially independent of grain filling duration. Breeders must also be aware that selecting for genotypes with high average grain growth rates can concomitantly cause a selection for fewer grains per ear (Shearman, 2001).

2.9 Grain filling duration

Final grain weight is determined by both grain growth rate and grain filling duration (Van Sanford, 1985, Shearman, 2001). The duration of grain filling is determined by such factors as plant health and nutrient status, reproductive sink demand for assimilate and air temperature. The duration of grain fill may also be determined by the capacity of the grain to utilise available assimilates, as determined shortly after anthesis by the number of endosperm cells and starch granules formed (Egli, 1994; Brocklehurst, 1977). Since grain growth rate is linear during most of the grain filling period, the duration of grain fill can be estimated by dividing grain dry weight at maturity by the rate of grain growth. This estimate of grain fill duration has been termed the effective filling period. The shorter the lag period before and after the period of linear growth, the more accurate the effective filling period is for estimating the actual grain filling period. Whan, Carlton and Anderson, (1996) showed genotypic variation in the duration of grain growth, but with greater environmental variation compared to genotypic variation. Moreover, Shearman (2001) reported genotypic variation in effective grain filling duration to be located in the range 545 – 708 °Cd in UK winter wheat. The duration of grain fill may be controlled by the rate of leaf senescence which, in turn, may be regulated by the N status of the plant and the rate of N demand by the developing grain (Frederick and Hesketh, 1993). As for grain dry weight accumulation, the rate of grain N accumulation is linear for most of the grain filling period (Sofield *et al.*, 1977).

Individual grain weight varies according to position within the ear (Calderini and Ortiz-Monasterio, 2003). Rawson and Evans (1970) found that grains in the second florets of the central spikelets maintain highest growth rates, whilst those in the first floret of the basal spikelets had the lowest. Grains in apical spikelets grow more slowly than those in central and basal spikelets and receive less assimilates from flag

and penultimate leaves. The preferential distribution of assimilate to the lower spikelets is accentuated when the source supply is reduced by defoliation or shading (Bremner, 1972). The systematic variation in mature grain weight occurring along both the length of the ear and within the spikelets is well documented (Slafer and Savin, 1994) and mainly reflects variation in rate of grain growth. At maturity, grains in the central spikelets are larger than the equivalent grains in basal spikelets, which are in turn, larger than the grains in the apical spikelets.

2.10 Use of simulation models for identify processes limiting grain growth

Crop simulation models provide useful information for predicting yield under a wide range of conditions. With the help of simulation models cultivar selection for a target location can be attempted (Stapper and Harris, 1989; Savin *et al.*, 1995). This is possible by including estimates of genotypic variation through the use of genetic coefficients for traits included in the model. Cultivars assessed in this way are calibrated within the model by assigning them characteristic values for the genotype-specific coefficients (Asseng *et al.*, 2002). The wheat simulation models APSIM (McGowan *et al.*, 1996) and CERES (Ritchie and Otter-Nacke, 1985) use genetic coefficients describing the number of grains per unit of stem mass at anthesis (Slafer, 2003). On the other hand, implicit assumptions about the trait interrelationships may bias the performance of the models in predicting changes in the various traits governing the grain yield. The genetic control of ear fertility that determines the final grain yield through various physiological processes appears to be very complex and there is significant G x E interactions. Nevertheless, data generated on the physiological basis of genetic variation in ear fertility will be of use to crop simulation studies in identifying quantitative frameworks to predict grain number and for quantitative analysis of grain sink strength in models.

2.11 Candidate traits for selection in breeding programmes to raise yield potential

As discussed above, HI is close to it is theoretical upper limit in many regions worldwide, so that future increases in yield potential will require the development of cultivars with greater biomass (Slafer, Araus and Richards, 1999). Because grain number is consistently related to yield in the UK environment, maximizing ear weight per unit area at anthesis should result in greater yields. The traits which would contribute to increasing ear dry weight at anthesis are: (i) increasing RUE and hence total biomass in the period before anthesis, (ii) additional improving partitioning to the ear without altering the distribution of the leaf layers, (iii) lengthening the ear growth period from GS31 to GS61 and (iv) improving the grain/ear DM ratio. RUE can potentially be improved by several means, e.g. optimising the distribution of incident radiation within the canopy (reducing K) through more erect leaf attitude. Smaller flag leaves may reduce light saturation of upper leaf layers also release some assimilates for partitioning to the growing ears. Further assimilates could be made available to the growing ears if the length of the peduncle was shortened or by increased tiller economy so that extra assimilates will be available for fertile tiller growth. Extending the duration of rapid ear growth phase (the period when the number of grains is determined) may also contribute to progress in grains m^{-2}. Novel CIMMYT spring wheat material has a large-ear phenotype (long rachis and high spikelet number), with potential for supporting many more individual grains per ear (Rajaram and Reynolds, 2001). So, introgressing the large-ear phenotype to the UK material could additionally gives a novel avenue to enhance grain yield.

Genetic manipulation of grain weight should be considered as a potential avenue to enhance yield potential. Since the physiological determination of individual grain weight potential is not completely understood, we can hypothesize some underlying mechanisms that may be useful. Knowledge of the physiological and genetic basis of this parameter is required before targeted grain size. If physiological traits are used

as selection criteria in breeding programmes, then simple high- throughput techniques are needed to screen for them that are quick and reliable. Not many traits fulfill these requirements. A number of potentially important traits can be easily selected. E.g. flag leaf dimensions, leaf erectness, peduncle length and ear to stem ratio. Others more complex traits such as RUE can be measured indirectly using remote-sensing techniques, e.g. canopy temperature depression using infra-red thermometry, stomatal conductance (air flow porometer; Rebetzke *et al.*, 2001).

2.12 Hypotheses

This thesis focuses on studying the physiological determinants of grain yield using doubled-haploid lines derived from a cross between winter wheat varieties Rialto and Spark, which contrast for source and sink traits. Lines of this population were examined in field experiments in three seasons under post-anthesis shading and post-anthesis de-graining treatments.

The hypotheses tested were:

- Grain growth of modern UK winter wheat is predominantly sink-limited during grain filling.

- There is genetic variation in thermal time from GS31 to GS61 amongst lines of the DH population. Greater thermal time between GS31 and GS61 will result in more radiation interception and anthesis biomass as well as an increased partitioning to the ears at anthesis.

- There is genetic variation in RUE amongst the lines of the DH population. Higher RUE pre anthesis will increase anthesis biomass, hence ear biomass and grains m^{-2}

- 1BL/1RS genotypes have greater pre-anthesis RUE than 1B lines and hence greater anthesis biomass and greater grains m^{-2} and yield

- Extent of sink limitation in DH population differs amongst the lines and this will be evidence in significant differences amongst genotypes in response to post-anthesis shading and de-graining.

- Grain source size and grain sink size can both be quantified on units of g m^{-2} , and can form components of a model to identify the source-sink balance in wheat. This model can be used to identify whether grain growth of crop is source or sink limited.

- Grain number and grain weight are partially independent, and there is a possibility to identify lines showing positive departures from the overall anticipated negative relationship between grains m^{-2} and grain weight.

The main objective in the present study is to identify important physiological traits to be used as selection criteria by plant breeders in future breeding programmes aimed at raising yield potential.

2.13 Thesis structure

The general materials and methods adopted in the present research are described in Chapter 3. The results and discussion of pre-anthesis development and growth as determinants of grains m^{-2} are presented in Chapter 4. In Chapter 5 an analysis of post-anthesis growth is presented to quantify the genetic variation in source-sink balance and the response of genotypes to post-anthesis shading. In Chapter 6, a detailed analysis of processes affecting the potential and final grain weight between anthesis and harvest for five pre-determined grain positions within the ear is presented for the two parents, Rialto and Spark. This includes an analysis of responses to shading for the five grain positions. In addition, the responses to sink manipulation, by removing all the spikelets from one side of the ear during the grain filling, for the ten lines and their parents for the five grain positions are presented. The overall conclusions from the present research are discussed in relation to genetic

improvement of yield potential in wheat in Chapter 7. This chapter will discuss genetic physiological mechanisms involved in determining grain number and grain yield and consider priorities for trait selection in breeding programmes and future research into the physiological basis of yield potential in wheat.

3 Materials and methods

Details of some specific methodologies will be given with the individual chapters.

3.1 Experimental site and plot management

Three field experiments were sown on in 23 September 2003, 28 September 2004 and 3 October 2005 at University of Nottingham Farm, Sutton Bonington, Leicestershire, UK (52°50' N, 1°15' W) on a medium stony loam to 0.80 m over clay (Dunington Heath series) with good drainage. A randomized block, split plot design was used. The previous crop was winter oats (*Avena sativa)* in each experiment. An Oyjard drill was used to sow seeds at 375, 250 and 300 seeds m^{-2} in each season, respectively into 12 rows of 12 cm row width. A prophylactic programme of fungicide at GS31, GS39 and GS61 was applied in all experiments, and herbicides and pesticides were applied depending on prevalent problems according to local conditions to control diseases, weeds and pest to minimum levels. Nitrogen fertilizer was applied as ammonium nitrate prill to ensure that N was not limiting. Plant growth regulators (PGR) were applied at GS31 in 2004 and 2006 but not in 2005. Full details of crop husbandry and the calendar of all operations are presented in Appendix I.

3.2 Experimental design and treatments

The 2003/4 and 2004/5 experiments both used a randomised split-plot design, with three replicates. In each case two post-anthesis shading regimes (unshaded and shaded) were fully randomised on main plots and within these, twenty five DH lines of the Rialto x Spark population and their parents were fully randomised on sub-plots (sub-plot size was 1.6 x 12 m and 1.6 x 18m respectively). Each of unshaded and shaded main plots contained 25 DH lines, but there was a capacity for shading only six genotypes and the other plots were not used for collecting experimental data. In the 2005/6 experiment the plot size was 1.6 x 18m and the ten DH lines and their parents were fully randomized in three replicates. There was no shading

treatment in 2005/6 experiment. From now here on the 2003/4, 2004/5 and 2005/6 experiments will be referred to 2004, 2005 and 2006, respectively.

Experimental plans are presented in Appendix II

3.2.1 Details of experimental treatments

3.2.1.1 Source manipulation (Main plots)

In the experiments in 2004 and 2005, the intention of the 'source manipulation' was to reduce source (assimilate) supply to grains during grain filling, without affecting the sink size (grains m^{-2} and individual potential grain weight) which is determined shortly after anthesis (Brocklehurst, 1978).

The two post-anthesis shading treatments were:

(1) Unshaded control and

(2) Shaded from GS61 + 220°Cd

In each sub-plot designated for shading, plastic netting (with a gauge size equating to approximately 9 mm^2 of netting per 20 mm^2 area that allow 50% light penetration, see figure 3.2) was placed over 6 m lengths of the subplots at GS61 + 220°Cd (base temp 0 °Cd). Shades were fixed to plastic frames which supported the netting approximately 0.5 m above the crop, and the plastic netting extended down the sides of outer rows to approximately 0.4 m from ground level (Figure 3.1). The shades were removed at complete green area canopy senescence.

Post-anthesis shading (decrease source):

3.2.1.2 Genotypes (sub-plot)

Twenty five doubled haploid mapping lines derived from Rialto x Spark cross and their parents were used in this study. This population was chosen because the parents contrast for tiller production and ear fertility (fertile florets ear $^{-1}$) hence grains per m^2 (Table 3.1). Spark has higher grains per m^2 than Rialto. They also contrast for individual grain weight, with Spark having lower individual grain weight. Rialto is a UK winter wheat which is characterised by

moderate tiller production, high fertile florets ear^{-1} and there is evidence that it has high pre-anthesis radiation-use efficiency and high stem reserves (Shearman *et al.*, 2005). While Spark is a tall (*Rht-D1a*) UK winter wheat which has high tiller production, but has small stem reserves (Foulkes *et al.*, 1998). Thus the population of lines should contrast in grains per m^{-2} (Table 3.1) but also importantly contrast markedly for individual grain weight. In addition Rialto and Spark contrast for possession of major gene(s) introduced into UK germplasm in recent decades, including the semi-dwarf *Rht-D1b* allele (Rialto semi-dwarf *Rht-D1b* ; Spark tall *Rht-D1a*) and the 1BL/1RS wheat-rye translocation (Rialto 1BL/1RS; Spark 1B). Spark is bread-making (NABIM Group 1) wheat, and Rialto (NABIM group 2) is feed wheat but has some potential for bread-making. Rialto was bred at the Plant Breeding Institute, Cambridge and Spark at Nickerson Ltd.

Twenty five DH lines derived from Rialto x Spark cross and their parents were randomized on sub-plots in 2004 and 2005. In 2006 ten DH lines and their parents were randomized in three replicates (Table 3.1)

3.2.2 Post-anthesis de-graining:

Post-anthesis de-graining was applied to the ten DH lines and their parents (in order to increase source supply relative to sink) in 2004 and 2005.Ten shoots in the control treatment (unshaded) were de-grained post-anthesis by removing 50% of the spikelets (i.e. all spikelets down one side of ear) at GS61 + 220°Cd (base temp, 0°C)). Ten out of the twenty five DH lines were chose to represent maximum contrast in grain m^{-2} from previous data for growth analysis and de-graining treatment. A further sub set of four out of the ten DH lines and their parents was chosen for the post-anthesis shading treatment (Lines 4, 12, 15, 26, Rialto and Spark). These genotypes were chosen not only because they represent the maximum contrasts in grains m^{-2} but also to provide contrasts in the presence/absence of the *Rht-D1b* semi-dwarf allele and the 1BL/1RS chromosome translocation.

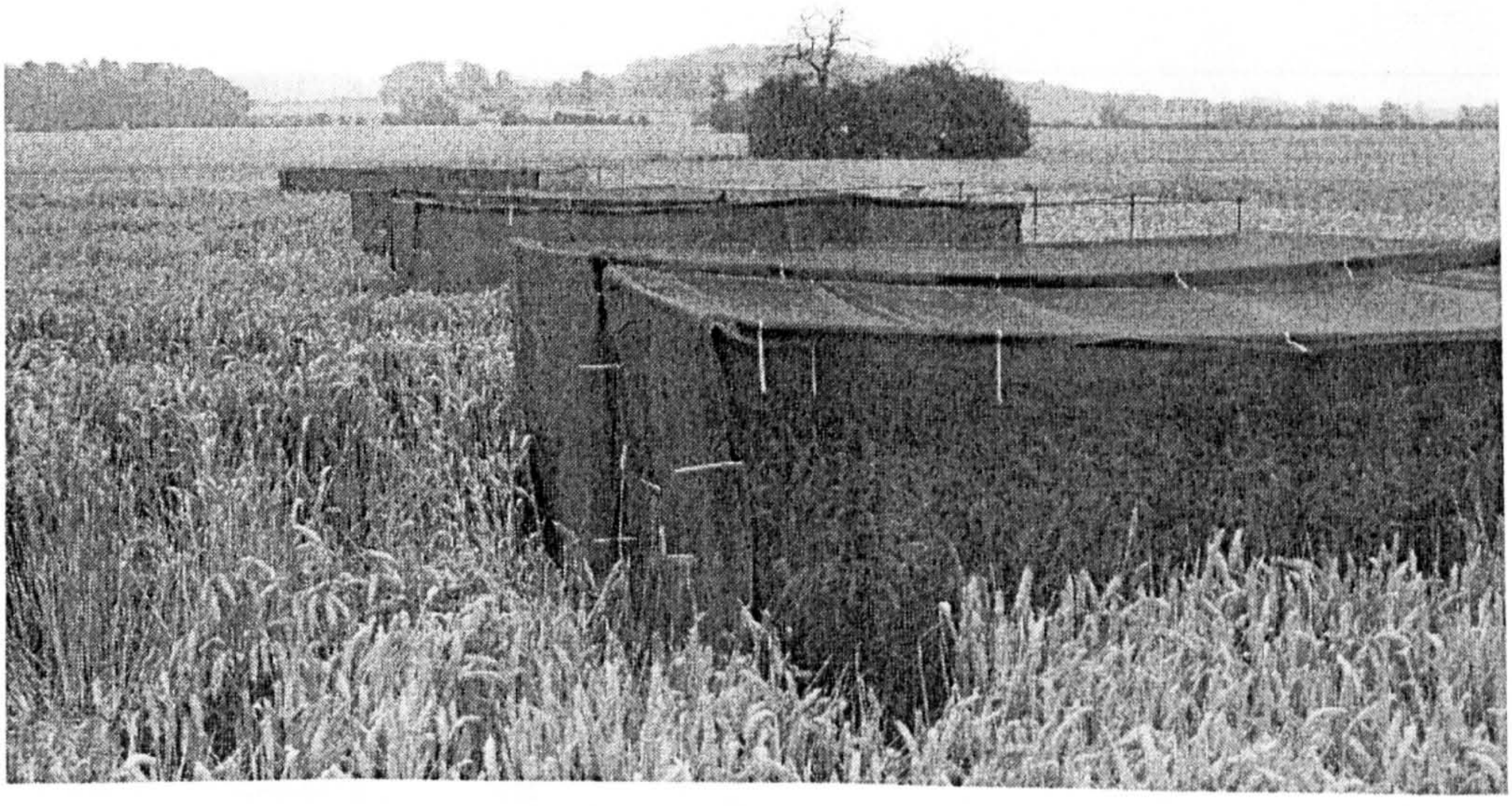


Figure 3.1 Photograph of the shades installed over the four lines and their Parents in 2004

3.3 Crop Measurements

All measurements from GS31 up to and including anthesis (GS61) were carried out in the unshaded treatment only, while measurements at anthesis and post-anthesis were carried out in both the unshaded and post-anthesis shaded treatments. In the unshaded sub-plots, 6 x 1.6 m was used for growth-analysis assessments, and the remaining sub-plot area for measuring combine grain yield, tiller production and survival on tagged plants and measurements of radiation interception, using hand-held ceptometers.

Unless stated otherwise, the following measurements were taken in the twelve genotypes (as indicated in Table 3.1) in unshaded treatment only in three replicates.

Table 3.1 A summary of the inclusion of DH lines in the respective treatments in the experiments in 2004-6. ✓ = genotype was characterised in that treatment. - = source/ sink manipulation treatment not imposed or in the case of the unshaded treatment in 2006, genotype not included in the field experiment. Data for grains m^{-2} refer to the mean of previous phenotyping data at ADAS Gleadthorpe, Nottinghamshire 2000/1 and 2001/2

Line no.	Rht-D1b/ Rht-D1a	*1BL/1RS /1B*	*Unshaded*		*Shaded*	*De-grained*	*Grains m^{-2}*
			2004 and 2005	2006	2004 and 2005	2004 and 2005	Mean 2001 and 2002
Line 1	*Rht-D1a*	1B	✓	✓	-	✓	13767
Line 2	*Rht-D1a*	1BL/1RS	✓	-	-	-	15474
Line 3	*Rht-D1a*	1BL/1RS	✓	✓	-	✓	22466
Line 4	*Rht-D1a*	1B	✓	✓	✓	✓	17964
Line 5	*Rht-D1a*	1BL/1RS	✓	-	-	-	14953
Line 6	*Rht-D1a*	1B	✓	-	-	-	16885
Line 7	*Rht-D1b*	1B	✓	-	-	-	15472
Line 8	*Rht-D1b*	1B	✓	-	-	-	16122
Line 9	*Rht-D1b*	1B	✓	-	-	-	16845
Line 10	*Rht-D1a*	1B	✓	-	-	-	14068
Line 11	*Rht-D1b*	1BL/1RS	✓	✓	-	✓	15458
Line 12	*Rht-D1a*	1BL/1RS	✓	✓	✓	✓	13983
Line 13	*Rht-D1b*	1BL/1RS	✓	✓	-	✓	19429
Line 14	*Rht-D1a*	1B	✓	-	-	-	17406
Line 15	*Rht-D1b*	1B	✓	✓	✓	✓	15734
Line 16	*Rht-D1a*	1B	✓	-	-	-	14131
Line 17	*Rht-D1a*	1B	✓	✓	-	✓	14122
Line 18	*Rht-D1a*	1BL/1RS	✓	-	-	-	17198
Line 19	*Rht-D1a*	1B	✓	-	-	-	10488
Line 20	*Rht-D1a*	1B	✓	-	-	-	14575
Line 21	*Rht-D1b*	1B	✓	✓	-	✓	17867
Line 22	*Rht-D1a*	1BL/1RS	✓	-	-	-	19883
Line 23	*Rht-D1a*	1BL/1RS	✓	-	-	-	15601
Line 24	*Rht-D1b*	1BL/1RS	✓	-	-	-	17742
Line 26	*Rht-D1b*	1BL/1RS	✓	✓	✓	✓	19930
Rialto	*Rht-D1b*	1BL/1RS	✓	✓	✓	✓	14195
Spark	*Rht-D1a*	1B	✓	✓	✓	✓	18710

3.3.1 Crop development

Developmental stages were recorded in unshaded sub-plots/ plots in all seasons in two replicates. This was done by observing sub-plots/ plots every 7d prior to GS31 and every 3-4 d thereafter. Growth stages were based on the decimal codes of the growth stages (GS) devised by Zadoks, Chang and Konzak (1974) as revised by Tottman and Broad (1987). For GS30 and GS31, 5 plants per sub-plot were pulled up, and the stage of the main shoot recorded. The sub-plot was considered to have reached GS30 or GS31 when more than 50% of main shoots were at the stage; it was considered to have reached GS39 (flag leaf emergence) and GS61 (anthesis) from a visual assessment of all shoots in the sub-plot, when more than 50% of all shoots were at the particular stage. Complete canopy senescence was taken in both shaded and unshaded plots as the date when all green lamina had senesced, and there was less than 10% of stem area remaining green.

3.3.2 Fertile shoot number, green canopy area and crop dry matter per m^2

Growth analysis at GS39 was carried out in the 2005 and 2006 only.

3.3.2.1 Sample time and sample area

Sample size was a 0.6 m length of eight adjacent rows avoiding outer two rows on each side of sub-plot .This equated to a sample area of 0.65 m^2. Final plant population was determined at GS31 by digging up and counting all plants contained in the sampled area. Growth of the above-ground plant material was analyzed at four stages: GS31, GS39 GS61 (unshaded subplots only, 12 genotypes) and harvest (unshaded and shaded subplots). At GS31, GS39 and GS61, the genotypes were sampled on the actual calendar dates that they reached the specific stage, that is, genotypes were sampled on different dates. At harvest, all genotypes were sampled on the same calendar date.

3.3.2.2 Growth analysis

At GS31 plants were dug up with their roots, which were later cut off in the laboratory at 'ground level' and discarded. Samples were stored in a cold room at 4 °C. At later growth stages, plants were sampled by cutting at ground level in the

field. Growth analysis was carried out within 4 d of sampling. All plants in the quadrat were counted at the first sampling (GS31) in each experiment. At GS31, GS39, and GS61, the total fresh weight of all the above-ground plant material sampled was recorded. The material was spread out on the bench, and an approximately 10% sub-sample (SS1) was randomly taken by fresh weight, and kept for further analysis. At GS31, the fresh weight of the remaining plant material (90%) was recorded, and it was then dried at 80°C for 48 hours. At GS39 and GS61, after the SS1 sub-sample had been taken, a second sub-sample (SS2) was taken as an approximate 20% sub-sample by fresh weight. After the SS1 and SS2 sub-samples had been taken the remainder of the original sample was discarded. Above-ground dry weight m^{-2} was calculated on the basis of the 90% sub-sample at GS31 and SS2 sub-sample at later stages, after drying at 80°C for 48 h to constant weight.

The SS1 sub-sample was split into three categories of shoots at GS31, GS39 and GS61, defined as: (i) Potentially fertile shoots (when the shoot showed no signs of yellowing at the tips of the youngest leaves at GS31 and GS39, and when the shoot had an ear at GS61) (ii) Dying shoots (when the newest expanding leaf had begun to turn yellow at the tip), and (iii) Dead shoots (when the shoot had no green material).

At each sampling time, for the potentially fertile shoot group, the material was separated into: (i) Green leaf lamina, (ii) Dead lamina, (iii) Green true stem with attached leaf sheath, (iv) Non-green stem with attached leaf sheath, (v) Ears (cut at the collar, once they had emerged). Ears were classified as 'emerged' if the ear was visible but less than 50% emerged. Partly green leaves were separated into green (living) and non-green (dead) parts, unless there was green lamina subtended by necrosis or physical damage across the whole width of the leaf in which case it was classed as non-green.

The fresh weight of each component was recorded, and dry weight recorded after drying at 80 °C for 48h to constant weight. The dry weights of the dead and dying shoots from the SS1 were also recorded.

For the green lamina, green stem (plus attached leaf sheath) and green ear fractions, projected areas were recorded using a LiCor 3100 leaf area meter (LiCor; Nebraska USA) before samples were dried. The green areas for the fractions were summed to

calculate green area index (GAI, defined as the green canopy area per unit area of ground). For green areas of ears, the projected area of the whole ear was recorded, and the percentage of the ears which was green was visually assessed.

At harvest, shoots from the 0.65 m^2 sample area were counted in two categories: (i) fertile shoots (those with an ear) and (ii) infertile shoots (those without an ear). For fertile shoots, the ears were cut off at collar, and the fresh weight of the straw was recorded. A 25% (by fresh weight) sub-sample of the straw was taken, and the fresh weight was recorded. The sub-sample of straw was separated into leaf dead lamina and stem plus leaf sheath. The dry weight of the components was recorded after drying for 48 hrs at 80 °C. The ears were counted and then threshed. The grain and chaff were collected, dried at 80 °C for 48 hrs and weighed. A 75g sub-sample of dried grain was taken, cleaned by removing all broken grains by hand, and the number of grains counted. This sample was weighted after drying at 80 °C for 48 h and the thousand grain weight calculated. The total dry weight of infertile shoots was recorded.

3.3.3 Percentage stem soluble carbohydrate content

At GS61+75 °Cd (base temp. 0 °C), stem soluble carbohydrate was assessed in the ten DH lines and their parents in 2005 and 2006 in the unshaded treatment. Ten fertile shoots were sampled per sub-plot/plot at random in the field between 11.00 and 14.00, to avoid fluctuations in stem sugar content due to the time of day. The shoots were placed in sealed plastic bags and were returned to the laboratory as quickly as possible. After the lamina and ears had been removed, the ten bulked stems (plus attached leaf sheaths) were placed, bending if necessary to fit, into gauze-bottomed drying trays, and 'flash dried' at 102 °C for exactly 2 hours to prevent respiration. The dry weight was then recorded. Dry matter percentage and the water soluble carbohydrate % (at 100 % DM) was determined using the anthrone method of Willis (1954). This was carried out in accordance with The Analysis of Agricultural Material MAFF ADAS 1985 Method 14. Soluble carbohydrates were first extracted with water. The concentration of carbohydrates expressed as glucose

was then determined spectrophotometrically as the blue-green complex formed when carbohydrates were heated with anthrone in sulphuric acid.

As the number of analysed shoots was recorded, WSC content per shoot was easily obtained. This was then multiplied by the total number of fertile shoot m^{-2} to give the stem carbohydrate content (g m^{-2}).

In 2004, the amount of stem reserves was not measured by chemical analysis.

3.4 Crop height

In 2004 and 2005, shortly before harvest, the height from ground level to the tip of the uppermost ear was recorded at 10 randomly located positions in each plot, avoiding any lodged areas of the plot.

3.5 Lodging and leaning assessment

When lodging was observed, assessments of its incidence and severity were made for all the 26 lines. A visual assessment of the percentage area of the crop which was standing, leaning or lodged was made of the whole subplot, including its edges, by walking around the subplot perimeter. The percentage of subplot area in the following classes was assessed:

Index 1 upright (crop leaning between 0 °C and 5° from the vertical)

Index 2 leaning (crop leaning between 5°C and 45° from the vertical)

Index 3 lodged (crop lodged between 45°C and 90° from the vertical)

3.6 Combine grain yield at harvest

Combine grain yield was measured in all unshaded sub-plots/plots in each of the three experiments i.e. for 25 lines and 2 parents in 2004 and 2005 and for 10 lines and 2 parents in 2006. Prior to harvesting, tramlines were cut out. The lengths of the resultant combine areas (approximately 10 m) were recorded accurately in the field. All combined grain from each sub-plot was weighed on the combine. Immediately after harvest, a 250 g sub-sample of grain was accurately weighed and dried to

constant weight to give the grain moisture content, allowing the sub-plot yield to be expressed as tonnes per hectare (t ha^{-1}) at 85% dry matter. A further 75 g sub-sample of grain was taken from each combine sample and cleaned by removing all broken grains. The number of grains and the dry weight of each sample were recorded. Thousand grain weight was then calculated for whole grains.

3.7 Statistical Analysis

Data were entered into EXCEL 2003 (Microsoft Corporation) spreadsheets, and analyzed using GENSTAT 8 (Lawes Agricultural Trust). Standard analysis of variance procedures for a split plot design in randomized blocks were used to calculate treatment means, standard errors and significant differences between treatments. Standard linear regression analysis was used to determine relationship between variables.

4 Effect of genotype on pre-anthesis processes as determinants of grain number m^{-2}

Grains per unit area is the end result of a process in which a significant number of floret primordia are generated during the initial phases of development, followed by abortion of most of these structures (Kirby, 1988; Miralles *et al.*, 1998b). The phase in which floret survival is determined is more important for yield formation than the initial phase of floret initiation (Kirby, 1988; Slafer and Savin, 1994; Reynolds *et al.*, 2005) and for this reason yield is generally well related to ear growth during stem elongation (Slafer, 1995; Miralles *et al.*, 1998b). This means greater growth during the rapid ear growth period (approximately 2-3 weeks before anthesis) is the major pre- requisite for increasing number of grains per unit area (Kirby, 1988; Reynolds *et al.*, 2000; 2005). Indeed, through this process the yield was increased as a result of the introduction of the semi-dwarfing genes (Gale and Youssefian, 1985). Because harvest index is approaching its theoretical upper limit, in the future, there seems to be relatively little opportunity for more improvement in biomass partitioning (Slafer *et al.*, 1999; Shearman *et al.*, 2005) through genetic manipulation of infertile tiller reduction, improved grain set, carpel size and phasic development (Richards, 1988; Fischer *et al.*, 1998; Calderini *et al.*, 1999b; Miralles, Richards and Slafer, 2000). So, the possibility of increasing ear growth at anthesis is rather restricted to enhancing the whole canopy growth and maintaining ear partitioning. This might be achieved by increasing the pre-anthesis RUE or by lengthening the ear-growth phase or by increasing fractional interception advancing canopy closure so that growth during the critical phase is greater. Moreover, the number of grains set per unit ear dry matter (grain-setting efficiency) may also be a genetic avenue for increasing grains per unit area (Abbate *et al.*, 1998).

The physiological basis of grains m^{-2} during the pre-anthesis period for Rialto, Spark and ten doubled haploid mapping lines derived from a Rialto x Spark cross will be analyzed in this chapter in terms of numerical (ear number m^{-2}, spikelet per ear, floret fertility/survival and grains per spikelet) and physiological (green area production, radiation capture, RUE and dry matter partitioning) components. Furthermore, the effects of the 1BL/1RS rye translocation and the *Rht-D1b* semi-

dwarfing allele on pre-anthesis AGDM, RUE and associated physiological traits in the UK's environment will be analyzed.

The specific objectives of the present chapter are to:

1. Identify the physiological basis of grains m^{-2} in the lines of the Rialto x Spark DH population (hence grain yield) by quantifying developmental rates, green area production, radiation capture, RUE and dry matter partitioning pre-anthesis.

2. Examine the effect of 1BL/1RS on pre-anthesis AGDM and associated physiological traits in the UK environment using the Rialto x Spark doubled-haploid mapping population derived from parents contrasting for presence of 1BL/1RS.

4.1 Materials and methods

4.1.1 Experimental design and treatments

The three field experiments were carried out as described in Chapter 3. In addition the following measurements were undertaken.

4.1.1.1 Genotypes

Ten doubled haploid mapping lines derived from Rialto x Spark cross and their parents were used in the analysis presented in this chapter (see Chapter 3- Table 3.1 for more details).

4.1.2 Crop Measurements

4.1.2.1 Fertile tiller number per plant

In December 2003 and 2004, five plants from each unshaded sub-plot were tagged. Number of tillers per plant was recorded every 100 °Cd (base temp 0°Cd) until anthesis on these plants.

4.1.2.2 Fertile shoot number, green canopy area and crop dry matter per m^2

In 2004, green area per shoot was measured for 6 genotypes (Lines 4, 12, 15, 26, Rialto and Spark) on samples of ten randomly selected shoots per sub-plot at GS39. At anthesis plant material from a 0.65 m^2 sample area was used to measure leaf lamina, stem and ear green area (see Chapter 3). However, during this period, there was a breakdown in the cold room leading to some slight deterioration of lamina tissue in some samples. So, green areas per shoot at GS61 were calculated on the basis of the green leaf lamina area per shoot from the ten randomly selected shoots at GS39 plus the green stem and ear areas from GS61. The total green area per shoot on this basis was multiplied by the fertile shoots m^{-2} from the 'quadrat' growth analysis at GS61.

4.1.3 Measurements of green area with canopy depth

These measurements were taken for Rialto and Spark only at GS39 in 2005 in three replicates. Stratified clips were taken in the field, every 10 cm from top of the crop

down, across a 0.25 m^2 area. A 0.25 m^2 quadrat was constructed from light aluminium, and held in position with the four vertical poles at the corners. The quadrat was adjusted to the top of the canopy and progressively lowered at 10 cm intervals. This upper layer (0-10 cm) was removed by cutting across at the quadrat level with scissors using two canes to guide the height when cutting into the centre. All the material from this layer was placed into a polythene bag and labelled. This was repeated down the whole canopy to the ground. This method was used to calculate the light extinction coefficient.

4.1.4 Fertile florets per ear

Measurements of floret fertility were carried out for the parents (Rialto and Spark) only in the three replications in 2004 and 2005. Four tagged main shoot ears were sampled 2-3 days prior to anthesis. All florets inside three central, one apical and one basal spikelet per ear were recorded as fertile or infertile. A floret was considered fertile when it had well developed lemmas, turgid green anthers, well grown carpels and large fluffy stigmas. A floret was considered infertile (aborted) when it had shrivelled and died or had flaccid discoloured anthers or withered carpels and stigmas (Calderini and Reynolds, 2000).

4.1.5 Leaf chlorophyll content (SPAD reflectance readings)

A SPAD meter (Minolta SPAD-502 Tokyo, Japan) was used to measure the flag leaf 'greenness' in all unshaded subplots in the 2004 and 2005 experiments. Ten measurements were made per flag leaf, on each of ten leaves per subplot, by clamping the SPAD sensor over the leaf lamina. Measurements were not taken over the midrib, as this could affect the readings due the thickness and paleness of the midrib.

4.1.6 Environmental measurements

4.1.6.1 Interception of radiation

Interception of photosynthetically active radiation (PAR, 400–700 nm) was measured using a handheld ceptometer (Delta-T Devices, Burwell, Cambridge, UK) in all unshaded sub-plots at GS39 in 2004 and at GS31, GS39 and GS61 in 2005 and

GS39 in 2006. Measurements were taken above the crop and at ground level diagonally across the rows, The extinction coefficient (K) was calculated from GAI and fractional interception using a modified version of Beer's Law (Eq. [1]), where Io is the incident radiation and I is the amount of radiation transmitted below a GAI value of L (Monsi and Saeki, 1953 cited by Saeki, 1960).

K = -ln (I/Io)/L.....Equation 4.1

For stratified clips of green canopy area, regression of cumulative green area against ln (1/1-F_{PAR}) measured at 10 cm depth intervals down the canopy profile using a hand-held ceptometer (10 cm layers from the top the crop to the ground level) allowing the calculation of K_{PAR} as the slope of ln (1/(1-F_{PAR}) on GAI.

4.1.6.2 Accumulated PAR interception

In each season, PAR interception accumulated during the GS31 to GS61 period was calculated by applying the K_{PAR} (at GS39) to incident PAR throughout this period, and assuming that GAI changed linearly with calendar time between sequential samplings.

4.1.6.3 Radiation-use efficiency

Radiation-use efficiency (g MJ^{-1}) was estimated during the GS31-GS61 period. In 2004, RUE was calculated by dividing cumulative biomass (GS31-GS61) over cumulative PAR interception over the same period in each plot. In 2005 and 2006, RUE was calculated by fitting a linear relationship between cumulative above-ground biomass (from measurements at GS31, GS39 and GS61) and cumulative PAR interception over the same period, with RUE calculated as the slope of the linear relationship. RUE values from each plot were analyzed using ANOVA. K_{PAR} from PAR measurements above and below the crop and not the slope in 2005 were used to calculate RUE for the parents.

4.1.6.4 Meteorological measurements

Meteorological data was collected at the meteorological station at Sutton Bonington campus, about 0.5 km from the site of the experiment and at a similar altitude. Total

daily solar radiation (MJ m^{-2}), rainfall (mm) and maximum and minimum air temperatures were recorded from sowing to harvest. Accumulated thermal time in degree days was calculated from maximum and minimum daily air temperatures (˚C) after Kirby and Weightman (1997) using a base temperature of 0 ˚C.

4.2 Results

4.2.1 Grain Sink Size

4.2.1.1 Number of grains m^{-2}

Genotypes differed in the number of grains m^{-2} in each of the three years ($P<0.001$; Table 4.1). Grains m^{-2} was lower in 2004 compared to 2005 and 2006. Averaging across years, grains m^{-2} was highest in lines 26 and 21 (26,452 and 24,916, respectively) and lowest in lines 4 and 12 (17,560 and 18,532, respectively). Cross-year analysis of variance showed differences between genotypes, seasons and their interaction ($P<0.05$). The DH lines showed transgresive segregation. For example lines 21 and 26 consistently produced more grains than Spark and lines 4, 12, and 17 produced fewer grains than Rialto Additionally, Line 4 produced heavier grains and lines 1, 21 and 26 produced lighter grains than Rialto in both years.

Table 4.1 Number of grains m^{-2} and spikelets per ear for the ten DH lines of the Rialto x Spark population and the two parents in 2004, 2005 and 2006

	Grains m^{-2}				Spikelets ear^{-1}	
	2004	2005	2006	Mean	2004	2005
Line 1	18599	21735	21539	20624	-	19.8
Line 3	21894	22493	20662	21683	-	21.8
Line 4	15285	18557	18839	17560	24.6	21.0
Line 11	16322	20760	21154	19412	-	21.0
Line 12	17636	18013	20426	18692	22.4	17.9
Line 13	20753	23240	24302	22765	-	21.0
Line 15	16009	22090	21164	19754	23.7	26.0
Line 17	18341	17784	19472	18532	-	18.0
Line 21	23809	25226	25714	24916	-	20.9
Line 26	25350	27461	26544	26452	25.1	21.7
Rialto	18620	21019	21382	20340	25.4	24.3
Spark	20951	24069	24614	23211	21.6	21.3
Mean	19464	21870	22151	21162	23.8	21.2
SED(df)						
Year				352.4 (69)		
Genotype	1417.3 (22)	561.4 (21)	844.2 (22)	704.8 (69)	0.783 (7)	1.225 (22)
Genotype x year				1220.8 (69)		

1BL/1RS positively affected grains m^{-2} in 2004 and 2006 but not 2005 ($P<0.05$) (Table 4.2). Cross-year analysis of variance showed that 1BL/1RS genotypes produced 685 grains more than 1B genotypes ($P=0.008$). *Rht-D1b* increased grains m^{-2} in 2005 and 2006 ($P<0.001$; Table 4.2) and the trend was also evident in 2004 ($P=0.056$). *Rht-D1b* genotypes produced relatively more grains in 2006 compared to 2004 and 2005. Cross-year analysis of variance showed a positive response to *Rht-D1b* ($P<0.001$) with an increase of 2140 grains.

Table 4.2 Effect of the 1BL/1RS translocation and Rht-D1b semi-dwarfing gene on grains m^{-2} and spikelets ear^{-1} in 2004 and 2005

	Grains m^{-2}				*Spikelets ear^{-1}*	
	2004	2005	2006	Mean	2004	2005
1BL/1RS	20096	21846	22412	21451	24.3	21.3
1B	18832	21577	21890	20766	23.3	21.2
Mean	19464	21712	22151	21109	23.8	21.2
P value						
1BL/1RS	0.04	0.10	<0.001	0.008	0.07	0.83
1BL/1RS x year				0.51		
Rht-D1b	20144	23049	23376	22190	24.8	22.5
rht	18784	20442	20925	20050	22.6	20.0
Mean	19464	21746	22151	21120	23.8	21.2
P value						
Rht-D1b	0.056	<0.001	<0.001	<0.001	0.002	<0.001
Rht-D1b x year				0.097		

4.2.1.2 Number of spikelets per ear and floret fertility

Significant differences among genotypes were identified in the total number of spikelets per ear (fertile and infertile) from samples taken prior to anthesis in 2004 and at harvest in 2005 ($P<0.05$; Table 4.1). Kemp and Whingwiri (1980) state that the initiation of spikelet primordia is not expected to be influenced by assimilate supply.

Floret fertility was measured as fertile florets per spikelet, for Rialto and Spark only, two days prior to anthesis in order to test the relationship with ear DM growth. Parents did not differ in the total number of florets (fertile and infertile) per spikelet in apical or basal spikelets in both years (Table 4.3), but there was a trend to have more fertile florets in central spikelets ($P<0.05$). Averaging across spikelet positions, differences in number of florets per spikelet were detected in 2004 ($P=0.057$) and in 2005 ($P=0.03$). Rialto had more fertile florets in the central spikelet in 2004 and in both apical and central spikelet in 2005 ($P<0.05$). Higher floret fertility for Rialto was associated with greater dry matter per spikelet of central and basal spikelets ($P<0.05$) in both years.

In general, results showed greater floret fertility for Rialto compared to Spark as an outcome of having heavier spikelets ($P<0.05$); i.e. the higher the amount of assimilates allocated to the ear during rapid ear growth phase, the higher the number of fertile florets. In the following section the physiological basis of these effects will be examined. 1BL/1RS did not affect spikelets ear^{-1} in either year. *Rht-D1b* increased number of spikelets per ear in both years ($P<0.05$; Table 4.2).

Table 4.3 Total number of florets per spikelet, number of fertile florets per spikelet and spikelet dry matter (mg) for apical, central and basal spikelets for the two parents (Rialto and Spark) in 2004 and 2005

		2004			2005		
Genotypes		Apical	Central	Basal	Apical	Central	Basal
Rialto	Total number of florets	2.92	4.47	3.92	2.92	4.89	3.92
	Fertile florets	1.83	3.47	2.92	2.00	3.92	3.33
	Spikelet DM (mg)	8.54	25.07	19.67	9.81	27.39	19.82
Spark	Total number of florets	2.92	3.69	4.08	2.33	4.25	3.75
	Fertile florets	1.25	2.75	2.17	1.33	3.25	2.67
	Spikelet DM (mg)	5.31	11.48	8.04	5.55	14.27	8.89

4.2.2 Dates of Growth Stages

Averaging across-years, durations from GS31-GS39, GS39-GS61 and GS31-GS61 were longer in 2005 (50, 22 and 72 d, respectively) compared to 2004 (42, 20 and 62 d) and 2006 (39, 18 and 57 d) (Table 4.4). The period from GS31 to GS39 on

average ranged from 48 d (Rialto) to 41 d (Lines 3, 4 and Spark). The period between GS39 and GS61 was on average 20 d over the three years, ranging from 18 d (Line 21) to 22 d (Line 26). The duration between GS31 and GS61 ranged from 69 d for Rialto to 60 d for Line 1. The parents differed in the length of this stem-elongation period by 8 d across the three years.

4.3 Tiller production and survival

4.3.1 Fertile shoots per plant

From shoot counts on tagged plants, genotypes reached their maximum fertile shoot number in April (Figures 4.1 a and b). Tiller death continued until anthesis (mid June). Spark had the highest maximum number of fertile shoots plant^{-1} and the highest number of fertile tillers at anthesis followed by Lines 1 and 21, while Lines 4, 12 and Rialto had the lowest number of fertile shoots in both years. In this chapter, data from 2005 and 2006 were used for resource capture data because these data are more reliable since there was a problem with leaf area measurements in 2004 which make data unreliable.

4.3.2 Shoot production per unit area

4.3.2.1 Tiller production

From measurements on quadrat samples, genotypes differed in fertile shoots m^{-2} at GS31, GS39 and GS61 in each of 2004, 2005 and 2006 ($P \leq 0.05$; Table 4.5). Averaging across 2005 and 2006, Lines 1 and 3 were among the top five ranking shoots m^{-2} at GS31 and GS61, whereas Rialto and Line 15 were among the bottom five ranking at both growth stages ($P<0.05$). Tiller production was lower in 2005 compared to 2006 ($P=0.032$) except for Lines 12 and 21. Total solar irradiance in March 2006 (246.9 MJ m^{-2}) was higher than that of 2005 (193.1 MJ m^{-2}), and tillering is enhanced by high light intensity (Whaley *et al.*, 2000). Mean temperature over winter (December to February) was similar in 2005 (5.38 °C) compared to 2006 (4.54 °C).

Table 4.4 Dates of growth stages (GS31, GS39 and GS61) and durations (days) from GS31 to GS39 and GS39 to GS61 for the ten DH lines of the Rialto x Spark population and the two parents in 2004, 2005 and 2006

	2004			Duration		2005			Duration		2006				
Genotype	GS31	GS39	GS61	GS31-GS39 (days)	GS39-GS61 (days)	GS31	GS39	GS61	GS31-GS39 (days)	GS39-GS61 (days)	GS31	GS39	GS61	GS31-GS39 (days)	GS39-GS61 (days)
Line 1	20-Apr	26-May	14-Jun	36	19	6-Apr	27-May	16-Jun	51	20	21- Apr	30-May	15-Jun	40	17
Line 3	13-Apr	23-May	10-Jun	40	18	7-Apr	24-May	15-Jun	47	22	21- Apr	25-May	12-Jun	35	18
Line 4	16-Apr	23-May	14-Jun	37	22	5-Apr	26-May	15-Jun	51	20	20- Apr	25-May	14-Jun	36	20
Line 11	8-Apr	22-May	9-Jun	44	18	3-Apr	22-May	13-Jun	49	22	15- Apr	24-May	11-Jun	41	18
Line 12	8-Apr	25-May	14-Jun	47	20	5-Apr	25-May	18-Jun	50	24	19- Apr	28-May	16-Jun	40	20
Line 13	16-Apr	27-May	15-Jun	41	19	7-Apr	28-May	18-Jun	51	21	25- Apr	31-May	16-Jun	41	17
Line 15	2-Apr	20-May	9-Jun	48	20	3-Apr	22-May	15-Jun	49	24	17- Apr	31-May	16-Jun	45	17
Line 17	11-Apr	22-May	8-Jun	41	17	2-Apr	22-May	15-Jun	50	24	21- Apr	25-May	13-Jun	35	19
Line 21	13-Apr	23-May	10-Jun	40	18	6-Apr	27-May	16-Jun	51	20	22- Apr	29-May	14-Jun	38	17
Line 26	8-Apr	23-May	14-Jun	45	22	4-Apr	25-May	17-Jun	51	23	18-Apr	26-May	14-Jun	39	20
Rialto	2-Apr	19-May	10-Jun	47	22	28-Mar	22-May	13-Jun	55	22	15- Apr	26-May	12-Jun	43	18
Spark	16-Apr	24-May	14-Jun	38	21	8-Apr	27-May	17-Jun	49	21	25-Apr	30-May	16-Jun	36	18
Mean				42	20				50	22				39	18

4.3.2.2 Tiller survival

Averaging across 2005 and 2006, the most efficient tillering genotypes were Line 17 and Rialto, losing an average of 280 and 377 shoots m^{-2} respectively ($P<0.001$; Table 4.5), while the least efficient ones were Line 13 and Spark, losing 1084 and 1035 shoots m^{-2}, respectively. There was a linear relationship between shoot production (number of shoots at GS31) and the number of non-surviving shoots in 2004 and 2005 ($P<0.001$; Figure 4.2) but not 2006. The genotypes such as Line 13 and Spark, which produced most shoots at GS31, lost most shoots during the tiller survival period. Cross-year analysis of variance for 2005 and 2006 showed no effect of season.

(a)

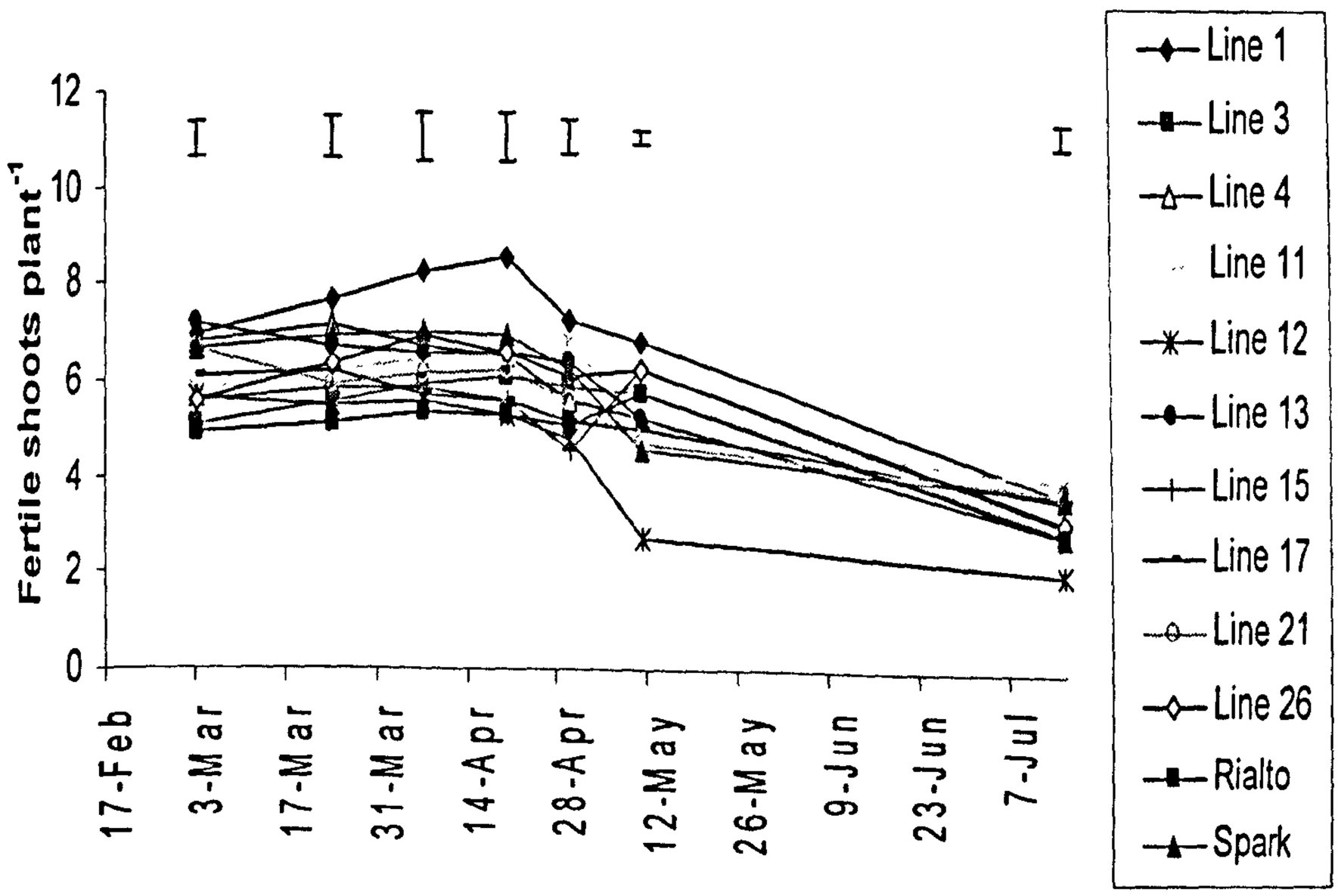


Figure 4.1 shoot number per plant for the twelve genotypes measured during the (a) 2004 and (b) 2005 growing season (bars show S.E.D, 11df).

(b)

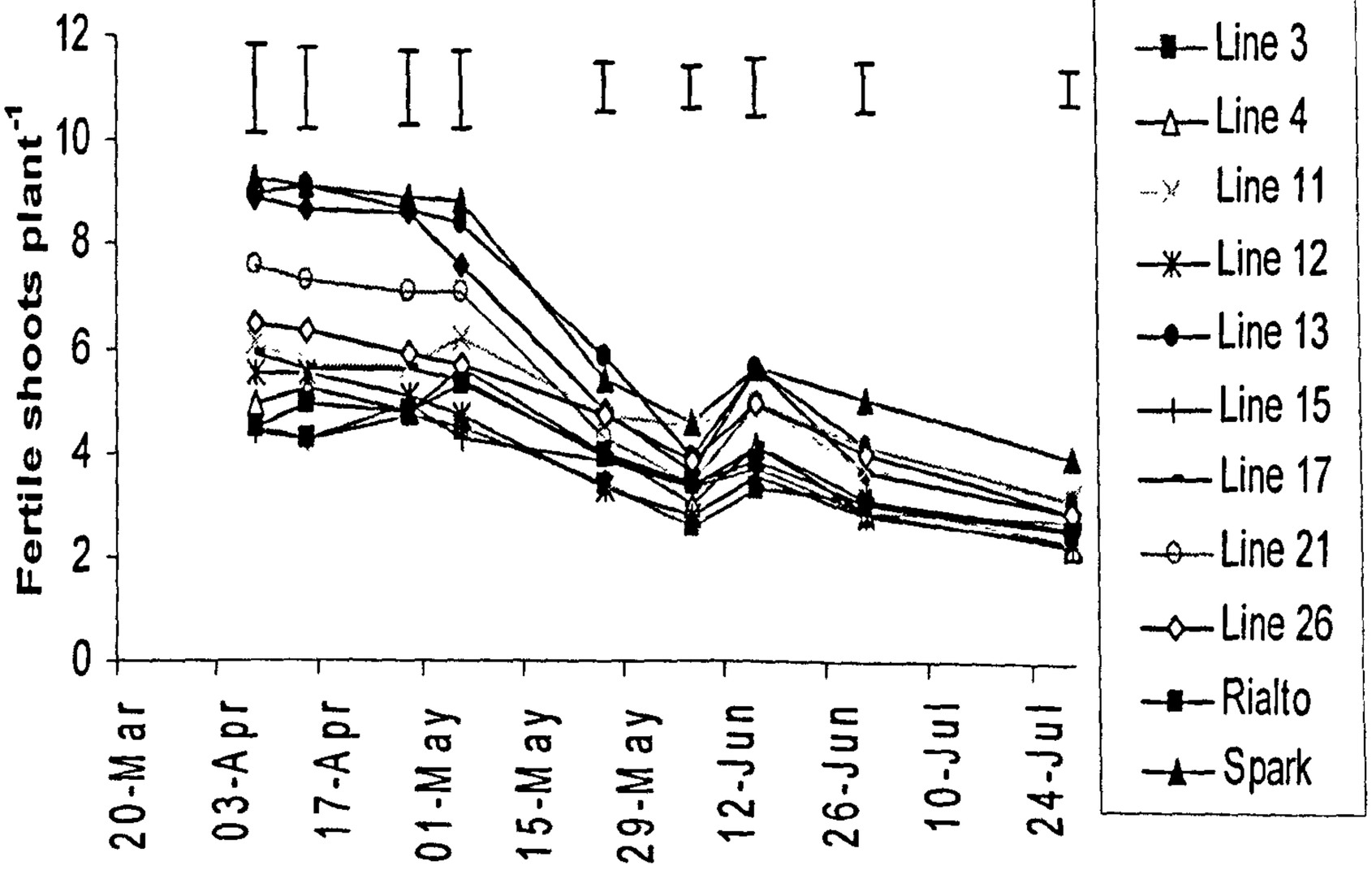


Figure 4.1 shoot number per plant for the twelve genotypes measured during the (a) 2004 and (b) 2005 growing season (bars show S.E.D, 11df).

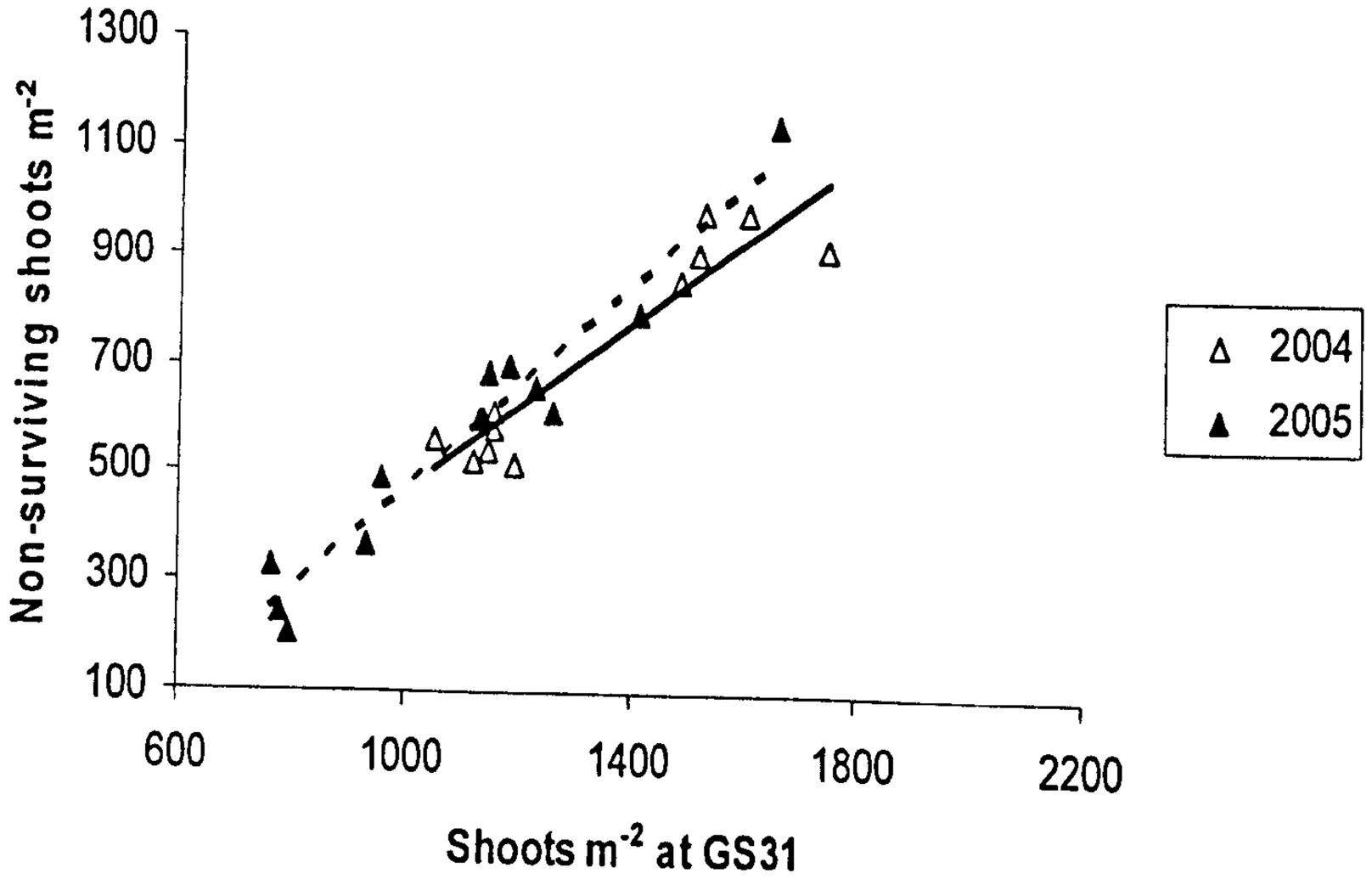


Figure 4.2 Regression of number of non-surviving shoots (GS31-GS61) on shoot production at GS31 in 2004 and 2005 seasons. 2004: $y=0.77x -296$; $R^2 =0.88$; 2005: $y=0.94x -475$; $R^2 =0.94$.

Table 4.5 Fertile shoot number m^{-2} (at GS31, GS39 and GS61) in 2004, 2005 and 2006 and above-ground dry matter (AGDM) for all shoots (ALL) and for non-surviving shoots (N-S) (g m^{-2}) for the ten DH lines of the Rialto x Spark population and the two parents

	Fertile shoot number m^{-2}												AGDM			
	GS31				GS39				GS61				ALL	N-S	ALL	N-S
Genotype	2004	2005	2006	2005-6 mean	2004	2005	2006	2005-6 mean	2004	2005	2006	2005-6 mean	2005	2005	2006	2006
Line 1	1519	1263	1391	1327	-	649	629	639	615	701	637	669	1771	93.3	1118	52.1
Line 3	1119	1139	1280	1209	-	630	735	603	594	472	734	604	1485	114.0	1030	25.9
Line 4	1484	1231	1326	1279	767	660	653	657	629	455	617	536	1419	105.0	1165	58.7
Line 11	1160	959	1079	1019	-	715	744	730	579	514	713	614	1487	116.1	1056	37.9
Line 12	1529	1147	1060	1104	851	674	714	694	545	540	623	581	1584	84.1	1099	37.2
Line 13	1608	1659	1719	1689	-	778	729	754	626	592	619	606	1649	82.0	1121	52.3
Line 15	1129	782	1168	975	725	595	652	623	529	489	548	519	1446	75.7	1270	43.3
Line 17	1146	799	910	855	-	736	638	687	607	569	579	574	1438	87.7	1219	47.9
Line 21	1195	1183	1027	1105	-	660	668	664	679	542	637	590	1250	43.3	1201	27.6
Line 26	1158	932	1186	1059	743	581	739	660	543	575	574	575	1720	65.7	1246	25.1
Rialto	1054	765	956	861	717	580	617	599	491	440	528	484	1510	79.0	1071	32.5
Spark	1745	1414	1816	1615	991	718	837	778	826	618	541	580	1457	64.9	1056	33.3
Mean	1320	1106	1243	1175	799	665		674	605	538	613	578	1518	84.2	1138	39.5
SED (df)																
Year				61.9 (46)				20.9 (46)				19.26 (45)	106.0 (22)	27.0 (22)		
Genotype	82.6 (22)	161.1 (22)	222 6 (22)	151.7 (46)	39.0 (10)	59.7 (22)	81 2	51 1 (46)	44.98 (21)	48.53 (21)	48.61 (22)	47.17 (45)			50.2 (16)	19.05 (22)
Interaction				214.6 (46)				72.3 (46)				66.71 (45)				

4.3.3 Green canopy area

Green area index (GAI) results are presented for six genotypes in 2004 and twelve genotypes in 2005 and 2006. For the twelve genotypes, mean values across 2005 and 2006 are also presented.

4.3.3.1 Green area index (GAI)

Significant differences in GAI ($P \leq 0.05$) existed among genotypes in 2004 at GS31 in the range 2.95 (Line 1) to 1.35 (Line 26) (Table 4.6). In 2004, GAI at GS31 for lines 1 and 13 was greater (2.95 and 2.72 respectively) associated with the production of more shoots m^{-2} (1519 and 1608 shoots m^{-2}, respectively) compared to other genotypes. Line 4 produced the greatest GAI at GS61 whereas Line 26 had the lowest value amongst the genotypes in 2004.

Significant differences ($P \leq 0.05$) existed among genotypes in GAI at GS31 in the range 2.99 (Line 12) to 1.40 (Line 17) in 2005 ($P<0.001$) but not in 2006 (Table 4.6). Cross-year analysis for 2005 and 2006 showed a trend for differences ($P=0.060$) in the range 1.56 (Line 17) to 2.39 (Line 12). GAI at GS31 for lines 12, Spark and 13 had greater values associated with the production of more shoots m^{-2} compared to other genotypes. There was a trend for interaction between year and genotype ($P=0.080$).

A trend for differences between the twelve genotypes in GAI at GS39 was observed in 2005 ($P=0.07$). However, there were no differences in 2006. Cross 2005 and 2006 ANOVA showed no effect for year, genotype and their interaction. The maximum GAI was reached at GS61 in both 2005 and 2006 (average value 7.11; Table 4.6) with no statistical differences among the genotypes.

In 2005, neither 1BL/1RS nor *Rht-D1b* affected GAI at GS61. However, in 2006, 1B-gentypes had higher GAI ($P=0.069$), while the semi-dwarfing allele showed a neutral effect on GAI. Cross-year analysis of variance showed a neutral effect for both 1BL/1RS and *Rht-D1b* on GAI (Table 4.7).

4.3.3.2 Resource capture

4.3.3.3 Light extinction coefficient

K_{PAR} calculated at GS39 did not differ significantly among genotypes in 2004 with values ranging from 0.42 (Line 26) to 0.49 (Rialto) (Table 4.8). K_{PAR} calculated at GS31, GS39 and GS61 in 2005 and at GS39 in 2006 showed no significant differences between growth stages, genotypes or for the interaction (P>0.05; Table 4.10). Averaging across the three growth stages in 2005, K_{PAR} ranged from 0.50 for Line 13 to 0.62 for Line 21. However, cross-year ANOVA for 2005 and 2006 for K_{PAR} at GS39 showed genotypic differences (P=0.046) in the range 0.54 (Line 15) to 0.72 (Line 1). There was no interaction between year and genotype. K_{PAR} in 2006 was greater than 2005 (P=0.001)

Table 4.6 Green area index at GS31, GS39 and GS61 for the ten DH lines of the Rialto x Spark population and the two parents in 2004, 2005 and 2006

Genotype	*GS31*				*GS39*				*GS61*			
	2004	2005	2006	2005-6 mean	2004	2005	2006	2005-6 mean	2004	2005	2006	2005-6 mean
Line 1	2.95	2.01	1.70	1.86	-	5.77	5.97	5 87	-	7 09	8 10	7 59
Line 3	2.33	2.01	1.96	1.99	-	6.51	5.64	6.06	-	7 27	6.49	6.88
Line 4	2.14	2.49	1.45	1.97	9.40	7.05	6.58	6 82	8 20	6.56	6.87	6.71
Line 11	1.45	2.04	1.72	1.88	-	7.12	6.91	7 02	-	7 17	6 90	7.03
Line 12	1.61	2.99	1.79	2.39	10.54	6.00	7.02	6.51	6.20	7.03	7.40	7.21
Line 13	2.72	2.94	1.69	2.32	-	7.63	6.74	7.18	-	7.53	6.85	7.19
Line 15	1.55	1.75	1.77	1.76	8.73	6.08	6.29	6.18	6.80	7.38	8.51	7.95
Line 17	2.11	1.40	1.72	1.56	-	6.63	6.11	6.37	-	6.80	6.90	6.85
Line 21	2.24	1.98	1.45	1.72	-	5.98	6.43	6 20	-	6.69	7 34	7.02
Line 26	1.35	2.04	1.65	1.84	8.12	6.06	6.43	6 25	5 90	7 01	7 15	7.08
Rialto	1.43	1.98	1.77	1.87	9.18	6.97	6.31	6.64	6.43	6.57	7.20	6.88
Spark	2.22	2.80	1.86	2.34	8.50	6.44	7.34	6.89	7.79	7 15	6.70	6.92
Mean	2.01	2.20	1.71	1.96	9.08	6.52	6.48	6.50	6.90	7.02	7.20	7 11
SED (df) Year				0.1090 (46)				0.185 (46)				0.177 (46)
Genotype	0.2036 (22)	0.2648 (22)	0.278 (22)	0.2670 (46)	0.702 (6)	0.561 (22)	0.638 (22)	0.452 (46)	0.517 (6)	0.691 (22)	0.4258 (22)	0 433 (46)
Interaction				0.3776 (46)				0.639 (46)				0.613 (46)

Table 4.7 Effect of 1BL/1RS translocation and *Rht-D1b* semi-dwarfing gene on GAI in 2005 and 2006

Gene	*GAI*		
	2005	2006	Mean
1BL/1RS	7.18	7.00	7.09
1B	6.79	7.40	7.10
Mean	7.08	7.20	7.14
P value			
1BL/1RS	0.49	0.069	0.48
Interaction			0.12
Rht-D1b	7.01	7.32	7.17
Rht-D1a	7.14	7.08	7.11
Mean	7.08	7.20	7.14
P value			
Rht-D1b	0.67	0.25	0.36
Interaction			0.63

Table 4.8 Light extinction coefficient (K_{PAR}) calculated at selected growth stages in 2004, 2005 and 2006 for the ten DH lines of the Rialto x Spark population and the two parents in 2004, 2005 and 2006

	2004	2005				2006	2005-6 mean
Genotype	GS39	GS31	GS39	GS61	Mean	GS39	GS39
Line 1	-	0 53	0.75	0.55	0.60	0.69	0.72
Line 3	-	0.62	0.61	0.55	0.59	0.70	0.66
Line 4	0.46	0.53	0.66	0.50	0.56	0.71	0.68
Line 11	-	0.62	0.53	0.51	0 61	0.62	0.57
Line 12	0.45	0.37	0.58	0.55	0.55	0.59	0.58
Line 13	-	0.49	0.66	0.51	0.50	0.70	0.68
Line 15	0.44	0.61	0.50	0.52	0.55	0.58	0.54
Line 17	-	0.71	0.58	0.57	0.54	0.84	0.71
Line 21	-	0.62	0.62	0.56	0.62	0.70	0.66
Line 26	0 42	0.56	0.51	0.56	0.54	0.67	0.59
Rialto	0.49	0.64	0.61	0.59	0.61	0.76	0.70
Spark	0.44	0.50	0.61	0.61	0.57	0.62	0.61
Mean	0.45	0.57	0.60	0.55	0.57	0.68	0.64
SED Genotype (df)	0.055 (10)	0 107 (22)	0.082 (22)	0.061 (22)	0.049 (70)	0.088 (21)	0.0241 (45)
Date (df)					0.024 (70)		0.0590 (45)
Interaction (df)					0.084 (70)		0.0835 (45)

Extinction coefficient measured using stratified samples for the parents (Rialto and Spark) at GS39 in 2005

K_{PAR} indicated by the slope of the regression line of GAI on ln (1/1-F $_{PAR}$) revealed differences between the parents ($P<0.001$; Figure 4.3). Rialto had a higher K_{PAR} value (0.68) than Spark (0.56), which was consistent with values presented in Table 4.9.

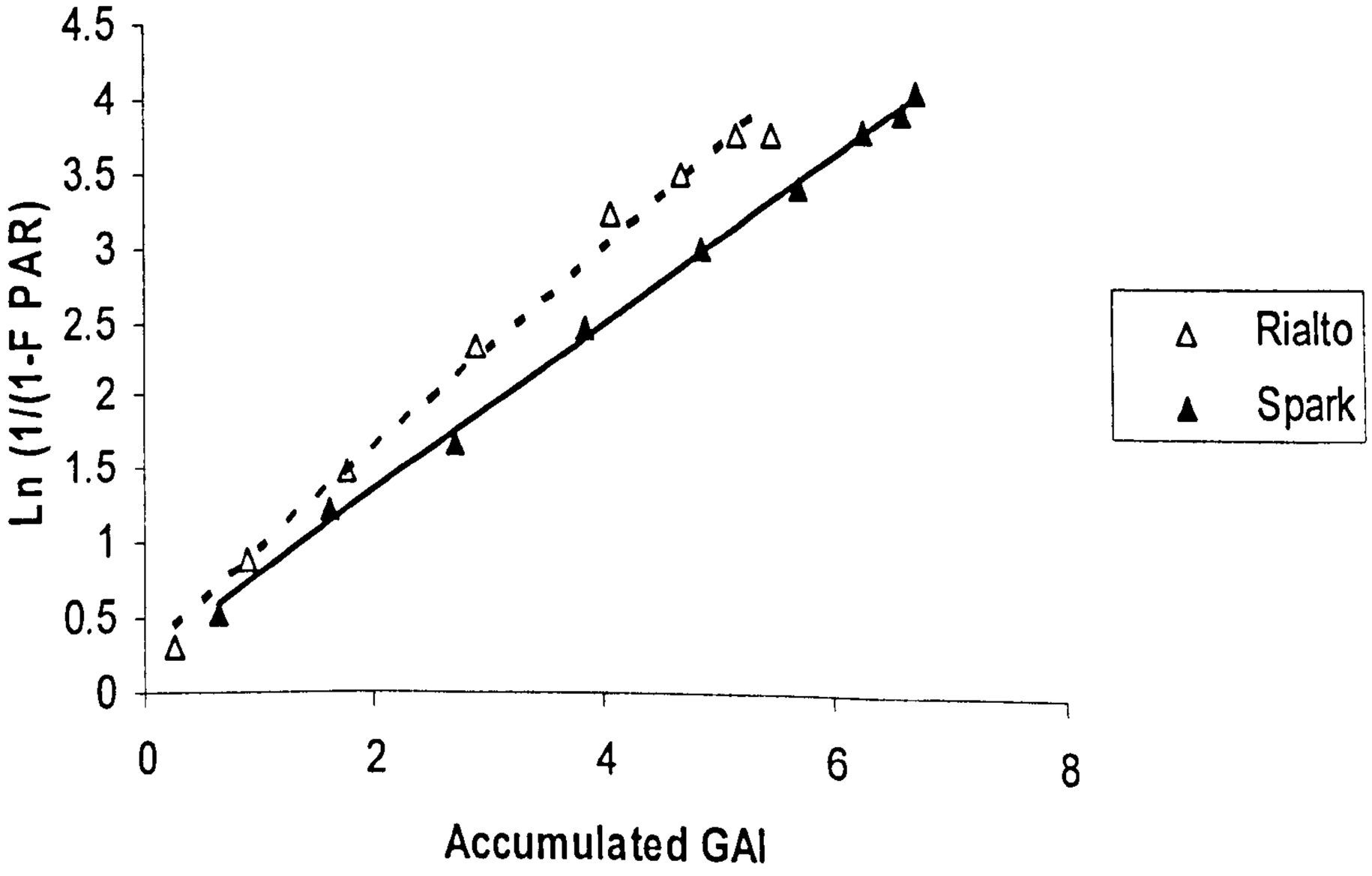


Figure 4.3 Regression of ln(1/(1-F PAR)) on accumulated GAI in 10 cm layers with canopy depth at GS39 in 2005. The slope of the regression line represents the PAR extinction coefficient (K_{PAR}). Rialto: y=0.68x +0.27; R^2= 0.991; Spark: y=0.56x +0.23; R^2=0.998.

4.3.3.4 Cumulative intercepted radiation

There were no significant differences amongst the 6 and the 12 genotypes in 2004 and 2005, respectively, in the amount of PAR intercepted between GS31 and GS61 (Table 4.9). However, differences existed among the 12 genotypes in 2006 ($P<0.001$). Cross-year analysis of variance for the twelve genotypes in 2005 and 2006 showed differences among genotypes in the range 669 (Spark) to 781 MJ m^{-2} (Line 15). Overall, the crop intercepted more PAR in 2005 compared to 2006 ($P<0.001$)

The amount of PAR accumulated showed a linear relationship with the duration from GS31 to GS61 for the twelve genotypes in 2006 (P=0.012; Figure 4.7), but not 2005. However for the mean across 2005 and 2006 there was a linear relationship (P=0.03, Figure 4.4). Moreover, there was a positive linear relationship between PAR accumulated between GS31 and GS61 in 2006 and GAI at GS61 (P=0.002; R^2=0.64).

Table 4.9 Accumulated photosynthetically active radiation (PAR) intercepted between GS31 and GS61 for the ten DH lines of the Rialto x Spark population and the two parents in 2004, 2005 and 2006

Genotype	Accumulated PAR (MJ m^{-2})			
	2004	2005	2006	2005 and 2006 mean
Line 1	-	793.4	736.8	765.1
Line 3	-	772.6	609.5	691.1
Line 4	662.3	690.8	687.7	689.3
Line 11	-	770.0	668.0	719.0
Line 12	637.7	804.5	739.7	772.1
Line 13	-	743.4	658.5	700.9
Line 15	622.3	728.2	756.4	742.3
Line 17	-	847.9	704.3	776.1
Line 21	-	769.9	700.6	735.3
Line 26	605.7	751.7	695.2	723.5
Rialto	690.3	816.3	742.2	779.2
Spark	625.0	723.7	613.6	668.7
Mean	640.6	767.7	692.7	730.2
SED (df)				
Year				10.72 (46)
Genotype	40.4 (10)	47.98 (22)	23.92 (22)	26.26 (46)
Year x genotype				37.14 (46)

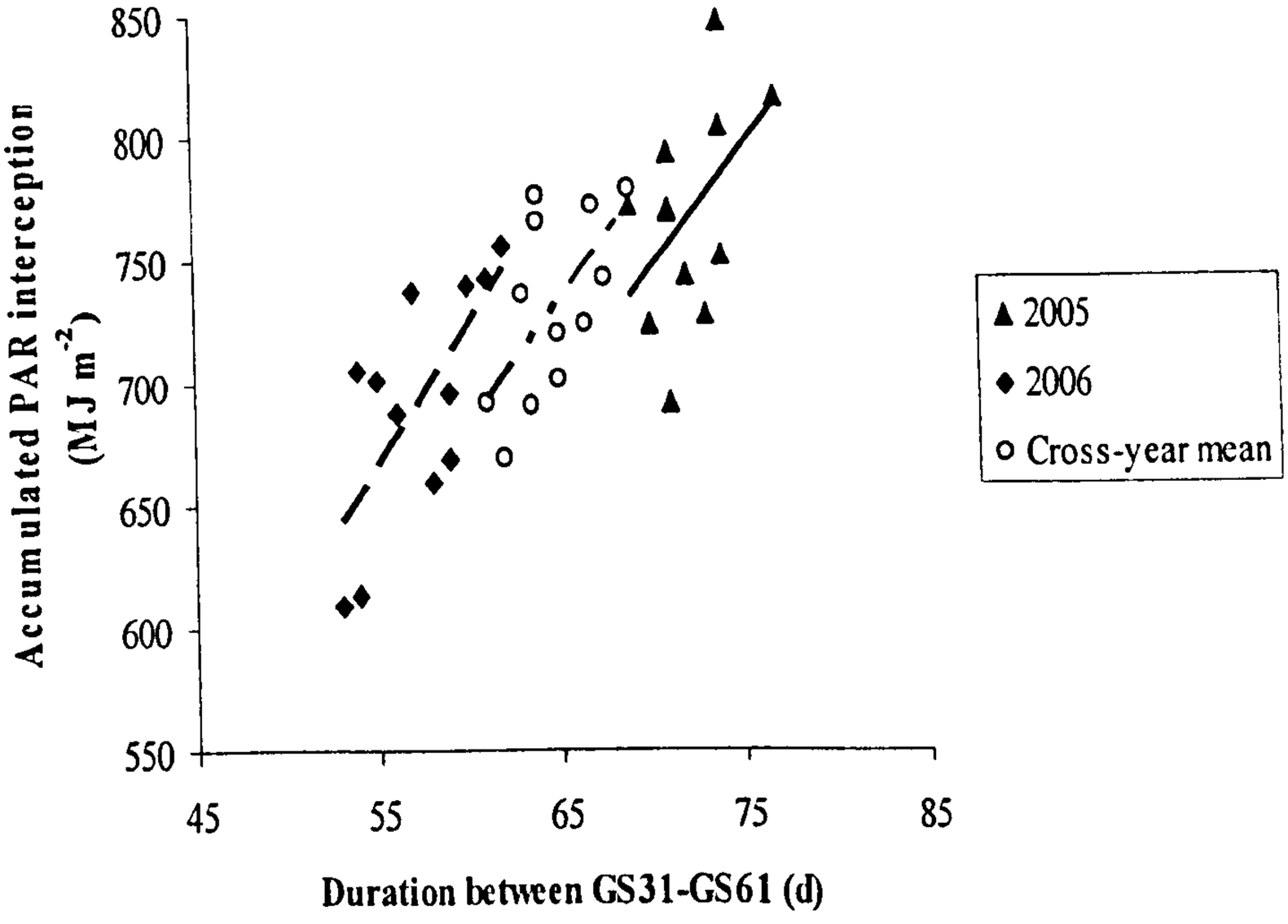


Figure 4.4 Regression of accumulated PAR intercepted between GS31 and GS61 on the duration between GS31 and GS61 in 2005 and 2006 and for the cross-year mean. 2005: y = 9.69x + 67.841; R^2=0.24; 2006: y = 11.414x + 38.298; R^2= 0.49; Cross-year mean: y = 9.98x + 83.358; R^2= 0.39

4.3.3.5 Above-ground biomass and ear DM partitioning

4.3.3.6 Biomass accumulation

The twelve genotypes differed in anthesis biomass, accumulated biomass during GS31-GS61 and ear index (ratio of ear DM to AGDM) at anthesis in 2005 and 2006 ($P \leq 0.05$; Table 4.10). Differences in ear DM m^{-2} observed in 2005 ($P<0.001$) but not in 2004 or 2006. Genotypes produced greater biomass between GS31 and GS61 in 2005 compared to 2006 ($P<0.001$). This could have been due to the longer period between GS31 and GS61 in 2005. Cross-year ANOVA for 2005 and 2006 showed differences in biomass accumulation between GS31 and GS61, anthesis AGDM, ear index and ear biomass among genotypes ($P<0.05$). There was a transgressive segregation in anthesis biomass. For example lines 26, 1 and 13 produced greater biomass than Rialto and Line 21 produced lower biomass than Spark. A linear relationship existed between grains m^{-2} and both ear index ($P \leq 0.05$; Figure 4.5) and ear DM m^{-2} at anthesis ($P \leq 0.05$; R^2=0.56, 0.63 and 0.53 in 2004, 2005 and 2006, respectively). In 2004, the above-mentioned

relationships were consistent with these relationships for the mean values across 2005 and 2006.

Genotypes differed in grain to ear DM ratio in 2005 and 2006 ($P<0.001$) but not in 2004 (Table 4.10). Cross-year ANOVA for 2005 and 2006 showed differences among genotypes in the range 81.3 (Line 12) to 114.2 (Line 3). Genotypes in 2006 had higher grain to ear DM ratio than 2005.

In 2005, 1BL/1RS did not significantly affect GAI at GS61 ($P>0.05$; Table 4.11), but increased AGDM by 121g m^{-2} ($P\leq0.05$). 1BL/1RS increased DM accumulation between GS31- GS61 and between GS39-GS61 by 90 ($P=0.058$) and 86 g m^{-2} respectively ($P=0.053$) and also increased ear DM m^{-2} by 25.3g m^{-2}. However, *Rht-D1b* did not have a significant effect on biomass accumulation and ear index (Table 4.11). However, in 2006, 1BL/1RS had a neutral effect on AGDM accumulation, stem BM, ear DW m^{-2} and ear index. The *Rht-D1b* allele showed increased AGDM by 42 g m^{-2} ($P=0.026$) and ear index by 0.01 ($P=0.007$) and decreased ear DM m^{-2} by 4 g m^{-2} ($P<0.001$) in 2006. Furthermore, the semi-dwarfing allele showed a neutral effect on the rest of the traits. Although there was significant effect in individual years, Cross-year analysis of variance showed a neutral effect for both 1BL/1RS and *Rht-D1b* on AGDM and ear index.

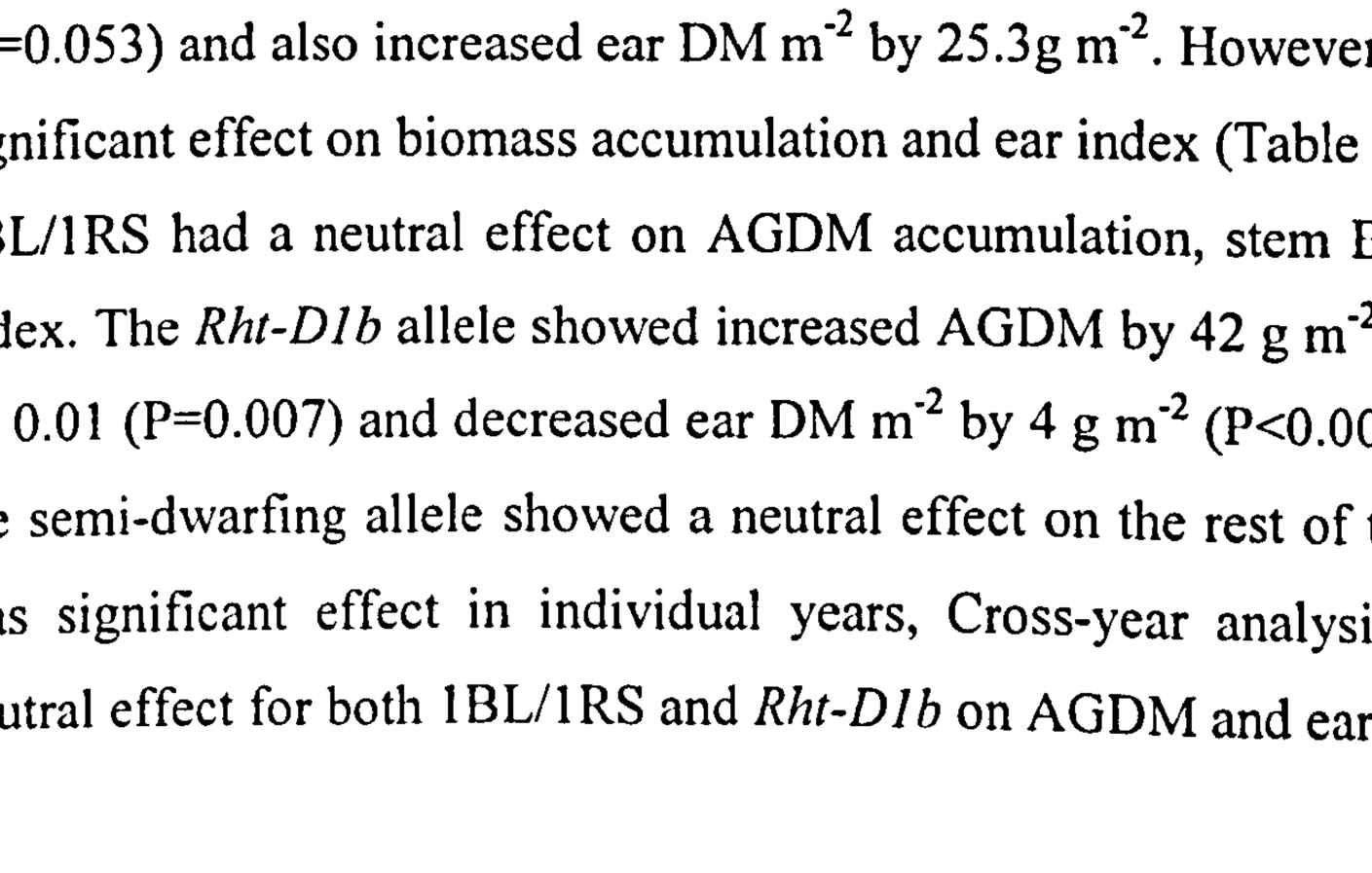


Figure 4.5 Regression of number of grains m^{-2} at harvest on ear index at anthesis in 2004, 2005 and 2006. 2004: y=113357x +2177, R^2 =0.43; 2005: y=100784x +7572, R^2 =0.73; 2006: y = 87028x + 6630.9, R^2 = 0.35

Table 4.10 Biomass accumulation and ear partitioning for the ten DH lines of the Rialto x Spark population and the two parents in 2004, 2005 and 2006

	Biomass accumulation ($g\ m^{-2}$) GS31-GS61			Above ground DM at GS61			Ear DM m^{-2} (ear biomass)				Ear index at GS61				Grains to ear DM ratio			
Genotype	2004	2005	2006	2004	2005	2006	2004	2005	2006	2005-6 mean	2004	2005	2006	2005-6 mean	2004	2005	2006	2005-6 mean
Line 1	1112	1562	1060	1367	1771	1118	218.1	290 7	188.4	239.6	0.16	0.16	0.15	0 16	87.6	64.2	126.2	95.2
Line 3	1268	1286	1038	1503	1485	1030	209.9	207.6	198.2	202.9	0.15	0.14	0.17	0.16	102	106.4	122	114.2
Line 4	1311	1224	1195	1497	1419	1165	217.0	140 6	216.0	178 3	0.13	0.10	0 16	0 13	60	108.8	103.6	106.2
Line 11	1232	1318	1105	1345	1487	1056	246 9	191.5	190 9	191.2	0.17	0.13	0 16	0.14	61.4	86.6	122.5	104.6
Line 12	1039	1312	883	1192	1584	1099	180.9	278.2	205 9	242.0	0 13	0.18	0 19	0.18	110	63 4	99 2	81.3
Line 13	1518	1320	968	1766	1649	1121	256.8	283 6	232 2	257.9	0.15	0.17	0.20	0.19	88 1	73 7	128.3	101
Line 15	1138	1245	954	1301	1446	1270	198 6	204 7	188.3	196.5	0.14	0.14	0.17	0 16	98.5	77.4	107	92.2
Line 17	1225	1311	835	1477	1438	1219	255 6	175 3	199 3	187 3	0.17	0.12	0.18	0 15	76.3	105.2	99.6	102.4
Line 21	1186	1051	904	1439	1250	1201	286 7	205 5	198 5	202.0	0.20	0.16	0 20	0 18	87 1	116 5	96.8	106.7
Line 26	1425	1488	930	1609	1720	1246	221.6	241.5	196.6	219.0	0.15	0.14	0.17	0.16	120.5	101 8	102.4	102.1
Rialto	1274	1330	853	1456	1510	1071	180.5	194.2	179.3	186.8	0.15	0 13	0.18	0.15	117 1	95.8	127.3	111.6
Spark	1130	1192	832	1348	1457	1056	294.6	241.0	202 0	221.5	0.14	0.17	0.20	0.18	67.8	86.9	137.2	112
Mean	1238	1303	963	1442	1518	1138	230 6	221.2	199 6	210.4	0.15	0 15	0.18	0.18	89.7	90.6	114.3	102.5
SED (df)																		
Year										7.90 (46)				0.0049 (40)				2.77 (46)
Genotype	281.1 (14)	117.5 (22)	62.2 (16)	299.9 (14)	106.0 (22)	50.2 (16)	40.9 (15)	16 02 (22)	35 0 (22)	19 35 (46)	0 0323 (12)	0 0096 (22)	0.0138 (16)	0.0121 (40)	22.65 (15)	10.17 (22)	9.24 (22)	6.79 (46)
Interaction										27.36 (46)				0.0171 (40)				9.60 (46)

Table 4.11 Effect of 1BL/1RS translocation and *Rht-D1b* semi-dwarfing gene on above ground dry matter, dry matter accumulation between GS31-GS61, ear dry weight m^{-2} in 2005 and 2006

Gene	Biomass accumulation ($g\ m^{-2}$) GS31-GS61			AGDM ($g\ m^{-2}$) at GS61			Ear DM ($g\ m^{-2}$) at GS61			Ear index		
	2005	2006	Mean	2005	2006	Mean	2005	2006	Mean	2005	2006	Mean
1BL/1RS	1354	975	1165	1585	1095	1340	235 0	200.5	217.8	0 15	0 17	0 16
1B	1264	986	1125	1464	1162	1313	209.7	198 7	204.2	0.14	0 18	0 16
Mean	1309	980	1145	1524	1128	1326	222.4	199.6	211.0	0.15	0.18	0.16
P value												
1BL/1RS	0.058	0.13	0 19	0.01	0.066	0.52	0.002	0.15	0.12	0.23	0.36	0.57
Interaction			0.18			0 008			0.18			0.70
Rht-D1b	1315	967	1141	1511	1144	1328	220 2	197 6	208.9	0 15	0.18	0.17
Rht-D1a	1292	992	1142	1526	1102	1314	222 2	201.6	211 9	0.14	0 17	0 16
Mean	1303	980	1142	1518	1123	1321	221.2	199.6	210.4	0 15	0 18	0 17
P value												
Rht-D1b	0.82	0 26	0.44	0.95	0 026	0.62	0 99	<0 00 1	0 70	0.68	0.007	0.57
Interaction		0 98		0.33			0 90				0 82	

4.3.3.7 Stem biomass accumulation

Genotypes accumulated more biomass in stems and leaf sheath in 2005 compared to 2004 and 2006 ($P<0.001$) (Table 4.12). An analysis of variance across years showed a trend for differences among genotypes ($P=0.072$). Moreover, there was a significant interaction between year and genotype ($P<0.001$).

The 6 1BL/1RS genotypes did not differ significantly when they were analysed separately from non-1BL/1RS genotypes in 2005 ($P=0.23$; S.E.D= 70.8; 10 df) and 2006 ($P=0.58$; S.E.D= 46.7, 9df). They accumulated 797 g m^{-2} compared to non-1BL/1RS genotypes (763 g m^{-2}) in 2005 ($P= 0.007$; S.E.D = 52.7, 9 df). These differences between the two groups show the importance of 1BL/1RS for biomass accumulation. The 6 semi-dwarf genotypes (*Rht-D1b*) differed significantly in the amount of biomass partitioned to

their stems in 2005 but not in 2006. They accumulated 768 g m^{-2} (P=0.048, S.E.D = 65.9; 9 df) compared to tall genotypes (790 g m^{-2}) (P= 0.06; S.E.D = 52.5; 10df).

Table 4.12 Stem and leaf sheath biomass accumulation for the ten DH lines of the Rialto x Spark population and the two parents in 2004, 2005 and 2006

	Stem biomass accumulation (g m^{-2}) (GS31-GS61)			
Genotype	2004	2005	2006	Mean
Line 1	498	898	676	691
Line 3	556	758	614	643
Line 4	688	710	712	704
Line 11	504	710	734	649
Line 12	525	829	525	626
Line 13	694	827	600	707
Line 15	627	775	588	663
Line 17	584	847	510	647
Line 21	622	641	527	597
Line 26	559	891	450	634
Rialto	504	765	530	600
Spark	953	701	512	722
Mean	610	779	582	657
SED(df)				
Year				21 76 (60)
Genotype	119.0 (14)	62.8 (21)	50 0 (21)	43.53 (60)
Interaction				75.39 (60)

4.3.3.8 Dry matter in non-surviving tillers

The proportion of dry matter of non-surviving shoots (N-S) was 6.2% at GS61 compared with 0.06% at GS31 in 2005, indicating that the N-S were much smaller in size compared to surviving shoots. Line 11 was potentially the most wasteful genotype whereas Line 21 was the least wasteful. No correlation was found between the number of N-S taken as the difference between shoot number at GS61 and GS31 and dry matter in non-surviving shoots at GS61. This indicated that the timing of tiller death was more important than the number of non-surviving tillers.

4.3.4 Pre-anthesis RUE

There were significant differences among genotypes in RUE in each of 2004, 2005 and 2006 ($P<0.05$; Table 4.13). RUE values in 2004 ranged from 1.66 g MJ^{-1} for Rialto to 2.77 g MJ^{-1} for Line 26. Higher values of RUE in 2004 than in 2005 and 2006 were probably related to overestimate GAI at GS61 in this year based on individual shoot samples (section 4.1.2.2). Cross-year analysis of variance for 2005 and 2006 showed genotypic differences ($P=0.014$) in the range 1.31 (Line 12) to 1.85 g MJ^{-1} (Line 26). The two parents contrasted in RUE. Moreover, RUE was higher in 2005 than 2006 ($P<0.001$). Furthermore, all genotypes had greater RUE in 2005 compared to 2006 except for Line 17 ($P=0.003$). A trend for linear relationship between grains m^{-2} and pre-anthesis RUE among the six genotypes in 2004 was found (Figure 4.3; $P=0.099$) and among the twelve genotypes in 2005 but was not significant in 2006 ($P<0.05$; Figure 4.6). However, for the mean values across 2005 and 2006 for the twelve genotypes there was a linear relationship between RUE and grains m^{-2} ($P<0.05$; $R^2=0.57$).

1BL/1RS increased RUE in 2005 by 0.15 g MJ^{-1} ($P=0.03$; Table 4.17) but not in 2006. Cross-year analysis showed a non-significant increase in RUE by 0.06 g MJ^{-1}. Furthermore, there was an interaction between 1BL/1RS and year.

4.3.4.1 Specific leaf weight (SLW)

Genotypic differences were shown in SLW at GS39 among the 6 genotypes in 2004 and the 12 genotypes in 2005 and 2006 ($P\leq0.05$; Table 4.14). SLW values ranged from 48 g m^{-2} for Line 15 to 76 g m^{-2} for Line 12 in 2004. Cross-years analysis of variance for 2005 and 2006 showed genotypic differences in the range from 65 (Line 4) to 83 g m^{-2} (Lines 26 and 12). Specific leaf weight was higher in 2005 compared to 2006 ($P<0.001$). Specific leaf weight was positively affected by 1BL/1RS in the three years ($P<0.05$; Table 4.15). Cross-year ANOVA showed that SLW was increased by 1BL/1RS by 5 g m^{-2} ($P<0.001$). A linear relationship was found in the regression between SLW and RUE for the six 1BL/1RS-genotypes in 2005 ($P=0.018$; $R^2=0.79$).

Table 4.13 Pre-anthesis RUE (g MJ^{-1}) for the ten DH lines of the Rialto x Spark population and the two parents in 2004, 2005 and 2006

Genotype	RUE (g MJ^{-1}) 2004	2005	2006	2005-6 mean
Line 1	-	1.98	1.24	1.61
Line 3	-	1.66	1.48	1.57
Line 4	2.17	1.67	1.52	1.60
Line 11	-	1.63	1.40	1.52
Line 12	1.82	1.37	1.24	1.31
Line 13	-	1.81	1.44	1.63
Line 15	2.48	1.57	1.46	1.52
Line 17	-	1.46	1.48	1.47
Line 21	-	1.87	1.47	1.67
Line 26	2.77	2.11	1.59	1.85
Rialto	1.66	1.62	1.25	1.44
Spark	2.58	1.70	1.40	1.55
Mean	2.25	1.71	1.42	1.56
SED (df)				
Year				0.0407 (40)
Genotype	0.284 (10)	0.160 (22)	0.0834 (16)	0.0996 (40)
Year x genotype				0.1409 (40)

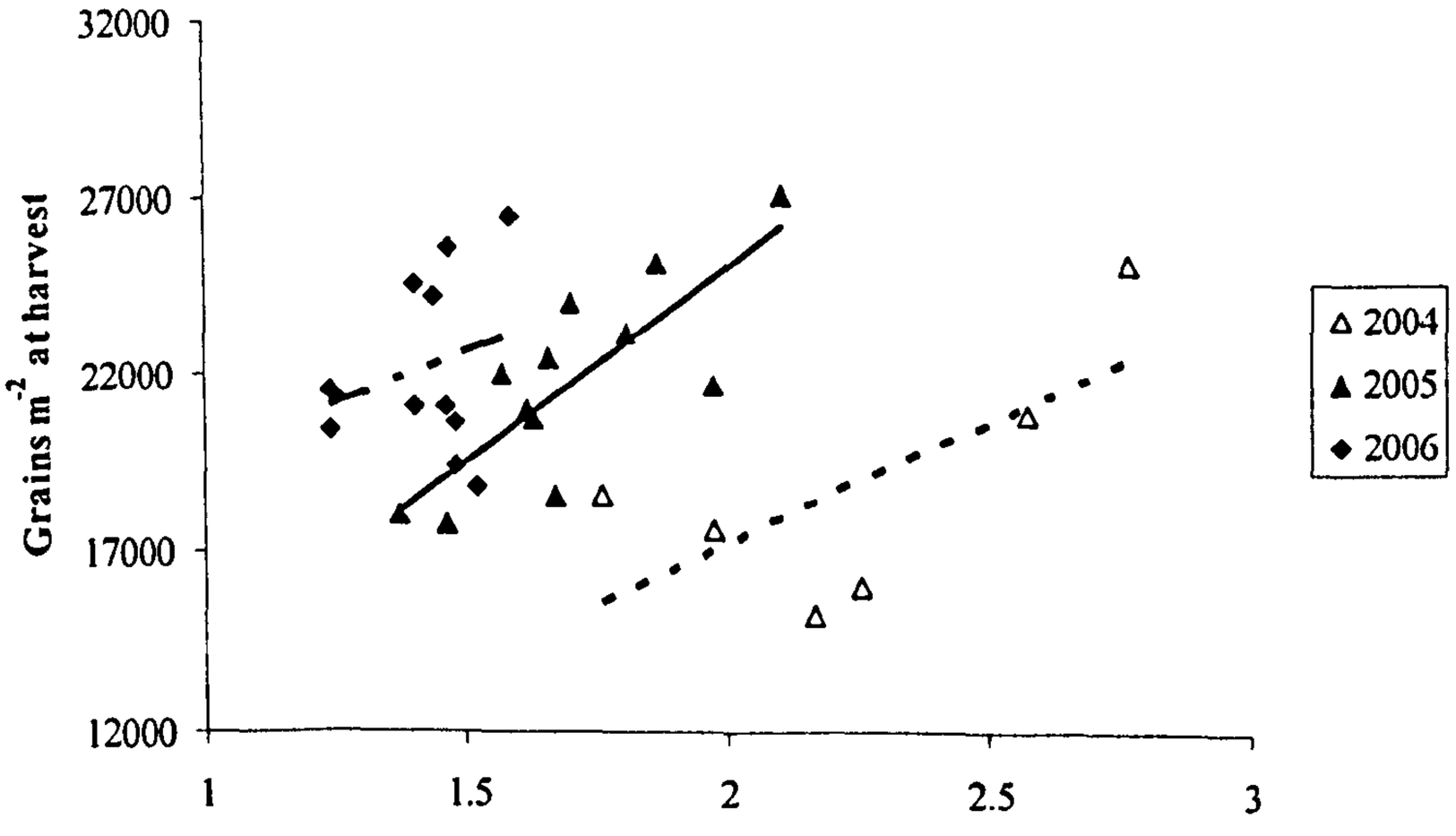


Figure 4.6 Regression of number of grains m^{-2} at harvest on RUE for the GS31-GS61 period (g MJ^{-1}) in 2004, 2005 and 2006 seasons. 2004: y=6824x +3599, R^2 =0.47; 2005: y=10978x +3141, R^2 =0.64; 2006: y = 6007.9x + 13655, R^2 = 0.10; 2005 and 2006 mean: y = 15134x - 1623.9, R^2 = 0.57

Table 4.14 Specific leaf weight (SLW; g m^{-2}) and leaf greenness (SPAD value) for the ten DH lines of the Rialto x Spark population and the two parents in 2004, 2005 and 2006

Genotype	SLW				SPAD		
	2004	2005	2006	2005-6 mean	2004	2005	Mean
Line 1	-	78	66	71	46.9	51.9	49.4
Line 3	-	79	73	76	48.0	53 1	50.6
Line 4	59	69	62	65	48.1	52.7	50.4
Line 11	-	74	65	69	47.4	52.0	49.7
Line 12	76	75	74	75	48.4	52.8	49.6
Line 13	-	92	72	82	48 9	52.9	50.9
Line 15	48	84	73	79	49.7	52.5	51.1
Line 17	-	73	63	68	47.1	51 9	49.5
Line 21	-	74	69	72	46.7	51.7	49.2
Line 26	72	97	68	83	47 9	53 1	50.5
Rialto	61	82	72	77	49.4	51.8	50.6
Spark	57	70	63	67	47 0	52 6	49 8
Mean	62	79	68	74	48.0	52.2	50.1
SED (df)							
Year				1.281 (46)			0.472 (46
Genotype	5 51 (5)	5 25 (22)	2 796 (22)	3.137 (46)	1 73 (22)	1.57 (22)	1 16 (46)
Year x genotype				4.44 (46)			1 64 (46)

The *Rht-D1b* semi-dwarfing allele did not affect pre-anthesis RUE in either year (Table 4.15). Cross- year analysis of variance for 2005 and 2006 showed no effect of *Rht-D1b* allele. Moreover, there was no interaction between *Rht-D1b* and year. The *Rht-D1b* allele positively affected SLW in 2005 (P=0.068) and 2006 (P=0.006). Cross-year analysis showed a trend (P=0.053) for SLW to be increased by 4 g m^{-2} as a result of the presence of the semi-dwarfing allele.

4.3.5 Leaf greenness

No significant differences existed among genotypes in 2004 and 2005 (P>0.05; Table 4.14). Leaf greenness was higher in 2005 compared to 2004 for all genotypes. This could have been due to the production of more shoots per m^2 in 2004 resulting in a larger green area, and thinner leaves containing less chlorophyll.

Table 4.15 Effect of the 1BL/1RS translocation and *Rht-D1b* semi-dwarfing allele on radiation use efficiency and specific leaf weight in 2005

	RUE ($g\ MJ^{-1}$)			SLW ($g\ m^{-2}$)			
	2005	2006	Mean	2004	2005	2006	Mean
Gene							
1BL/1RS	1.78	1.39	1.59	71	81	71	76
1B	1.63	1.43	1.53	56	75	66	71
Mean	1.71	1.41	1.56	63.5	79	68	74
P value							
1BL/1RS	0.03	0.64	0.13	0.003	<0.001	<0 001	<0.001
Interaction			0.025				0.64
Rht-D1b	1.69	1.42	1.55	63	81	70	76
Rht-D1a	1.72	1.39	1.56	62	77	66	72
Mean	1.71	1.41	1.56	62.5	79	68	74
P value							
Rht-D1b	0.60	0 17	0.97	0.31	0 068	0 006	0 053
Interaction			0.37				0 49

4.3.6 Crop height

Lines were on average 4 cm taller in 2005 than 2004 (Table 4.16). Cross-year analysis of variance showed genotypic differences in the range 78 (Line 26) to 92 cm (Line 1) ($P<0.001$).

Crop height was significantly affected by the presence of 1BL/1RS translocation and *Rht-D1b* semi-dwarfing allele in both years ($P<0.001$). 1BL/1RS decreased the crop height by 5 cm in both years (i.e. the height reduced from 86 to 81cm in 2004 and from 90 to 85 cm in 2005. Whereas, *Rht-D1b* reduced the crop height by 6 cm (86-80cm) and 9 cm (92-83 cm), respectively.

Table 4.16 Crop height (cm) for the ten DH lines of the Rialto x Spark population and the two parents in 2004 and 2005

	Crop height (cm)		
	2004	2005	Mean
Line 1	89	94	92
Line 3	80	89	85
Line 4	87	94	91
Line 11	81	78	80
Line 12	88	94	91
Line 13	78	84	81
Line 15	86	88	87
Line 17	85	88	87
Line 21	81	84	83
Line 26	76	79	78
Rialto	79	85	82
Spark	86	92	89
Mean	83	87	85
SED(df)			
Year			0.613 (46)
Genotype	2.365 (22)	1.544 (22)	1.502 (46)
Year x genotype			2.124 (46)

4.4 Discussion

The experimental results presented in this chapter relate to the investigation of crop growth and development during the pre-anthesis period for ten doubled-haploid lines derived from the Rialto x Spark cross and their parents. The discussion will revolve around mechanisms underlying the genotypic differences in grains m^{-2} considering the final ear number m^{-2} and floret fertility/survival determining the number of grains per spikelet. Following this, the physiological basis of grains m^{-2} (sink strength) will be discussed in terms of developmental rates, green area production, radiation capture, RUE and dry matter partitioning pre-anthesis. A detailed account of the effects of the 1BL/1RS rye translocation and the *Rht-D1b* semi-dwarfing allele on pre-anthesis AGDM and underlying physiological traits in the UK's environment will be presented.

4.4.1 Components of yield

A numerical approach to understanding genetic determination of grains m^{-2} (and yield potential) is first outlined in terms of the genetic variation in ears m^{-2}, spikelets per ear and grains per ear. The present study showed a linear relationship between grains m^{-2} at harvest and ear biomass per unit area as well as the ear index at anthesis, confirming that ear growth pre-anthesis strongly influences the final grain number. Such a relationship in spring wheat is supported by Slafer *et al.* (1990) who found that ear dry weight m^{-2} at anthesis accounts for the majority of the genetic differences in grains m^{-2}. In the present study, final ear number per unit area was primarily related to the tiller production rather than tiller survival i.e. greater shoot production was not entirely counteracted by effects of greater shoot death, so that differences established between genotypes earlier in the season in ears m^{-2} were still evident at flowering.

4.4.1.1 Floret fertility/survival and ear growth

In two out of the three seasons, Rialto had a longer duration between GS39 and GS61 by 1 d compared to Spark. Such a small difference could not account for the greater floret fertility for Rialto compared to Spark. Rather, the period between GS31 and GS61 (stem elongation period) which was longer by 8 days for Rialto than Spark, together with the higher assimilate partitioning to the ears as a result of the presence of *Rht-D1b* semi

dwarf allele, probably explained why Rialto produced heavier ears and more fertile florets per ear than Spark. In addtion to this, Rialto produced fewer ears per unit area. Numerous studies have revealed that semi-dwarf wheat genotypes generate heavier ears at anthesis, and thus more fertile florets per ear due to more partitioning of assimilates toward ears (Fischer, 1985; Miralles and Slafer 1995b; Miralles *et al.*, 1998b; Gonzalez, *et al.*, 2003).

The higher number of fertile florets per spikelet at anthesis in central and apical positions in Rialto compared to Spark probably related to the higher dry weight per spikelet for Rialto. Thus florets in distal positions may have received more assimilate enabling them to develop to competent florets.

The ten lines and their parents in 2004, 2005 and 2006 showed that ear index and ear biomass at anthesis was correlated with grains m^{-2} at harvest. Relevant literature argues that the heavier the ears, the more fertile florets or grains per ear. For example, the number of grains per spikelet at maturity depends largely on the amount of assimilates allocated to the ears when the crops are shaded during the ear growth phase (Thorne and Wood, 1987; Slafer, 1995). The increase in the spikelet fertility is likely linked with an increase in the survival of more distal florets. This effect of floret survival is normally greater than any effect on the maximum number of floret primordia, e.g. Craufurd and Cartwright (1989) did not detect any photoperiodic effect on the number of floret primordia per spikelet. Instead, the increase in floret fertility (survival) was related to changes in the length of the ear-growth phase, which might be associated with increased partitioning of assimilates to the ear. This coincides with findings of Gonzalez *et al.* (2003) who showed that extending the duration of the late reproductive phase (stem elongation phase) leads to an increase in floret fertility. Because increases in grain number have been largely responsible for genetic yield progress in wheat, then increasing floret fertility (and hence survival) offers a potential mechanism to increase yield. Lines 21 and 26 showed transgressive segregation and produced more grains m^{-2} than Spark. Moreover, grains per ear accounted for 22% of the variation in grains m^{-2} ($P=0.07$). There was a genetic difference in spikelets ear^{-1} amongst the genotypes.

In general, genotypes with a longer stem-elongation period (e.g. Rialto and Line 26) had higher ear DM due to more intercepted radiation during that period. Differences in dry matter partitioning to the ears additionally contributed to differences in ear dry weight at anthesis. This, in turn, resulted in more fertile florets than those with shortened stem elongation period (e.g. Line 3 and Spark). These relationships will now be discussed in more detail.

4.4.2 Shoot and canopy production

The slower developing genotypes, e.g. Spark and Line 1, showed a trend to have greater shoot production than the more rapid developing genotypes, e.g. Rialto. Thus, slower developing genotypes had more time for tiller production. Additionally, the maximum tiller number may be linked to phyllochron. Genotypes with the shorter phyllochrons tend to produce the most tillers. At the onset of stem extension, further development of tiller buds into tillers is suppressed by competition for assimilates from extending stems, which shade the younger tillers and lead to a reduction of red light at the base of the canopy that could decrease tiller production (Skinner and Simmons, 1993; Sparkes *et al.*, 2006).

In the present study, a strong relationship existed for greater shoot production to be associated with greater shoot death. This can be explained by a greater competition between the main shoot and the younger tillers for resources (Friend 1965; Shearman *et al.*, 2005). Younger and smaller tillers (late tillers) were less likely to survive since young tillers rely on the main shoot for resources until they are capable of absorbing enough nitrogen and radiation to support their own growth. Darwinkel (1978) observed that earlier produced tillers had better growth and reproductive behaviour than late-formed tillers. This is due to late-formed tillers being positioned at the base of a well-developed plant, which constrains light interception, resulting in a low carbohydrate production. These tillers are also affected by apical dominance that results in a very short time for growth between initiation and maturity. Thus, though the survival and ear growth of late-formed tillers is poor, they develop more rapidly in a way that restricts the size of the ear.

Line 4 and Spark had more dead and dying shoots at anthesis. This could mean that tillers were dying late when they are larger and were using a large amount of resources. Therefore the amount of resources wasted in their production was potentially more than the other genotypes for which the phase of tiller death occurred relatively earlier. Nonetheless, the exact causes of shoot death across genotypes cannot be identified based on the available data (N, Light or other nutrients) in the present study. At high N availability, large canopies might shade younger tillers resulting in their death. The extent of shading will be smaller when N is limited. Shoot survival, however will also be limited by N availability. Severe shading reduces the amount of light intercepted by the non-surviving shoots (Berry *et al.*, 2003). The greatest number of dying shoots was noticed in the slow developing genotypes (e.g. Spark) since they produced more tillers. These genotypes also tended to have a larger maximum GAI that could have resulted in greater dry matter wastage through increased shading of non-surviving shoots. Greater shading minimises the amount of dry matter that non-surviving shoots may supply to the surviving shoots.

Despite the higher number of dying shoots observed in slower developing genotypes, maximum GAI in 2005 and 2006 were not significantly different amongst the genotypes. This was because the low tillering genotypes compensated by producing greater green area per shoot ($P<0.05$). Calderini *et al.* (1996) reported a decrease in leaf size in wheat varieties containing *Rht-D1b* alleles, which was compensated by a higher SLW (Leaf dry weight per unit green area). Such a trend was also demonstrated in this study that showed an increase in SLW was ascribed to the effect of *Rht-D1b* allele. Furthermore, the present study showed that the 1BL/1RS translocation increased SLW. Such increases in SLW could be an indicator of an increase in photosynthetic tissues per unit leaf area and hence improved RUE (Morgan *et al.*, 1990; Shearman *et al.*, 2005).

The introduction of 1BL/1RS in the late 1980s in UK wheat varieties may have played a role in raising stem reserve capacity and RUE in modern cultivars hence avoiding source limitation in cultivars with improved grain sink size (Shearman *et al.*, 2005). This effect of higher RUE might be partly related to the positive effect of 1BL/1RS on SLW as shown in this study. The improvement of SLW with 1BL/1RS is also consistent with potential mechanisms for improving yield stability, and it may be that 1BL/1RS has

contributed to improved yield stability in the UK in recent years. Indeed, greater yield stability with 1BL/1RS has been widely observed in spring wheat worldwide (Rajaram *et al.*, 1990).

4.4.3 Radiation capture

Present results inform on the extent to which genotype affects the capture of resources and their conversion to dry matter, and the extent to which such differences may explain the differences in grains m^{-2}. The rate at which light is intercepted per unit canopy surface area is described by the constant K_{PAR}, as affected principally by the canopy architecture. Shearman *et al.* (2005) reported a range of K_{PAR} at GS39 of 0.44 to 0.57 for 10 UK winter wheat genotypes. Similarly, Foulkes *et al.* (1998) reported a range of 0.47 to 0.66 for 17 UK winter wheat genotypes and showed that the lax-leafed variety, Avalon, had a higher K_{PAR} (0.61) whilst the erect-leafed variety, Brigadier, had a low value of 0.49. In the present study, stratified measurements of green area and radiation interception showed that Rialto (erect flag leaf) had higher values of K_{PAR} than Spark (also erect flag leaf). So, leaf angle did not explain the current differences in K. This finding corresponds with the results of Foulkes, Sylvester-Bradley, and Scott, (2001) which showed that genotypes with small canopies were predisposed to have higher K through thicker and less transmissible leaves. Indeed, present results showed that Rialto had thicker leaves than Spark. Duncan (1971) stated that crop assimilation rates could be, theoretically, increased by erectophile leaf canopy. This suggestion is supported by several investigations, for example, in wheat under fully irrigated conditions in the UK, more erect leaf lines were linked with up to 11% more biomass and 4% more yield when comparing sister lines in the trait (Innes and Blackwell, 1983). Similarly, a comparison of two barley cultivars in this trait revealed that net photosynthesis measured at different heights within the canopy was more evenly distributed in the more erect leaf cultivar. It also showed that leaf sheath photosynthesis was greater than in the lax leaf canopy (Angus, Jones and Wilson, 1972 ; Reynolds *et al.*, 2000). Nevertheless, a low K value does not necessarily imply more erect leaves, as recurved leaves may also show lower fractional interception per unit area similar to leaves with low N concentration and hence greater transmission of radiation through leaves.

In the context of the present study, development stage did not significantly affect the light extinction coefficient (K) at GS31, GS39 and GS61 indicating that the calculation of extinction coefficient at GS39 gives a good estimation of K for the pre-anthesis period. This indicates that several measurements for this character taken pre-anthesis conferred no advantage over one set of data collected at GS39 (Shearman *et al.*, 2005). Such a result also agrees with the findings of Thorne *et al.* (1988) that indicated that K did not significantly vary with growth stage from the unfolding of the first leaf to ear emergence, at which time it increases, due to radiation interception by the ears. The fact that no differences were found among the genotypes in this study does not preclude the existence of genotypic variation in K_{PAR} in these genotypes. Methodology may have been an issue, since differences between Rialto and Spark were identified using the stratified measurements.

Averaging over 2005 and 2006, accumulated intercepted radiation between GS31 and GS61 differed between genotypes in the range 669 to 781 MJ m^{-2}. These differences were caused principally by differences in the length of the stem-elongation phases between genotypes rather than by differences in canopy architecture or leaf traits associated with K. Indeed, the correlation between the amount of PAR accumulated between GS31-GS61 and the duration of that period in calendar days was significant in 2005 and 2006 (Figure 4.4). Longer stem elongation period in 2005 compared to 2006 and 2004 enabled the crop to intercept more radiation and produce greater biomass in this year. Present results demonstrate that variations in pre-anthesis radiation interception amongst the twelve genotypes in 2005 and 2006 were more governed by differences in the duration of pre-anthesis period than differences in fractional interception.

4.4.4 Biomass production and radiation-use efficiency

The hypothesis that higher RUE pre-anthesis increases above-ground biomass amongst genotypes in the DH population hence grains m^{-2} was tested in the present study. Principles of resource capture show that radiation capture generally closely correlates with biomass production of a crop. Various studies have confirmed these principles and their application in a wide range of cropping systems (Sinclair and Muchow, 1999;

Kemanian, Stockle and Huggins, 2004; Muurinen and Peltonen-Sainio, 2006). Evidence for differences in RUE between genotypes within species is more limited. Muurinen and Peltonen-Sainio (2006) reported pre-anthesis differences in RUE in the range 1.45 to 2.04 g MJ^{-1} for three modern wheat cultivars. Moreover, Shearman *et al.* (2005) reported significant differences in RUE_{PAR} for eight winter wheat cultivars released between 1972 and 1995 to be in the range 2.33 to 2.64 g MJ^{-1}. In the current study, RUE was calculated between GS31-GS61 and the correlation between RUE and AGDM at anthesis was examined to see whether differences in RUE were associated with improved performance. Genetic differences in pre-anthesis RUE were observed in the range 1.31 to 1.85 g MJ^{-1}. Genotypic differences in anthesis AGDM in 2005 and 2006 might result from significant improvement in the efficiency of conversion of solar energy in to AGDM (i.e. improvement in assimilate production per unit of solar energy intercepted by the crop).

RUE was positively correlated with both ear biomass at anthesis and grains m^{-2} in 2004 and in 2005. Such relationships were stronger in 2005, when experimental errors and lodging were not severe. RUE did not correlate with anthesis biomass in 2005 because Line 1 (had high RUE) produce small biomass. When regression was performed without Line 1, a positive linear relationship appeared ($P=0.013$; $R^2= 0.51$). Similarly, there was a trend for relationship between anthesis biomass and RUE in 2006 ($P= 0.085$; $R^2= 0.27$). It is possible that the differences in RUE highlighted in this study could be attributed to differences in partitioning between root and shoot. This is supported by the findings of Foulkes *et al.*, (1998) which revealed a reduction in partitioning to root with date of introduction of UK winter wheat varieties released during 1970s and 1980s. This suggests that modern varieties (such as Rialto and Spark) have a less extensive root system than old cultivars.

With regard to the SLW, significant differences among genotypes in SLW suggest that genotypes with higher SLW (thicker leaves) coupled with higher leaf greenness may contain more chlorophyll. It is also implied that there is an increase in the concentration of the photosynthetic tissue (Morgan, *et el.,* 1990; Shearman *et al.*, 2005). Indeed, SLW showed a trend for linear relationship with RUE in 2005 ($P=0.087$; $R^2= 0.27$). There

were another possible mechanisms for RUE differences such as dark respiration and Rubisco specificity to CO_2.

At anthesis, 1BL/1RS increased above ground dry matter by 0.76 g m^{-2} (in 2005). 1BL/1RS had an effect on dry matter accumulation between GS31 and GS61 and between GS39 and GS61. Since there was no effect of 1BL/1RS on green canopy area, the physiological basis of the biomass increase probably related to greater RUE in 1BL/1RS genotypes compared to non 1BL/1RS genotypes, discounting a decrease in root: shoot partitioning. Ehdaie Whitkus and Waines (2003) showed that root: shoot ratios are largely unaffected by 1BL/1RS. A tendency for higher RUE has been reported for modern UK 1BL/1RS cultivars (e.g. Haven) compared to their 1B predecessors (e.g. Maris Huntsman and Mercia) (Foulkes *et al.*, 2001). Similarly, Shearman *et al.*, (2005) reported indirect evidence for increased RUE in modern UK 1BL/1RS cultivars compared to their earlier 1B predecessors amongst a set of ten examined historic cultivars introduced between 1964 and 1994. Present results suggest that genes on the short arm of 1R of rye may play some role in the reported increase. 1BL/1RS genotypes accumulate more ear weight by anthesis, and a lower proportion of the total biomass accumulated over the pre-anthesis period being allocated to the stems. This was supported by the significant effect of the 1BL/1RS on grains m^{-2} at harvest. As well as 1BL/1RS, genotypes were shown to be part of the trend for increasing RUE. They appear to behave in a predicted manner in the partitioning of pre-anthesis assimilates.

Several researchers (Gale and Youssefian, 1985; Miralles and Slafer, 1995a) have shown that the presence of *Rht-D1b* alleles led to a larger number of grains per ear and per unit area. Similarly, in this study, semi-dwarfing genes resulted in an increased number of grains set per unit biomass at anthesis. Genetic reduction in height due to the action of *Rht-D1b* allele did not affect the crop capacity to intercept incoming radiation. However, semi-dwarfing gene had a neutral effect on RUE.

4.4.5 Summary

A number of features could probably increase ear growth during the later stages of the pre-anthesis period and hence increased yield potential. Certainly in this study, significant differences between genotypes in radiation interception were found to be associated with differences in GS31 to GS61 duration. Furthermore, genetic differences in RUE have been found in this study, resulting in grains m^{-2} increase being associated with increased pre-anthesis biomass (due to improved RUE). In addition, differences in partitioning to the ear which had a positive impact on floret survival were attributed to the semi-dwarf gene. The hypothesis that 1BL/1RS-genotypes have greater RUE than 1B-genotypes was confirmed. Moreover, grain m^{-2} was correlated with ear index at anthesis and accounts for 23% of the variation in grain m^{-2}.

5 Effect of post-anthesis processes on grain yield potential

Determining physiological factors limiting yield is important in improving genetic yield potential. So, it is important to know if grain filling is limited by photosynthate availability (source-limited) or by the capacity of the grains to accommodate the available assimilates (sink-limited). Attempts to identify physiological factors limiting yield must account for source-sink interactions. Manipulations of grain source size by post-anthesis shading (50% radiation reduction) have been used in the present study to investigate source- sink relationships in winter wheat. A 50% radiation restriction reduces total source size and increases the grain sink size to reproductive source ratio.

Both source and sink sizes were calculated using a quantitative model which has been derived to consider post-anthesis radiation capture by green canopy area, pre-anthesis RUE and the contribution of stem carbohydrate reserves as the determinants of the source, and grain number and grain potential size as determinants of the sink size. Potential yield will be the lower of source and sink sizes, and the difference between them will be used to define the extent of source or sink limitation.

Potential grain source size was calculated using the growth analysis data. The estimated potential source size (g m^{-2}) was calculated as:

Source size = (PAR_{INT} x RUE_{PRE}) + WSC_{MAX}.....Equation 5.1

Where, PAR_{INT} = the amount of post-anthesis PAR interception (MJ m^{-2})
RUE_{PRE} = Pre-anthesis RUE (g MJ^{-1}) from GS31 to GS61
WSC_{MAX} = the maximum stem water soluble carbohydrate reserves measured at GS61 + 75 °Cd (g m^{-2})

Pre-anthesis RUE was used because modern cultivars were reported to maintain close the same level of RUE pre-anthesis and during grain-filling (Calderini *et al.*, 1997)
Potential sink size was calculated from the potential individual grain weight (measured at 28d post-anthesis), and the final grain number per unit area at harvest.

Sink size = PGW x GN..... Equation 5.2

Where, PGW = potential grain weight at 0% moisture
GN = grain number m^{-2} at harvest.

The objectives of this chapter were:

1. To characterize physiological processes defining post-anthesis source and sink size and source-sink balance by quantifying components of the model for Rialto, Spark and four DH lines derived from the Rialto x Spark cross.

2. To quantify the inter-relationships between physiological processes occurring during the post-anthesis period that affect potential grain size and grain growth.

3. To identify the underpinning physiological processes determining components of source and sink production and thereby suggest targets for further genetic improvement.

4. To compare yield responses to post-anthesis shading with predicted responses according to the quantitative model in units of dry weight per unit area

Specific hypothesis:

1. Yield potential of modern wheat genotypes is sink-limited by grain number and potential grain weight during grain-filling.

2. There is genetic variation amongst modern wheat genotypes in the extent of sink limitation i.e. some genotypes are closer to source limitation than others.

5.1 Materials and methods

Three field experiments were conducted in 2004, 2005 and 2006. The experimental site, design and treatments as well as genotypes used were described in Chapter 3. Source manipulation by post-anthesis shading was imposed at GS61+ 20 d (for full details see section 3.2.1)

5.1.1 Crop measurements

Harvest parameters (grain yield, grains m^{-2} and MGW) were measured for 25 DH lines and the two parents. For the subset of ten lines and the parents, measurements of grain

yield, harvest AGDM, HI, yield and yield components were carried out in the unshaded and shaded treatments (see Chapter 3). In addition, stem WSC were measured in the unshaded treatment. Yield from shaded sub-plots was measured by quadrat sampling only, but from unshaded sub-plots by quadrat sampling and by combining (machine harvested).

5.1.1.1 Potential individual grain weight

Potential grain weight was measured for four DH lines (Lines 4, 12, 15 and 26) and their parents in both unshaded and shaded sub-plots in 2004 and for ten DH lines (Lines 1, 3, 4, 11, 12, 13, 15, 17, 21, 26) and their parents in unshaded plots and for four lines plus their parents in shaded sub-plots in 2005. At GS61+ 28 days, ten ears were randomly selected per sub-plot and cut below the collar. The ears were immediately wrapped in 'Clingfilm', sealed in a labelled polythene bag, and taken back to the laboratory. In the laboratory, the most proximal grains (G1, G2 and G3) were immediately excised from the central spikelet (Figure 5.1). The most proximal grain was also excised from one basal and one apical spikelet (G4 and G5), respectively (Figure 5.1). The second fertile spikelet from both the top and bottom of the ear were considered as apical and basal spikelets, respectively, while central spikelets were identified as the one located exactly in the middle of the ear. The total number of excised grains per10-ear sample was recorded. Then the fresh weight of the bulked grains was recorded and the grains dried at 80°C for 48 hrs and the dry weight recorded. The average moisture content of the grains from G1 to G5 was taken as an indicator of potential grain weight. The results were then used to calculate the potential maximum grain weight.

Potential mature grain weight (mg) was calculated using the equation developed by Macbeth (1996) from:

$PGW=44.02 + (0.51 \times GWC)-(0.24 \times GNNO)- (0.01 \times S\ m^{-2})$

Where PGW= potential grain weight (mg)

GWC = grain water content (mg)

GNNO = grain number per ear

$S\ m^{-2}$ = shoots m^{-2}

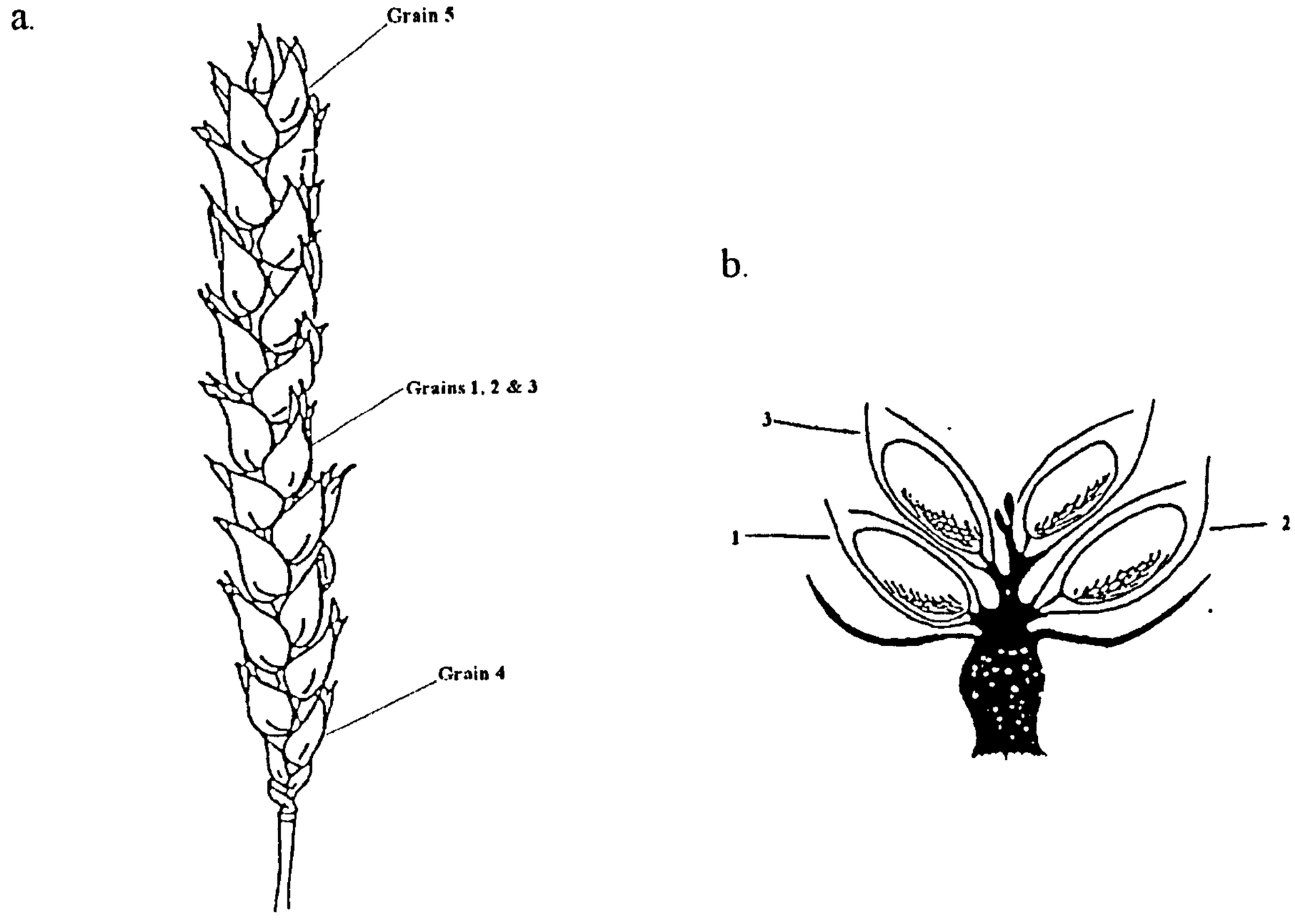


Figure 5.1 diagrammatic representation of an ear to show both the spikelets sampled (a) and a spikelet with six florets (b), two of which are infertile, to show grain positions sampled (adapted from Macbeth, 1996)

5.1.1.2 Green area per shoot

Green area per shoot was measured for the four DH lines and their parents in unshaded and shaded sub-plots. Ten fertile shoots were selected at random avoiding the outer two rows on either side of the sub-plot (to avoid edge effects) from sub-plots at around GS61 + 4 weeks (10 July 2004 and 12 July 2005) and GS61 + 6 weeks (27 July 2004 and 19 July 2005). Shoots were separated into leaf lamina, stems with attached leaf sheaths and ears. The stems (plus attached leaf sheaths) and leaf lamina were separated into green and non-green components. Projected areas of green lamina and green stem plus sheath and all the ears were recorded using a LiCor 3100 Leaf Area Meter (LiCor; Nebraska USA). The percentage of 10 ears that was green was visually assessed.

5.2 Results

This chapter firstly analyses the harvest results in 2004 and 2005. In addition, combine yield and individual grain weight are reported in 2006. Crop growth is characterized in terms of grain yield, yield components, harvest index and above-ground harvest biomass. This will be followed by a description of the physiological processes affecting the development and subsequent filling of grains between anthesis and harvest in 2004 and 2005. The last part of this chapter focuses on source-sink balance, including a quantitative consideration of radiation capture by the green canopy, RUE and accumulation of stem carbohydrate reserves as determinants of the source, and grain number and potential size as determinants of the sink size.

5.2.1 Combine grain yield and individual grain weight

In 2004 and 2005, genotypic differences were found ($P<0.001$) in grain yield, grains m^{-2} and mean grain weight (MGW) amongst the 25 DH lines and their parents. In 2006, the ten genotypes and the two parents also differed in grain yield, grains m^{-2} and MGW. Cross-year analysis of variance for 2004 and 2005 for the 27 genotypes showed that lines 26, 9 and 8 produced the highest yields (10.88, 10.38 and 10.17 t ha^{-1}, respectively) while Lines 1, 14 and 17 produced the lowest yields (7.60, 7.79 and 7.86 t ha^{-1}, respectively). When confidence interval at 95% was applied to test for transgressive segregation amongst the DH lines with respect to the parents, results showed that Lines 7, 9, 21 and 26 consistently produced more grains than Spark. Additionally, Lines 4, 6, 16, 19 and 23 produced heavier grains than Rialto in both years. Cross-year means indicated that Line 8 was the only genotype that consistently showed a positive departure from the overall negative relationship between grain number and MGW ($P<0.001$; $R^2= 0.46$). A significant positive relationship was found between grain yield and grains m^{-2} in both years ($P<0.001$; Figure 5.2). However, grain yield was not correlated with MGW. As expected, grains m^{-2} was negatively correlated with MGW in both years ($P<0.005$; Figure 5.3). Averaging over 2004 and 2005, there was a positive linear relationship between yield and grains m^{-2} ($P<0.001$; $R^2= 0.77$) but not between yield and MGW.

Though the group of 1BL/1RS Lines (11 lines plus Rialto) produced greater yield than the group of 1B Lines (14 lines plus Spark) in 2004 ($P<0.001$), a neutral effect of the translocation was observed in 2005 ($P=0.84$; Table 5.1). The 1BL/1RS group (six genotypes) produced 7.0 t ha^{-1} compared to 6.43 produced by the 1B group ($P<0.001$) in 2006. Cross-year analysis of variance for 2004 and 2005 showed that 1BL/1RS genotypes produced 9.05 t ha^{-1} compared to 8.79 t ha^{-1} by the 1B genotypes ($P=0.014$). The interaction between 1BL/1RS and year was significant ($P=0.007$).

The effect of the 1BL/1RS translocation on grains m^{-2} was not significant in 2004 and 2006, whereas there was a trend in 2005 ($P=0.051$) for more grains for the 1B genotypes (21743 versus 21092). A positive effect of 1BL/1RS on MGW was present in each of the three years ($P<0.05$). Cross-year analysis of variance for 2004 and 2005 showed that MGW improved by 1.12 mg.

In 2004, 2005 (9 lines plus Rialto) and 2006 (5 lines plus Rialto), the *Rht-D1b* semi-dwarf allele had a positive effect on combine grain yield ($P<0.001$; Table 5.1). Averaging across 2004 and 2005, *Rht-D1b* increased grain yield by 1.08 t ha^{-1}, ($P<0.001$) compared to the tall *Rht-D1a* group (16 lines plus Spark). The *Rht-D1b* allele had a positive effect on grains m^{-2} in each of the three years ($P<0.001$; Table 5.1). Cross-year analysis of variance for 2004 and 2005 showed that *Rht-D1b* genotypes produced 1543 grains more than the tall genotypes ($P<0.001$). There was no effect of the semi-dwarf gene on MGW in 2004 and 2006. However, *Rht-D1b* reduced the MGW by 0.79 mg in 2005 ($P<0.001$). Cross-year analysis of variance for 2004 and 2005 indicated a non-significant effect of the semi-dwarf allele on MGW ($P=0.23$).

With regard to the ten DH lines and their parents examined in each of 2004, 2005 and 2006, genotypes differed ($P<0.001$) in combine yield, grains m^{-2} and MGW in 2004, 2005 and 2006 (Table 5.2). Cross-year analysis of variance showed Line 26, Rialto and Line 21 produced greater yields than Lines 1, 17 and 12. Averaging across genotypes, yields were lower in 2006 (6.70 t ha^{-1}) compared to 2005 and 2004 (9.37 and 8.25 t ha^{-1},

respectively) ($P<0.001$). Lower yield in 2006 was related to lower MGW in the same year ($P<0.001$; Table 5.3). This, in turn, was probably associated with higher mean air temperature during July (20.1 °C) compared to the long-term mean (15.4 °C). Higher temperatures in 2006 reduced the duration of the grain filling period and accelerated canopy senescence. As for the 25 genotypes, there was a positive linear relationship among the twelve genotypes between combine yield and grains m^{-2} in 2004 and 2005 ($P<0.001$; $R^2= 0.75$ and 0.47 respectively) but not in 2006. Furthermore, there was a positive linear relationship between combine yield and MGW in 2006 ($P=0.42$; $R^2=0.35$; Figure 5.4) but not in 2004 or 2005.

As expected, there was a negative relationship between grains m^{-2} and MGW in 2004 ($P=0.092$; $R^2=0.26$). However, the negative relationship was stronger in 2005 and in 2006 ($P<0.05$; $R^2=0.62$ and 0.67 respectively; Figure 5.5). Averaged across the three years, there was a positive relationship between grain m^{-2} and grain yield ($P=0.003$; $R^2= 0.60$; Figure 5.6) and a negative relationship between grain m^{-2} and MGW ($P=0.008$; $R^2= 0.52$; Figure 5.5). However, there was no relationship between grain yield and MGW.

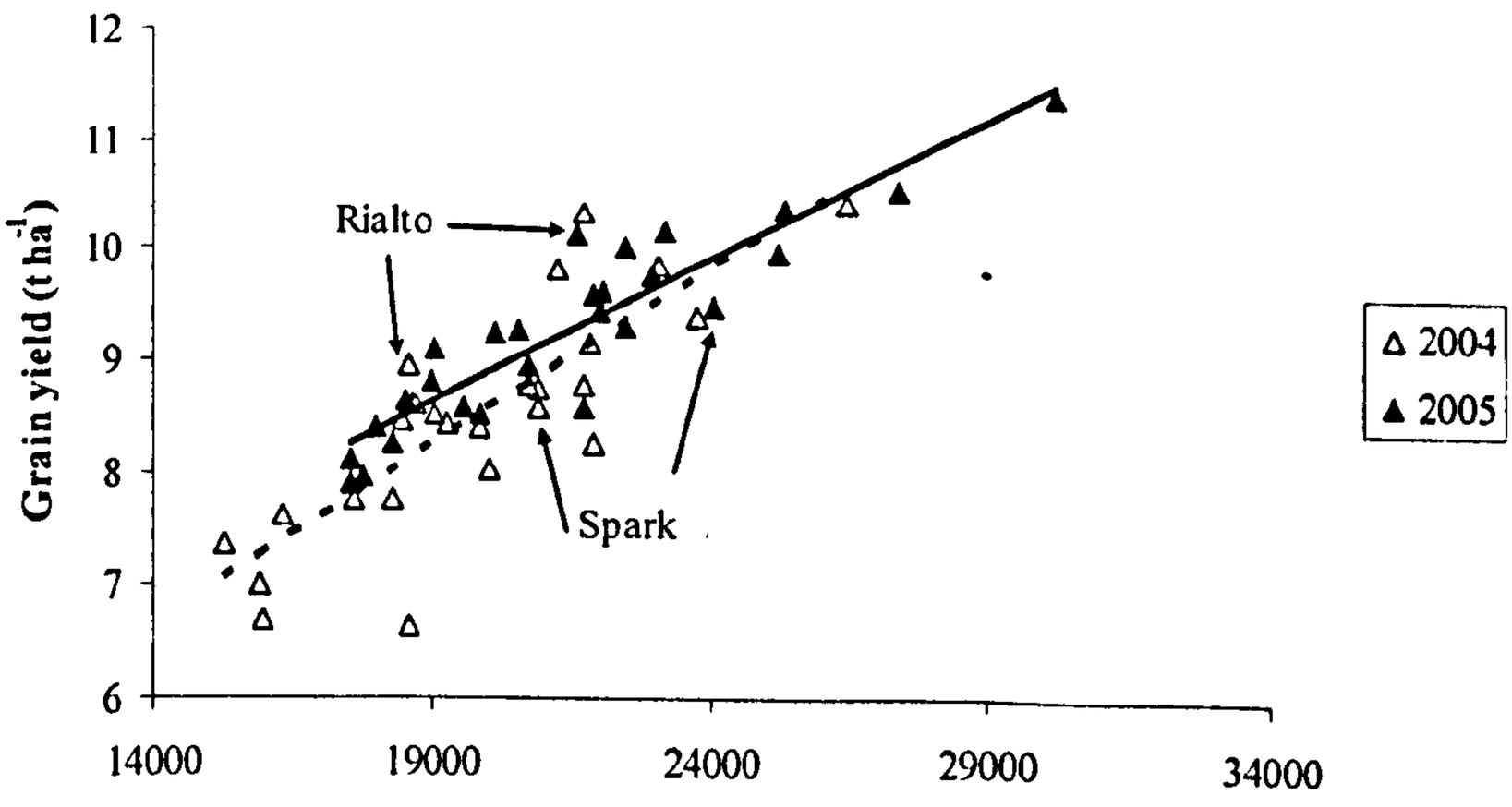


Figure 5.2 Regression of grains m^{-2} on combine grain yield (t ha^{-1}; 85% DM) for 25 lines of Rialto x Spark DH population and their parents in 2004 and 2005. 2004: $y = 0.0003x + 2.2196$; $R^2 =0.71$; 2005: $y = 0.0003x + 3.7742$; $R^2 =0.84$

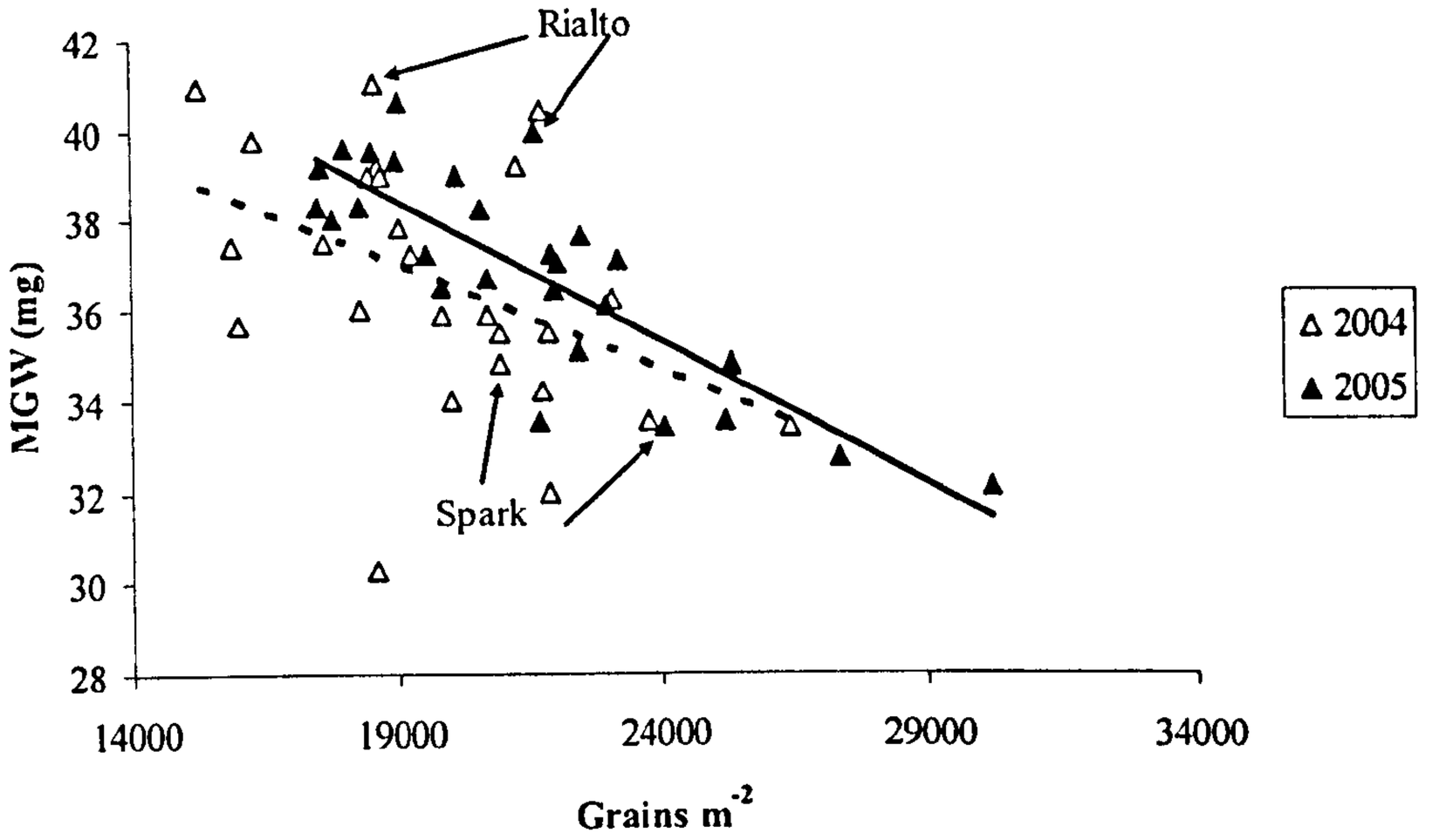


Figure 5.3 Regression of grains m^{-2} on mean grain weight (MGW; mg) for 25 lines of Rialto x Spark DH population and their parents in 2004 and 2005. 2004: $y = -0.0005x + 45.895$; $R^2 = 0.22$; 2005: $y = -0.0006x + 50.398$; $R^2 = 0.68$

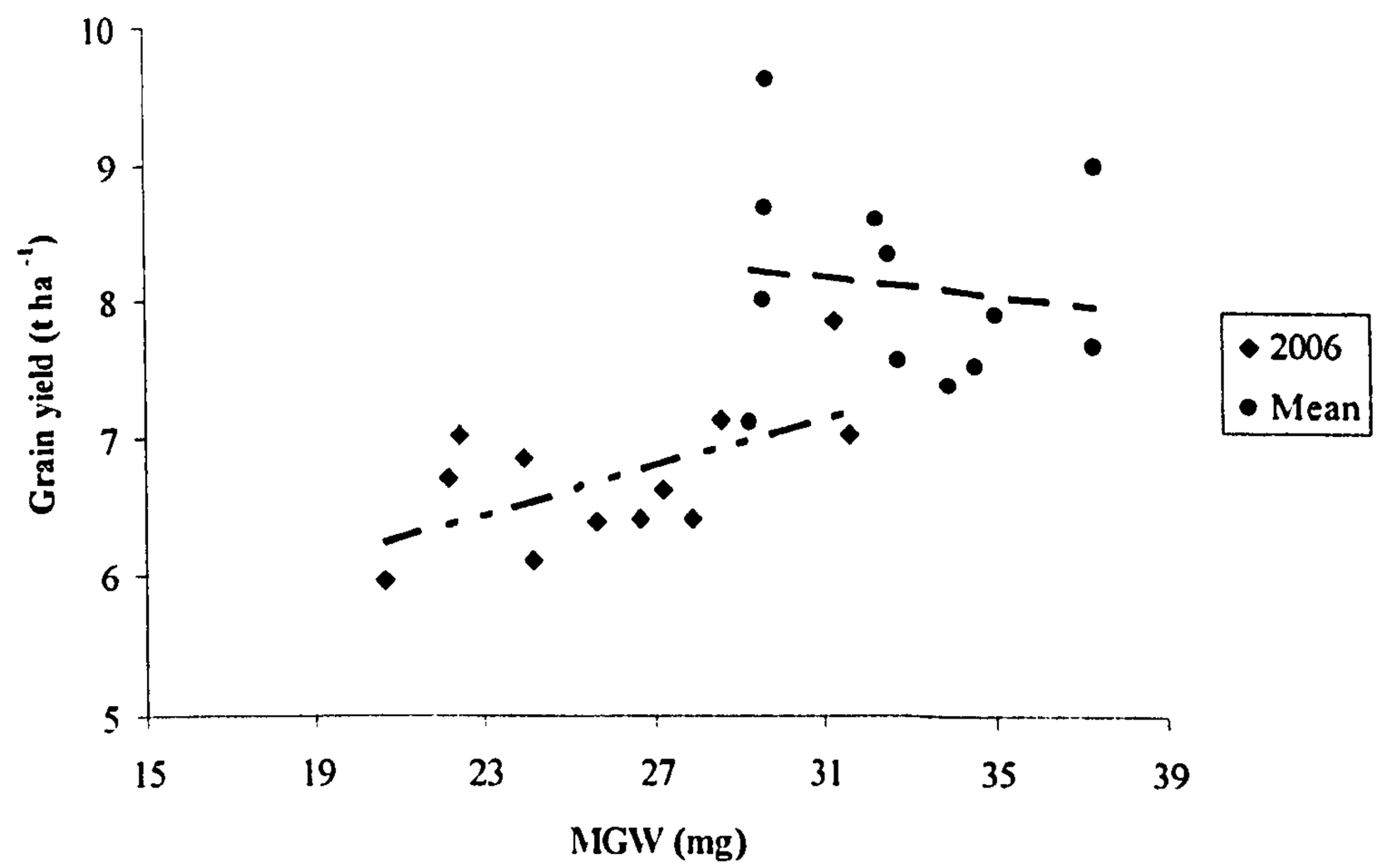


Figure 5.4 Regression of mean grain weight (MGW; mg) on grain yield (t ha^{-1}; 85% DM) for 10 lines of Rialto x Spark DH population and their parents in 2006 and mean across 2004, 2005 and 2006; 2006: $y = 0.0872x + 4.4345$; $R^2 = 0.36$; Mean: $y = -0.0332x + 9.1995$; $R^2 = 0.02$

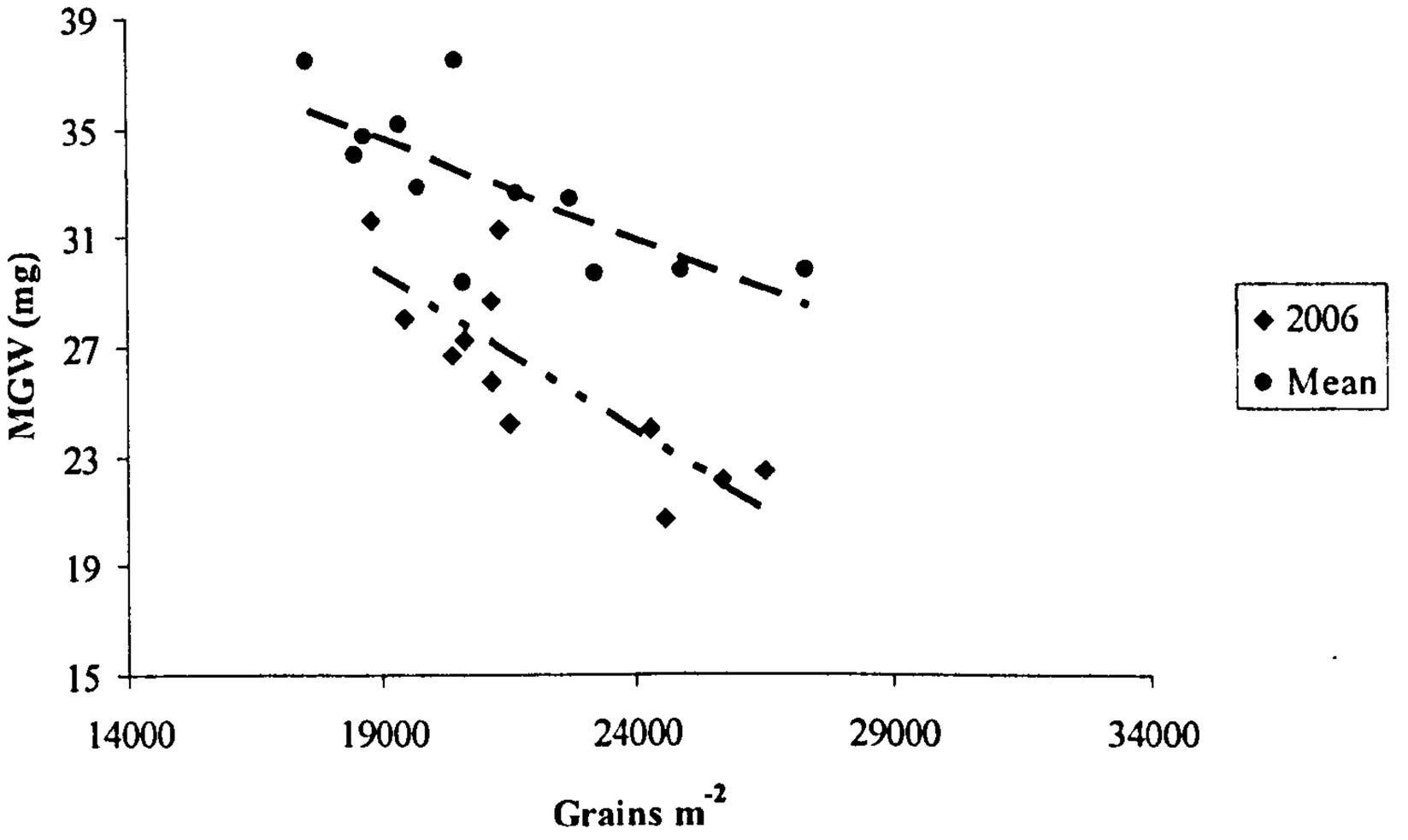


Figure 5.5 Regression of grains m^{-2} on mean grain weight (MGW; mg) for 10 lines of Rialto x Spark DH population and their parents in 2006 and mean across 2004, 2005 and 2006; 2006: $y = -0.0011x + 51.405$; $R^2 = 0.67$; Mean: $y = -0.0007x + 48.372$; $R^2 = 0.52$

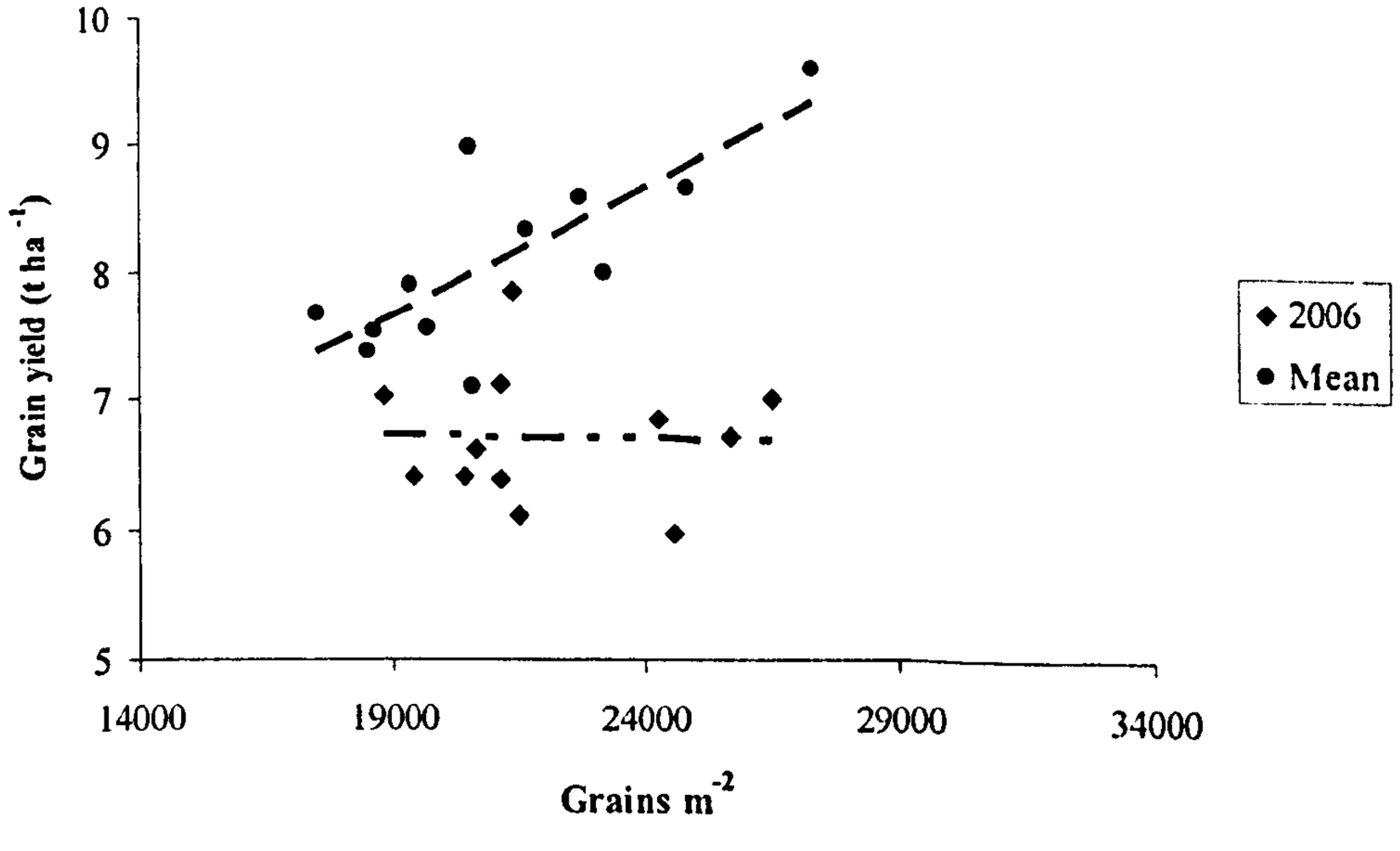


Figure 5.6 Regression of grain m^{-2} on grain yield (t ha^{-1}) for 10 lines of Rialto x Spark DH population and their parents in 2006 and mean across 2004, 2005 and 2006; 2006: $y = -6E\text{-}06x + 6.8351$; $R^2 = 0.01$; Mean: $y = 0.0002x + 3.8861$; $R^2 = 0.60$

Table 5.1 Effect of the 1BL/1RS translocation and the *Rht-D1b* allele on grain yield, grains m^{-2} and mean grain weight (MGW) for 25 lines of Rialto x Spark DH population in 2004, 2005 and 2006

	Yield 15% moisture (t ha^{-1})				Grains m^{-2}				MGW (mg)			
	2004	2005	2006 *	2005-6 mean	2004	2005	2006	2005-6 mean	2004	2005	2006	2005-6 mean
1BL/1RS	8.86	9.26	7.00	9 05	20349	21092	22412	20721	37.19	37.54	26.71	37.36
1B	8 32	9 24	6.43	8.79	19782	21743	21890	20763	35.99	36.49	25.37	36.24
Mean	8.56	9.25	6.70	8.91	20034	21454	22151	20744	36.52	36.96	26.04	36.74
Rht-D1b	9 11	10 06	7.00	9.59	21396	23977	23376	22686	35.34	35.96	25.69	35.65
Rht-D1a	8.24	8.78	6.42	8.51	19233	19970	20925	19601	36.54	37.54	26.38	37.04
Mean	8.68	9.42	6 70	9.05	20315	21974	22151	21144	35.94	36.75	26.07	36.35

* Data in 2006 obtained from the 12 genotypes

5.2.2 Hand-harvested grain yield and yield components

Hand-harvested samplings were carried out in 2004 and 2005 only for ten DH lines and their parents. Genotypes differed in hand-harvested yield in both years ($P<0.05$; Table 5.2). In unshaded conditions, hand-harvested yield was 2% greater than combine yield in 2004. In 2005, however, it was reduced by about 24% compared to combine yield. Averaged across years, hand-harvested yield was lower than the combine yield by 12%, but the genotype rankings were similar (Table 5.2). As for the combine yield, a linear relationship ($P<0.001$) was found between grain yield and grains m^{-2} in both years (R^2 = 0.75 and 0.74 in 2004 and 2005, respectively).

Shading was in 2004 and 2005 imposed on four DH lines (Lines 4, 12, 15 and 26) and their parents at GS61 + 20 d. Shading reduced the hand-harvested yield by 1.78 and 0.41 t ha^{-1} in 2004 and 2005, respectively ($P<0.05$; Table 5.2). Cross-year analysis of variance showed a reduction in yield with shading of 1.10 t ha^{-1} ($P<0.001$), and there was a strong trend for a shading x genotype interaction ($P=0.054$). Lines 26, 15 and Spark were most affected by shading losing 1.44-1.82 t ha^{-1} while Line 4, Line 12 and Rialto were least affected losing 0.37- 0.75 t ha^{-1}.

Genotypic differences in grains m^{-2} were accounted for by variation in both ears m^{-2} and grains ear^{-1} ($P<0.001$; Table 5.3). Regression analysis using mean values across 2004 and 2005 indicated that 21% of the genotypic variation in grains m^{-2} was accounted for by grains ear^{-1} ($P=0.076$). Cross-year analysis showed that Spark and Line 1 produced most ears m^{-2} and Rialto and Line 26 had the most grains ear^{-1} ($P<0.001$). However, Lines 4 and 17 had low values for both ears m^{-2} and grains ear^{-1}. There was a significant effect of year for both ears m^{-2} and grains ear^{-1}($P<0.05$), but the year x genotype interaction was not significant.

Yield components were calculated for the four lines and their parents from hand-harvested data for the shading treatment. As expected, ears m^{-2} was not affected by shading in either year, since shading was imposed after this yield component had been determined. Cross-year analysis of variance showed no shading effect on ears m^{-2}, grains ear^{-1} or grains m^{-2}. However, MGW was reduced by shading in both years ($P<0.05$; Table 5.3) and on average by 2.4 mg ($P=0.006$). There was no shading x genotype interaction in either year.

In the three years in unshaded conditions for the ten lines and their parents, 1BL/1RS had a positive effect on grains ear^{-1} ($P<0.005$) and a negative effect on ears m^{-2} ($P<0.05$; Table 5.4). Averaging across years, 1BL/1RS increased grains ear^{-1} by 4.6 grains and reduced ears m^{-2} by 57 ears m^{-2} ($P<0.005$). Cross-year analysis of variance showed that *Rht-D1b* genotypes produced more grains ear^{-1} and fewer ears m^{-2} compared to tall genotypes ($P<0.05$) (Table 5.4). Grains m^{-2} accounted for 81% and 57% of the variation in grain yield between semi-dwarf and non semi-dwarf genotypes ($P<0.05$) in 2004 and 2005, respectively.

Table 5.2 The effect of post-anthesis shading and genotype on combine and hand-harvested grain yield (t ha^{-1}; 85%DM) for ten lines of Rialto x Spark DH population and the two parents at harvest in 2004 and 2005.

Genotype	Combine yield (t ha^{-1} ;85%DM)				Hand- harvested yield (t ha^{-1} ;85%DM)					
	Unshaded				Unshaded			Shaded		
	2004	2005	2006	Mean	2004	2005	Mean	2004	2005	Mean
Line 1	6.63	8.57	6.11	7 11	5.73	6.15	5.94	-	-	-
Line 3	9.15	9.29	6.61	8.35	10.15	6.65	8.40	-	-	-
Line 4	7.36	8.63	7.02	7.67	7.75	6.13	6.94	6.93	6.22	6 57
Line 11	7.62	8.95	7.12	7 90	7 90	6 93	7.42	-	-	-
Line 12	7.77	8.39	6.41	7 52	7.17	6.92	7.05	6.04	6.72	6.38
Line 13	8.77	10.14	6 85	8.59	8.62	8.03	8.32	-	-	-
Line 15	6 68	9.60	6.39	7.56	7.93	7.80	7 87	5 08	7.60	6 34
Line 17	7.76	7.96	6.40	7 37	8.03	6.59	7 31	-	-	-
Line 21	9.38	9 95	6 69	8.68	9 61	7 26	8 44	-	-	-
Line 26	10 37	11.40	7.01	9.59	9.71	8 62	9 17	7.42	7.27	7.35
Rialto	8.95	10 12	7 86	8.97	9.58	7 01	8.30	8 15	6.94	7.55
Spark	8.58	9.46	5.97	8 00	8.90	6 94	7 92	6 73	6.24	6 48
Mean	8.25	9 37	6 70	8.11	8.42	7.09	7 76	6.72	6 83	6.78
SED (df)										
Year				0 1506 (70)			0.1838 (38)			0 266 (2)
Shade								0 282 (21)	0 257 (14)	0 3428 (4)
Genotype	0.555 (22)	0 565 (22)	0 2619 (22)	0 3013 (70)	0 721 (21)	0 539 (15)	0 4501 (38)	0 488 (21)	0 446 (14)	0 3032 (31)
Year x shade										0 485 (4)
Year x genotype				0 5218 (70)			0 6366 (38)			0 473 (15 44)
Shade x genotype								0 691 (21)	0 630 (14)	0 520 (17 41)
Year x shade x genotype										0 736 (17 41)
1BL/1BR	8.77	9.71			8.86	7 39	8.12			
1B	7.73	9.03			8.01	6.76	7 38			
Mean	8.25	9 37			8.43	7.07	7.75			
P-Value	<0.001	0.007			0 008	<0.001	<0 001			
Rht-D1b	8.63	10 03			8 89	7.59	8.24			
Rht-D1a	7.87	8.72			7.97	6.57	7.27			
Mean	8 25	9.37			8.43	7.08	7.76			
P-Value	0.003	<0.001			0.005	<0.001	<0.001			

Table 5.3 Effect of post-anthesis shading and genotype on ears m^{-2}, grains ear^{-1}, grains m^{-2} and mean grain weight (MGW) for the ten lines of Rialto x Spark DH population and their parents in 2004 and 2005

	Ears m^{-2}		*Grains ear^{-1}*		*Grains m^{-2}*			*MGW (mg)*						
			Hand-harvested					Hand-harvested			Combined-sampled			
	2004	2005	2004	2005	2004	2005	Mean	2004	2005	Mean	2004	2005	2006	Mean
Unshaded														
Line 1	660	807	28.1	26.2	17295	14519	15907	29.6	35.9	32.8	30.3	33 5	24.2	29.3
Line 3	618	549	41.4	41.6	24476	15180	19828	37.0	37.2	37 1	35.5	35.1	27.2	32 6
Line 4	629	418	26.1	32.9	16780	12310	14545	41.6	42.4	42.0	41.0	39 5	31.6	37.4
Line 11	615	686	28.7	31.1	16617	15037	15827	43.6	39.3	41.5	39.8	36.7	28.6	35.0
Line 12	545	477	30.5	33.7	16540	14080	15310	40.0	41.1	40.6	37.5	39.6	26.7	34.6
Line 13	656	682	31.6	34.1	19804	18118	18961	38.8	37.8	38.3	35 9	37.1	24.0	32.3
Line 15	529	453	37.3	41.6	19708	17429	18569	35.8	37.8	36 8	35 7	37 0	25 7	32.8
Line 17	638	631	31.8	28.3	19298	14220	16759	39.2	39.4	39.3	36 0	38.0	28 0	34.0
Line 21	694	689	38.3	37.3	25959	17869	21914	33.9	34.6	34 3	33 5	33.5	22 1	29 7
Line 26	543	520	43.9	45.4	23877	20695	22286	36.1	35.2	35.6	34.8	32.3	22 4	29.8
Rialto	492	356	41.1	42.1	20112	13990	17051	44.3	42 4	43.3	41 0	41 0	31 3	37 8
Spark	826	603	26.3	30.0	21594	16578	19086	36.3	35 6	36 0	34 8	33 4	20 7	29.6
Mean	620	573	33.8	35.4	20194	15867	18030	38.0	38.2	38 1	36 3	36 4	26 0	32 9
Shaded														
Line 4	609	487	24.2	29.6	16464	13746	15105	39.7	37 4	38 56				
Line 12	546	480	28.5	32.1	16724	13472	15098	38 0	38 3	38 2				
Line 15	540	477	33.2	37.3	19110	16905	18008	32 1	36 4	34 3				
Line 26	556	474	41.1	39.4	24914	17082	20998	32 4	32 9	32 7				
Rialto	477	354	37.2	42.4	18627	13574	16101	41.5	39 2	40 4				
Spark	820	609	21.7	25.2	18760	15716	17238	32.4	39 5	36 0				
Mean	591	480	31.0	34 3	19100	15083	17091	36.0	37 3	36 7				
SED (df)														
Year							511.1 (2)			0.395 (2)				0.261 (70)
Shade	11.69 (15)	15 16 (22)	0.750 (12)	1.715 (14)	494.0 (10)	484.5 (18)	241.9 (4)	0.48 3 (20)	0.733 (17)	0.456 (4)				
Genotype	47.07 (22)	58.6 (22)	2.021 (20)	2.752 (15)	1760. 9 (20)	1237. 8 (15)	1152.6 (37)	1.12 1 (20)	0.560 (15)	0 664 (37)	1 128 (18)	0 499 (22)	1 02 (22)	0.522 (70)
Year x shade							565.5 (2.92)			0.645 (4)				
Year x genotype							1630.1 (37)			0.939 (37)				0.905 (70)
Shade x genotype	28.64 (15)	37.12 (22)	1 836 (12)	4.201 (14)	1210. 1 (10)	1186. 8 (18)	750.1 (24)	1.18 4 (20)	1.795 (17)	1.068 (33)				
Year x shade x genotype							1121.4 (22.1)			1.511 (33)				

Table 5.4 Effect of the 1BL/1RS translocation and the *Rht-D1b* allele on ears m^{-}, grains ear^{-1} and mean grain weight (MGW) for 25 lines of Rialto x Spark DH population in 2004, 2005 and 2006

	Ears m^{-2}				Grains ear^{-1}				MGW (mg) Combined sampled		
	2004	2005	2006	Mean	2004	2005	2006	Mean	2004	2005	Mean
1BL/1RS	563	522	593	559	36.2	38.7	39.1	38.0	39.96	38.94	39 45
	647	569	632	616	32.0	34.0	34.4	33.4	35.7	37.98	36.84
1B											
Mean	605	545	613	588	34.1	36.4	36.8	35.7	37.83	38.46	38.15
P value											
1BL/1RS	<0.001	<0.001	0.007	0.048	<0.001	<0.001	0.001	<0.001	<0.001	<0.001	<0.001
1BL/1RS x year				0.003				0.99			<0.001
Rht-D1b	574	525	603	568	36.8	38.4	38.8	38.0	38.74	38.14	38 44
Rht-D1a	636	565	622	608	31.3	34.4	34.7	33.4	37.07	38.73	37.90
Mean	605	545	613	588	34 0	36.4	36.8	35.7	37.91	38.44	38.17
P value											
Rht-D1b	0.001	<0 001	0.89	0.009	<0 001	<0.001	0.004	<0.001	0 005	0.004	0 23
Rht-D1b x year				0.45				0 48			<0 001

5.2.3 Lodging

In 2004, following heavy rainfall on 30 July, some genotypes lodged during ripening (Table 5.5; P<0.001). Lodging at this stage of the growing season would not be expected to reduce the grain growth of affected sub-plots. When lodging was used as a covariate in the analysis of variance of yield, there was no significant effect of lodging on yield (P=0.68). In 2005, lodging occurred on 29 July and was observed only for Lines 1 and 13. Therefore, it was not possible to perform ANOVA on the lodging data, due to large number of plots in which there was no lodging.

Table 5.5 Mean percentage area of sub-plot lodged, recorded on 30 July in 2004 and 29 July in 2005. Figures quoted are the sum of the percentage areas 'lodged' and lodged flat

Genotype	% lodged area			
	Unshaded	Shaded	Unshaded	Shaded
	2004		2005	
Line 1	90	-	38	-
Line 3	15	-	0	-
Line 4	5	0	0	0
Line 11	0	-	0	-
Line 12	35	5	0	0
Line 13	72	-	3	-
Line 15	45	78	0	0
Line 17	24	-	0	-
Line 21	0	-	0	-
Line 26	0	0	0	0
Rialto	8	0	0	0
Spark	0	0	0	0
Mean	24 5		3 8	0
SED (df)	12 76 (11)			

5.2.4 Harvest index and above-ground biomass

Harvest index was measured for the ten unshaded lines plus their parents and for the four shaded lines and their parents in 2004 and 2005. Genotypes differed in HI in both years ($P<0.05$; Table 5.6). Cross-year analysis of variance showed differences in HI in the range 0.371 (Line 12) to 0.473 (Line 26) ($P<0.001$). The genotype rankings were consistent across years, and there was no interaction between year and genotype. A positive linear relationship was observed between HI and combine yield in both years ($P<0.05$; Figure 5.7), and for the mean values across years ($P=0.003$; $R^2 = 0.59$).

Harvest biomass was calculated by two methods. For the ten unshaded DH lines and their parents values were calculated from (1) measurements of combine yield and harvest index (HI) from growth analysis and (2) directly from the harvest growth analysis. For the four shaded lines and their parents values were calculated from growth analysis data since there was no combine harvesting for the shaded genotypes. Therefore method (2) will be used to compare between shaded and unshaded genotypes. For

consideration of the ten unshaded lines and the parents in unshaded conditions alone, method (1) is preferred because it relates to a larger sample area. There were no significant differences in AGDM between genotypes in either year (Table 5.6). Cross-year ANOVA showed no differences between genotypes (P=0.13). There was an effect of year (P<0.001) but the genotype x year interaction was not significant. AGDM, however, showed a positive genetic correlation amongst genotypes with combine yield in 2004, although not in 2005 (P=0.013; Figure 5.8). Pooling the data over years, AGDM was positively correlated with combine yield and accounted for 28% of the phenotypic variation in grain yield (P=0.045; R^2 = 0.35). The effect of 1BL/1RS on AGDM was neutral in either year, but the cross-year ANOVA showed 1BL/1RS increased AGDM on average by 116 g m^{-2} (P<0.05; Table 5.6).

1BL/1RS improved HI in 2004 by 3.1% (P= 0.051) and had a neutral effect in 2005 (P=0.40). Cross-year analysis of variance showed that 1BL/1RS improved HI by 0.011 (P=0.051).

Rht-D1b increased HI in both years, and harvest biomass in 2005 (P<0.05; Table 5.6). Cross-year analysis showed that *Rht-D1b* increased AGDM by 69 g m^{-2} (P=0.088) and HI by 0.038 (P<0.001).

With regard to the shading of the four DH lines and their parents, shading decreased AGDM and HI of the six genotypes in both years (P<0.05; Table 5.6). However, there was no interaction between shading and genotype. Cross-year analysis of variance showed that, on average, shading reduced AGDM and HI by 229 g m^{-2} and 0.055, respectively (P<0.05).

Table 5.6 The effect of post-anthesis shading and genotype on AGDM and HI for four lines of Rialto x Spark DH population and the two parents in 2004 and 2005

	AGDM ($g\ m^{-2}$)						*HI*		
	Method 1 (combine			Method 2 (hand-harvested)					
	2004	2005	Mean	2004	2005	Mean	2004	2005	Mean
Unshaded									
Line 1	1514	1813	1663	-	-	-	0.381	0.392	0 386
Line 3	1563	1800	1682	-	-	-	0.499	0.431	0.465
Line 4	1556	1844	1700	1362	1713	1538	0.404	0.399	0 402
Line 11	1491	1894	1692	-	-	-	0.434	0 415	0 425
Line 12	1792	1943	1867	1346	1843	1594	0 370	0.372	0 371
Line 13	1699	1967	1833	-	-	-	0.440	0.439	0 439
Line 15	1292	1792	1542	1275	1744	1510	0.446	0.458	0 452
Line 17	1537	1721	1629	-	-	-	0.429	0 395	0.412
Line 21	1655	1980	1818	-	-	-	0.482	0.421	0.451
Line 26	1786	1847	1817	1447	1823	1635	0.493	0 453	0.473
Rialto	1652	2055	1854	1463	1867	1665	0.462	0.419	0 440
Spark	1674	1943	1809	1454	1773	1614	0.436	0 417	0.427
Mean	1601	1883	1742	1391	1794	1593	0.440	0.417	0.429
Shaded									
Line 4				1091	1557	1324	0.371	0 357	0 364
Line 12				1126	1703	1415	0.372	0.372	0 372
Line 15				924	1191	1058	0.380	0 427	0 403
Line 26				1337	1494	1416	0 391	0 361	0 376
Rialto				1288	1671	1480	0 459	0 304	0 381
Spark				1206	1782	1494	0.394	0 273	0 333
Mean				1162	1566	1364	0.394	0 349	0 372
SED (df)									
Year			46.5 (33)			90.7 (2)			0.0258 (2)
Shade				39.6 (21)	91.7 (18)	46 8 (4)	0.013 8 (19)	0.014 89	0.0150 (4)
Genotype	162.2 (18)	121.3 (13)	114.0 (33)	71.6 (9)	281.9 (8)	136.4 (19)	0.023 6 (18)	0.022 8 (14)	0 0170 (34)
Year x shade						66 2 (4)			0.0298 (3.39)
Year x genotype			161.2 (33)			120 5 (35)			0.0241 (34)
Shade x genotype				97.0 (21)	224.7 (18)	119.6 (37.9)	0.033 7 (19)	0.036 5 (14)	0.0263 (23 7)
Year x shade x genotype						169.1 (37.9)			0.0427 (12 6)
Unshaded									
1BL/1BR	1666	1870	1768				0.445	0 423	0.434
1B	1537	1767	1652				0.431	0 412	0.421
Mean	1601	1819	1710				0.438	0.417	0 428
P-Value	0.092	0.22	0.016				0.051	0 40	0 051
Rht-D1b	1591	1899	1745				0.459	0.433	0.446
Rht-D1a	1614	1738	1676				0.415	0 502	0 408
Mean	1603	1818	1710				0.437	0 417	0.427
P-Value	0.82	0.015	0.088				<0.001	0.019	<0.001

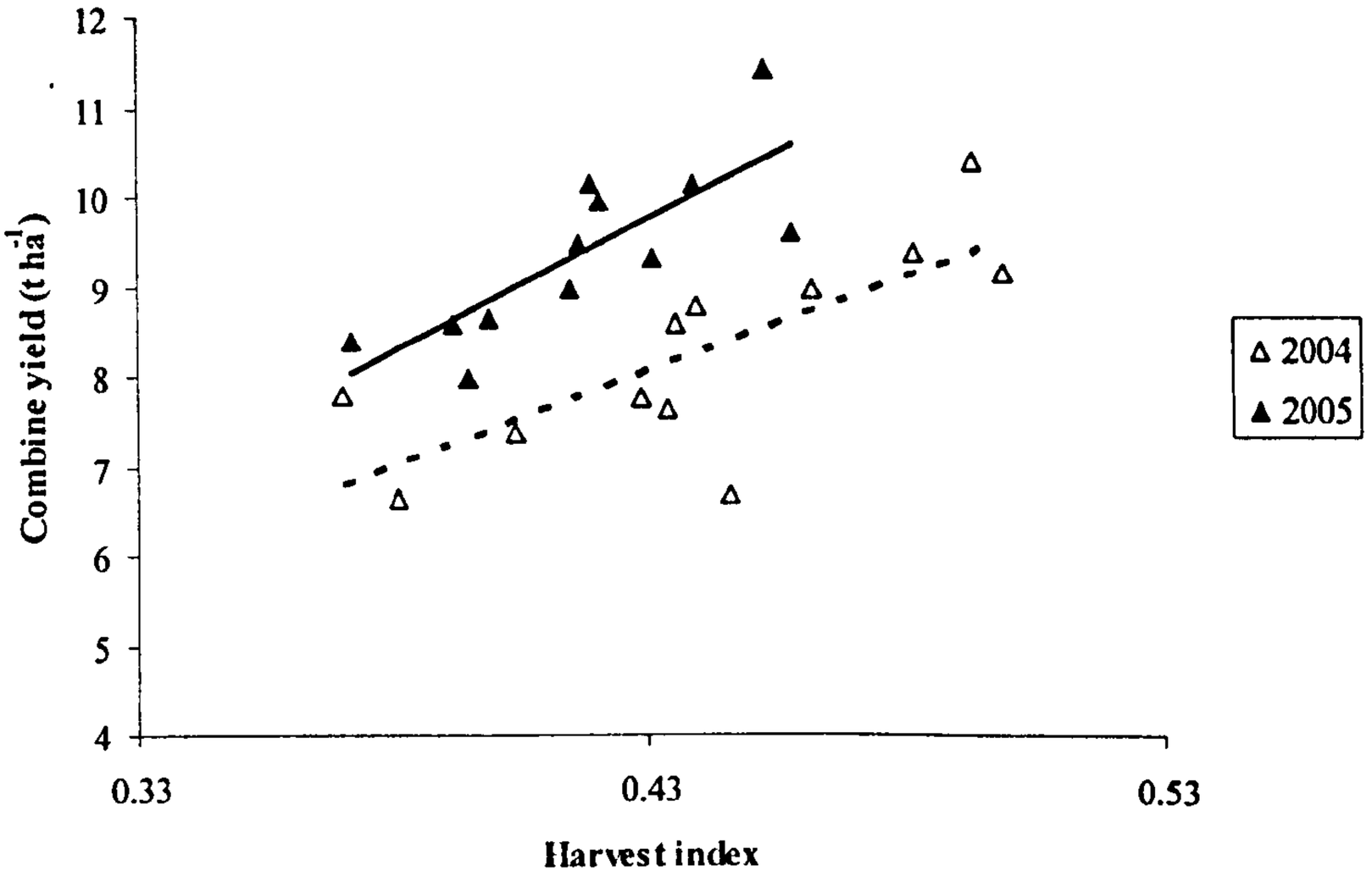


Figure 5.7 Regression of harvest index (HI) on combine yield (85% DM) in 2004 and 2005 for ten DH lines of the Rialto x Spark population and the two parents. 2004: y = 21.06x - 1.08; R^2= 0.57; 2005: y = 29.63x - 3.00; R^2= 0.63 for ten genotypes and the two parents

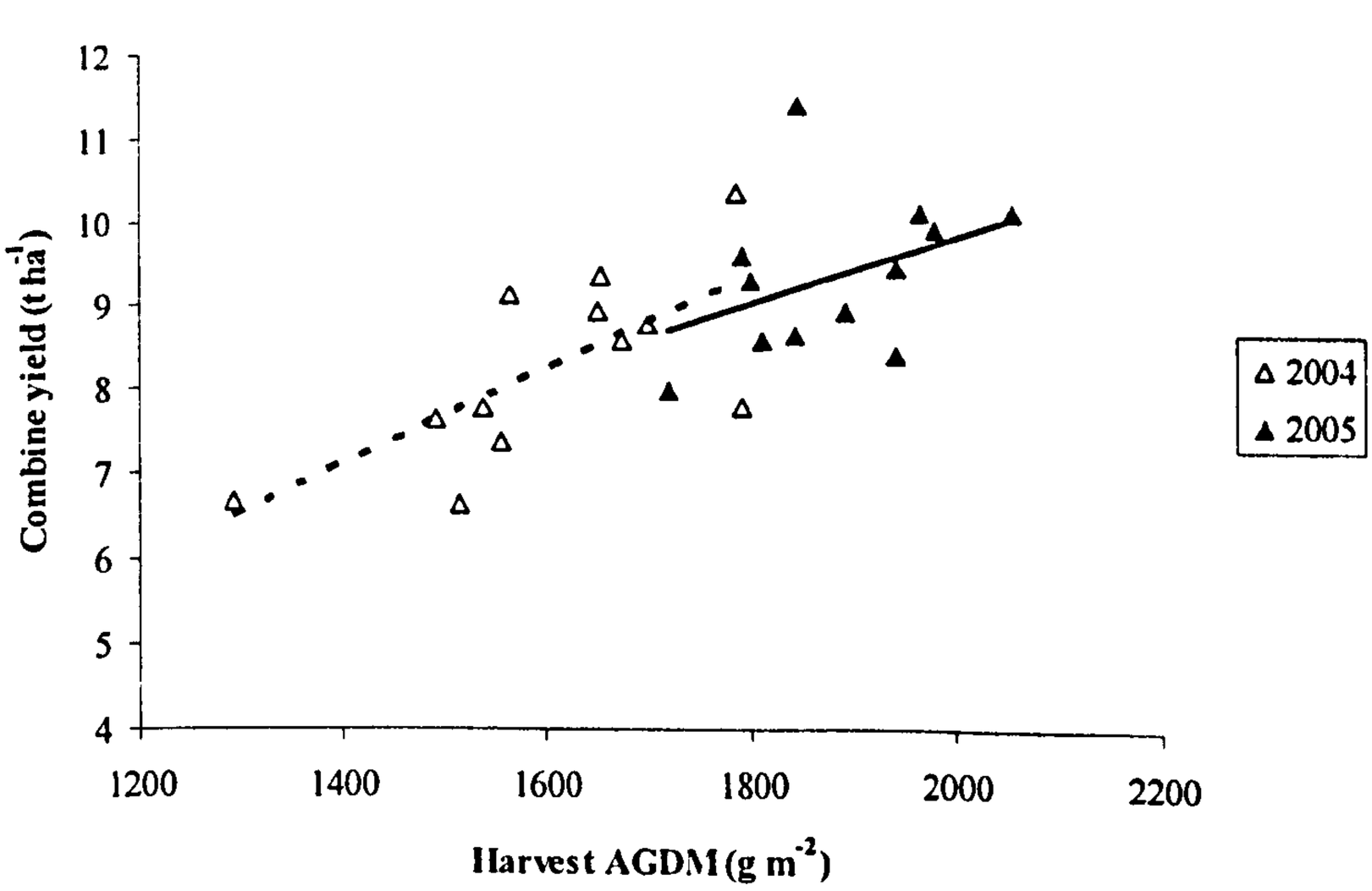


Figure 5.8 Regression of harvest above-ground dry matter on combine yield (85% DM) in 2004 and 2005 for ten DH lines of the Rialto x Spark population and the two parents. 2004: y = 0.0056x - 0.7673; R^2= 0.48; 2005: y = 0.0041x + 1.6949; R^2= 0.17

With regard to dry weight of the non-grain plant organs at harvest, genotypes differed ($P<0.05$) in chaff dry weight m^{-2} in both years (Table 5.7). Cross-year analysis of variance showed that chaff dry weight ranged from 107.3 g m^{-2} (Line 1) to 157.3 g m^{-2} (Line 26). Genotypes also differed in stem and sheath DM m^{-2} in 2004 ($P<0.001$), in the range 506.8 (Line 1) to 741.8 g m^{-2} (Line 12). However, there were no differences ($P=0.13$) between genotypes in 2005. Cross-year analysis showed genotypic differences in the range 509.8 (Line 15) to 687.1 g m^{-2} (Line 12) ($P<0.001$), but there was no year x genotype effect. There were no significant differences in lamina DM m^{-2} in either year, but average lamina DM was lower in 2004 (141.3 g m^{-2}) than 2005 (169.7 g m^{-2}) ($P<0.001$).

Stem and sheath DM m^{-2} was not significantly affected by shading in either year, but when data were pooled over years shading significantly reduced stem and sheath DM at harvest ($P=0.025$; Table 5.7). There was no effect of shading on lamina DM m^{-2} in either year.

5.2.5 Potential grain weight

The potential mean grain weight (PGW) was predicted from measuring the water content of grains four weeks after anthesis (section 5.2.2). Potential grain weight was measured in 2004 for four lines plus their parents in shaded and unshaded conditions. In 2005, it was measured for ten lines plus their parents in unshaded conditions and for the four lines and their parents in shaded conditions. There were differences amongst the 6 and the 12 genotypes in 2004 and 2005, respectively ($P<0.05$; Table 5.8). PGW ranged from 41.3 (Spark) to 48.1 (Rialto) mg in 2004 and from 43.4 (Line 1) to 47.4 (Rialto) mg in 2005. Cross-year analysis of variance showed an effect of year ($P<0.001$), genotype ($P<0.001$) and year x genotype ($P<0.001$). Averaged across years, Rialto had the highest PGW (47.8 mg) and Spark the smallest (41.8 mg). Shading had no effect on PGW in either year (Table 5.8). In both years for both the shaded and unshaded treatments, the linear regression of PGW on grain yield amongst genotypes was not significant.

Table 5.7 The effect of post-anthesis shading and genotype on DM for chaff, stem plus leaf sheath and lamina (g m^{-2}) for ten lines of Rialto x Spark DH population and the two parents in 2004 and 2005

	Chaff DW m^{-2}						Stem DW m^{-2}						Lamina DW m^{-2}					
	Unshaded			Shaded			Unshaded			Shaded			Unshaded			Shaded		
Genotype	2004	2005	Mean	2004	2005	Mean	2004	2005	Mean	2004	2005	Mean	2004	2005	Mean	2004	2005	Mean
Line 1	115.8	98.8	107.3	-	-	-	506 8	572.7	539.8	-	-	-	143.1	158.1	150.6	-	-	-
Line 3	166 4	107.3	136.8	-	-	-	536 4	492.4	514.4	-	-	-	141 6	138.1	139.8	-	-	-
Line 4	155.0	85.8	120.4	134.6	91.1	112 8	651 3	486 6	569.0	404	509	456	149.9	141.7	145.8	133 2	137.8	135 5
Line 11	155.6	116.0	135.8	-	-	-	571 9	478 6	525.3	-	-	-	124.4	183 5	153.9	-	-	-
Line 12	146.2	116.8	131 5	122.2	111 7	116 9	741 8	632 3	687.1	599	573	586	132 9	189.0	161.0	124 4	181.0	152 7
Line 13	158.9	110.5	134.7	-	-	-	570 9	526 4	548 6	-	-	-	153 3	204.1	178 7	-	-	-
Line 15	147.2	129.4	138 3	107.2	118 9	113.1	563 2	456 4	509.8	462	436	449	114.2	150.2	132.2	117.4	141.2	129.3
Line 17	157.8	108 7	133 2	-	-	-	649 7	552 5	601 1	-	-	-	146.3	161.5	153.9	-	-	-
Line 21	163.5	107.5	135.5	-	-	-	563.6	473 8	518 7	-	-	-	139.3	195.0	167.2	-	-	-
Line 26	158.4	156.1	157.3	134.1	123 2	128.7	519 6	517 6	518 6	721	503	612	131.2	166.8	149.0	124.6	191.3	157.9
Rialto	183.3	124.3	153.8	156.1	124.7	140.4	573.3	481 4	527.4	503	437	470	163.8	168.7	166 3	143.1	172.8	157.9
Spark	151.0	101.0	126.0	131.8	112.0	121 9	643 3	503 1	573.2	581	572	577	156 1	179.3	167 7	132.7	166.0	149.3
Mean	154.9	113 5	134.2	131.0	113.6	122.3	591 0	514 5	552.7	545	505	525	141.3	169.7	155 5	129 2	165.0	147.1
SED (df)			3.59 (38)						13 89 (43)						6.30 (43)			
Year	10.76 (21)	14.08 (15)	8.80 (38)			5 27(2)	42 89 (20)	54 0 (21)	34 02 (43)			5.0 (2)	13.84 (20)	27 0 (21)	15.44 (43)			3.97 (2)
Shade			12.44 (38)	4.42(21)	5 48(18)	4 55(4)			48 11 (43)	48.4(22)	19 89(21)	11.1 (4)			21 83 (43)	6.23 (22)	9.29 (21)	6.52 (4)
Genotype				7.66(21)	9.49(18)	5 87(35)				83 8(22)	34.45(21)	48.3 (38)				10.79 (22)	16 09 (21)	9.67 (38)
Year x shade						6 96 (4 76)						15.7 (4)						9.22 (4)
Year x genotype						9.23 (15.1)						68.3 (38)						13.68 (38)
Shade x genotype				10.83(21)	13 42(42)	8 84 (30 34)				118 5(22)	48.72(21)	68.3 (38)				15.26 (22)	22.75 (21)	14.09 (36.08)
Year x shade x genotype						12.78 (30 64)						96 6(38)						19.92 (336 08)

There was positive linear relationship between PGW and mean grain weight from the combine grain samples in 2004 (P= 0.029, R^2= 0.74) and in 2005 (P<0.001, R^2= 0.85; Figure 5.9). A negative linear relationship was evident between PGW and grain m^{-2} in 2005 (P≤0.05, R^2 =0.48). However, there was no relationship in 2004. There was no effect of shading on PGW

1BL/1RS had a neutral effect on PGW in both years, with an average PGW for both the 1BL/1RS and 1B groups of 45.1 mg. In 2004, the *Rht-D1b* semi-dwarf gene increased the PGW by 1.54 mg, but in 2005 it had no significant effect.

Table 5.8 Effect of post-anthesis shading and genotype on potential mean grain weight for ten DH lines of Rialto x Spark population and the two parents in 2004 and 2005

Genotype	Potential grain weight (mg)				
	Unshaded		Shaded		
	2004	2005	2004	2005	Mean
Line 1	-	43.4	-	-	-
Line 3	-	43.7	-	-	-
Line 4	45.2	46.7	47.6	47 7	47 7
Line 11	-	46.4	-	-	-
Line 12	44.3	47.3	45.8	48.9	47 3
Line 13	-	46.4	-	-	-
Line 15	46.2	46.8	46.6	44 3	45 5
Line 17	-	46 5	-	-	-
Line 21	-	44.1	-	-	-
Line 26	41.4	43.6	43.3	41.2	42.3
Rialto	48.1	47.4	47.2	46 2	46 7
Spark	41.3	44.2	43.1	43 7	43.4
Mean	44.4	45.6	45.6	45 3	45.5
SED (df)					
Year					0 431(2)
Shading			0.313(2)	0.591(2)	0.335(4)
Genotype	1.15 (8)	0.919 (12)	0.970(13)	0 691(9)	0 613(22)
Year x shading					0.473(4)
Year x genotype					0.867(22)
Shading x genotype			1.372(13)	0 977(9)	0.867(22)
Year x shading x genotype					1.215(26)

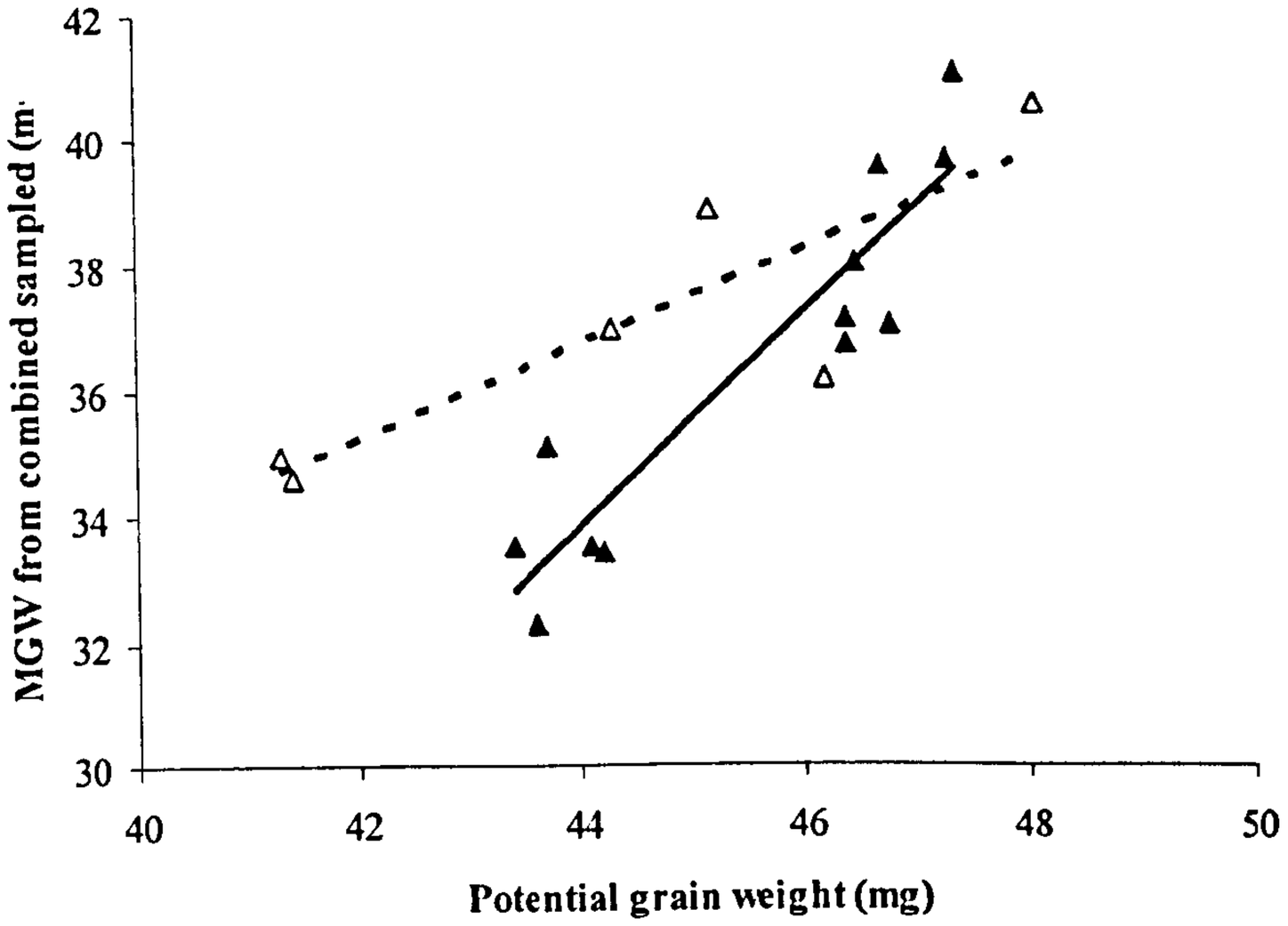


Figure 5.9 Regression of potential mean grain weight on combine mean grain weight (MGW) in 2004 and 2005 for ten DH lines of the Rialto x Spark population and the two parents. 2004: y = 0.7319x + 4.5024; R^2= 0.74; 2005 y = 1.6619x - 39.294; R^2= 0.85.

5.2.6 Green area persistence

Ten-shoot samples were taken for the four lines and their parents in 2004 and 2005 at around GS61+ 4 weeks and GS61+ 6 weeks. The genotypes were divided into two groups according to their flowering date; the early group (Lines 4, 15 and Rialto) were sampled two days earlier than the late group (Lines 12, 26 and Spark) in both years. Genotypes differed in green area fertile shoot^{-1} on both sampling dates in 2004 ($P<0.05$; Table 5.9) in the range 61.6 (Spark) to 112.0 cm^2 (Rialto) on 10 July and from 11.1 (Line 26) to 44.1 cm^2 (Rialto) on 27 July (Table 5.9). Similarly, genotypic differences ($P<0.001$) were observed in 2005 on 12 July in the range 32.7 (Spark) to 64.1 cm^2 (Rialto). A strong trend for differences was observed on 19 July 2005 in the range 30.6 cm^2 (Spark) to 56.3 cm^2 (Line 4; $P=0.055$). Shading did not affect green area shoot^{-1} in 2004. In 2005, however, shading increased green area shoot^{-1} on 12 July by 19.9 cm^2 ($P<0.001$). There was no interaction between shading and genotypes in all the samplings in the two years except for the second sample in 2005 ($P=0.054$).

Table 5.9 The effect of shading and genotype on shoot green area fertile shoot^{-1} for four lines of Rialto x Spark DH population and the two parents during grain filling in 2004 and 2005

	Green area fertile shoot^{-1} (cm^2)											
	2004						2005					
	10 July			27 July			12 July			19 July		
	Unshaded	Shaded	Mean	Unshaded	Shaded	Mean	Unshaded	Shaded	Mean	Unshaded	Shaded	Mean
Line 4	71.8	72.1	72.0	36.1	29.6	32.8	47.7	72.3	60.0	42.6	56.3	49.4
Line 12	67.1	77.1	72.1	18.2	21.2	19.7	34.7	66.4	50.5	26.4	39.1	32.7
Line 15	92.0	95.4	93.7	21.0	28.5	24.8	55.2	58.6	56.9	49.9	33.6	41.8
Line 26	66.4	65.0	65.7	11.1	19.9	15.5	38.7	62.0	50.3	24.2	40.7	32.4
Rialto	112.0	114.6	113.7	44.1	44.0	44.0	64.1	80.8	72.5	35.8	50.3	43.1
Spark	61.6	61.1	61.3	25.9	28.2	27.1	32.7	46.9	39.8	25.2	30.6	27.9
Mean	78.5	80.9	79.7	26.1	28.6	27.3	45.5	64.5	55.0	34.0	41.8	37.9
SED (df)												
Shading			4.79 (20)			3.71 (22)			2.62 (20)			4.27 (21)
Genotype			8.29 (20)			6.43 (22)			4.55 (20)			7.40 (21)
Shading x genotype			11.73 (20)			9.09 (22)			6.43 (20)			10.46 (21)

5.2.7 Stem water soluble carbohydrate reserves

Stem water soluble carbohydrate reserves were measured directly through chemical analysis in 2005 and 2006 (Table 5.10). Stem reserves from chemical analysis showed genotypic differences in both years ($P \leq 0.05$; Table 5.10). Cross-year analysis of variance showed differences ($P<0.001$) ranging from 243 (Spark) to 487 g m^{-2} (Rialto). There was no effect for either year or the year x genotype interaction. There was a positive linear relationship between stem reserves and grain yield in 2006 ($P<0.001$; Figure 5.10) but not in 2005. There was a positive linear relationship ($P=0.013$) between stem WSC reserves and the loss in stem DM between GS61+ 5d and harvest in 2005 amongst the ten DH lines and their parents (Figure 5.11). The slope was below 1:1 perhaps because some soluble sugars were respired to provide energy for breaking down fructan and transport. Therefore, stem DM loss was used as an indicator of the stem water soluble content accumulated in 2004 (see chapter 3). The stem DM loss differed between genotypes in 2004 ($P<0.05$; Table 5.10). Cross-year analysis of variance for 2004 and 2005 showed genotypic differences in stem reserves ($P<0.05$) whereby Rialto produced the highest stem reserves (576 g m^{-2}) and Line 12 produced the least (274g m^{-2}).

1BL/1RS genotypes accumulated more stem reserves than 1B genotypes in both years ($P \leq 0.05$) (Table 5.10). Cross-year analysis showed that 1BL/1RS genotypes produced 96 g m^{-2} more stem reserves than 1B genotypes. The interaction between 1BL/1RS and year was not significant. There was a tendency for more stem reserves to be accumulated by genotypes possessing the semi-dwarf gene *Rht-D1b* in both years (Table 5.10). Cross-year analysis showed that semi-dwarf genotypes accumulated 381 g m^{-2} and tall genotypes 303 g m^{-2} ($P=0.017$).

Table 5.10 Effect of genotype on stem WSC reserves at GS61+ 75 °Cd and the estimated stem DM loss from GS61+ 75 °Cd to harvest for ten DH Lines of Rialto x Spark population and the two parents in 2004 and 2005 in unshaded treatment

	Stem WSC reserves (chemical analysis) g m^{-2}			Estimated Stem reserves by DW loss (GS61 +75 °Cd to harvest) g m^{-2}		
Genotype	2005	2006	Mean	2004	2005	Mean
Line 1	310	203	256	371	420	395
Line 3	352	301	327	323	333	328
Line 4	330	374	352	225	371	298
Line 11	416	539	478	445	500	473
Line 12	314	217	265	245	304	274
Line 13	424	365	395	457	347	402
Line 15	378	211	294	409	523	466
Line 17	406	311	359	407	393	400
Line 21	247	272	260	323	307	315
Line 26	297	391	344	472	358	415
Rialto	490	484	487	589	562	576
Spark	217	269	243	361	263	312
Mean	348	328	338	386	390	388
SED(df)						
Year			21.3 (44)			34.3 (33)
Genotype	71.4 (21)	77.1 (21)	52.2 (44)	86.0 (15)	142 4 (16)	83 9 (33)
Interaction			73.8 (44)			118 7 (33)
1BL/1RS	382	397	390			
1B	315	273	294			
Mean	348	335	342			
P value	0.029	0.04	0 004			
Rht-D1b	375	386	381			
Rht-D1a	322	284	303			
Mean	349	335	342			
P value	0.074	0.085	0.017			

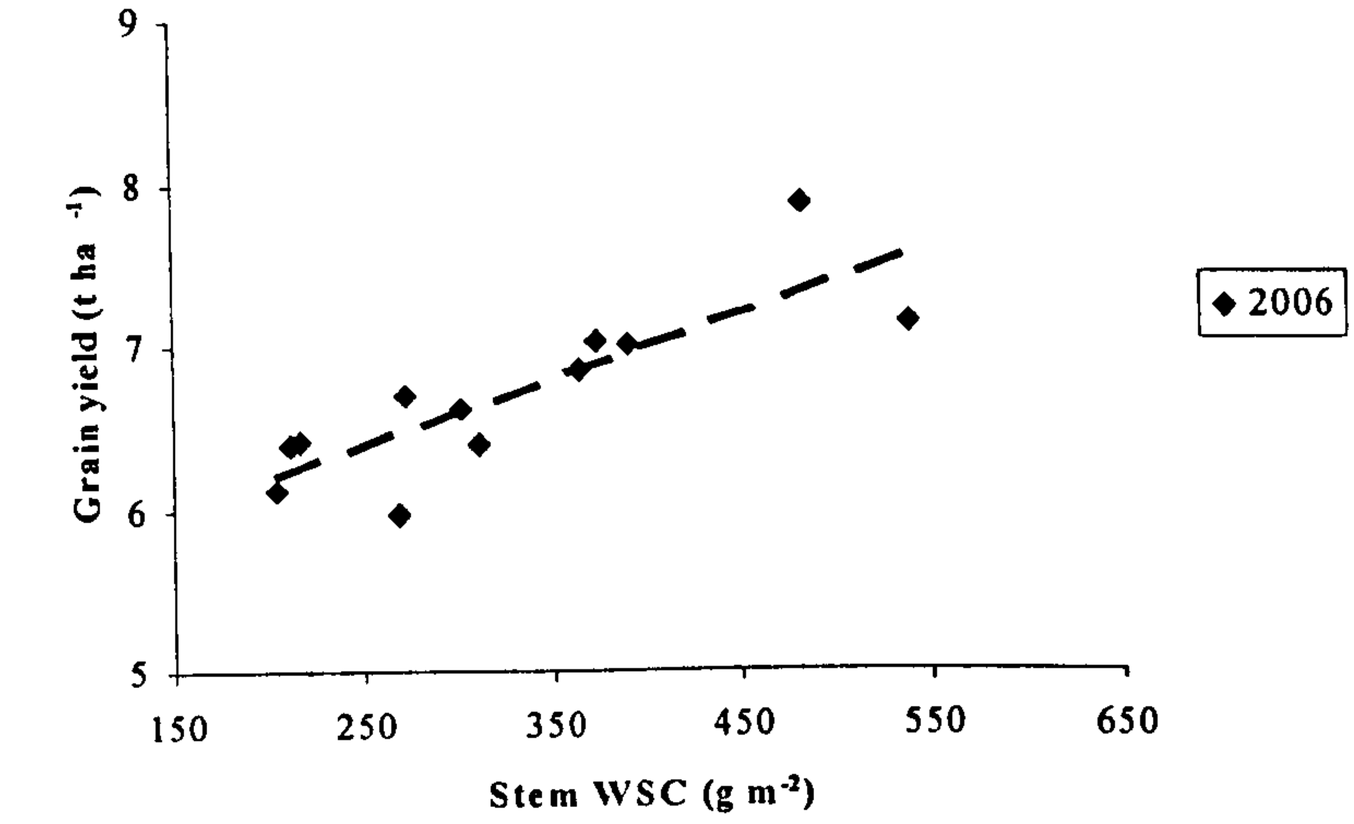


Figure 5.10 Regression of grain yield on stem WSC reserves at GS61+ 75 °Cd in 2006 for ten DH lines of the Rialto x Spark population and the two parents. y = 0.004x + 5.3899; R^2= 0.69.

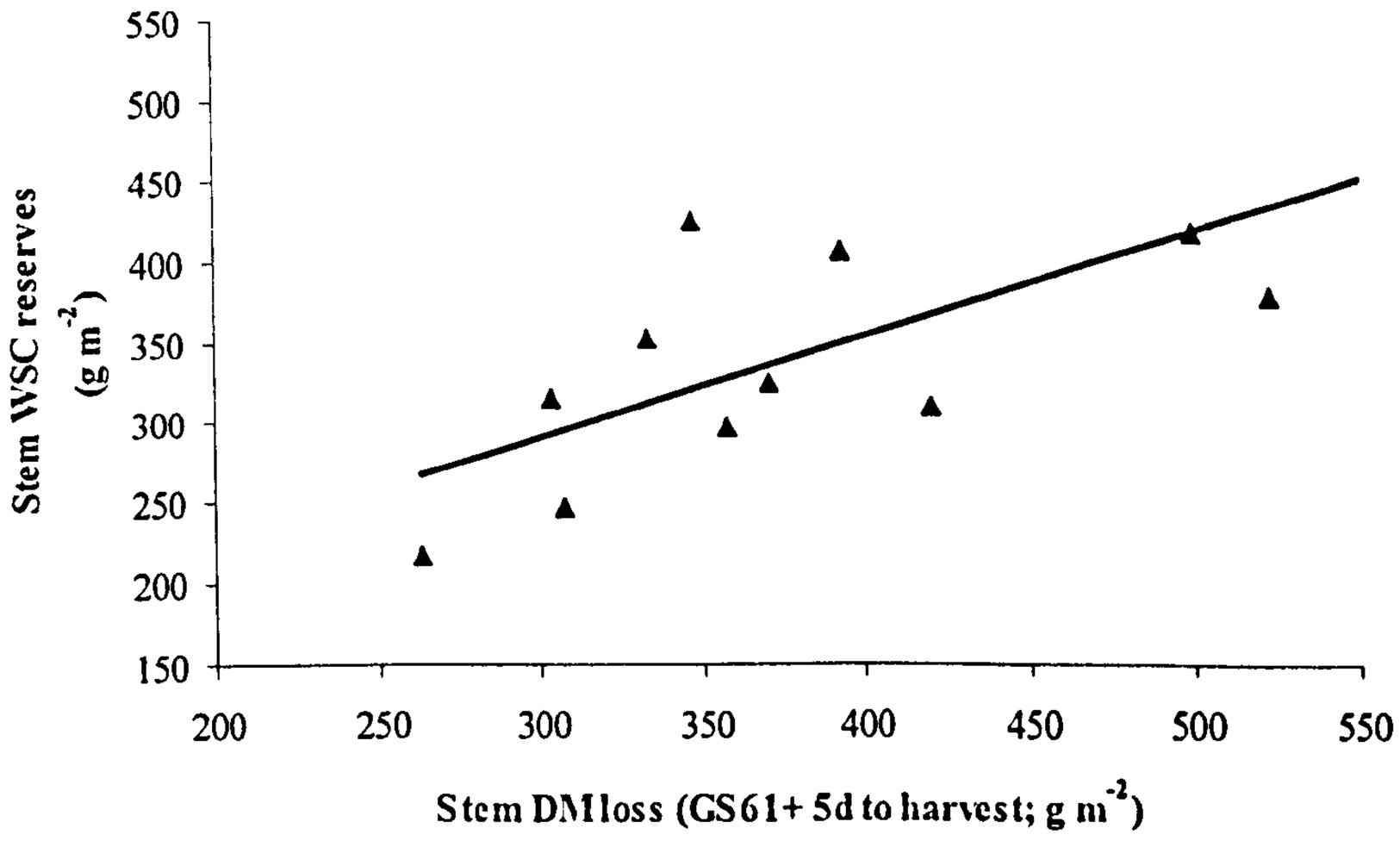


Figure 5.11 Regression of estimated stem DM loss (GS61+ 75 °Cd to harvest) on stem WSC reserves in 2005 for ten DH lines of the Rialto x Spark population and the two parents. y = 0.6325x + 101.2; R^2= 0.57.

The relative contribution from pre-anthesis stem reserves to grain DM at harvest was calculated indirectly as the ratio of the change in stem and sheath DM loss between

anthesis and harvest to the change in ear DM over the same period (Table 5.11). Cross-year analysis of variance showed a trend for shading (P=0.07) to result in a greater contribution (34.5%) of the stem reserves to the grain growth compared to unshaded genotypes (31.9%). Furthermore, averaged across years, differences among genotypes existed in the range 18.4 (Line 12) to 40.1% (Spark) and 22.1 (Line 12) to 41.1% (Line 15) in unshaded and shaded genotypes, respectively (P=0.001).

Table 5.11 The effect of post-anthesis shading and genotype on percentage contribution of pre-anthesis stem reserves to ear DM growth during grain filling for four lines of Rialto x Spark DH population and the two parents in 2004 and 2005

	Contribution of pre-anthesis stem reserves to ear DM growth during grain filling (%)					
	Unshaded			Shaded		
	2004	2005	Mean	2004	2005	Mean
Line 4	25.7	40.2	32.9	24.3	35.3	29.8
Line 12	6.1	30.6	18.4	7.0	37.2	22.1
Line 15	18.9	43.7	31.3	36.1	46.1	41.1
Line 26	26.4	43.8	35.1	24.6	48.5	36.5
Rialto	25.0	41.9	33.4	29.9	47.2	38.5
Spark	42.9	37.2	40.1	49.7	28.5	39.1
Mean	24.2	39.6	31.9	28.6	40.5	34.5
SED (df)						
Year			3.72 (2)			
Shading	3.48 (8)	2.95 (20)	1.08 (4)			
Genotype	6.03 (8)	5.10 (20)	4.05 (24)			
Year x shading			1.53 (4)			
Year x genotype			5.72 (24)			
Shading x genotype	8.52 (8)	7.22 (20)	5.72 (24)			
Year x shading x genotype			8.09 (24)			

5.2.8 Estimated source-sink balance

The following section will focus on the calculation of the source-sink balance, and analyzing the genotypic differences. These findings will then be used to predict the effect of source manipulation on yield for the six shaded genotypes. These predictions will be compared with the observed responses to source manipulation.

5.2.8.1 Potential source size post-anthesis

Source size post-anthesis was estimated for four DH lines plus their parents, as post-anthesis green area measurements were only assessed for these genotypes. Accumulated PAR interception from GS61 to complete canopy senescence was calculated by applying K_{PAR} measured at GS61 to daily values of interpolated GAI and incident PAR. Daily values of green area fertile shoot^{-1} were calculated by linearly interpolating between values at sequential samplings during grain-filling. Daily GAI was calculated by multiplying the number of fertile shoots at GS61 by the daily green area fertile shoot^{-1} (Section 5.2.3). In the unshaded treatment, post-anthesis PAR interception did not differ between the genotypes in either year. Cross-year ANOVA showed no differences between genotype, but there was an interaction effect with year (P=0.02) (Table 5.12).

For shaded treatments, measurements of PAR above the crop, both outside and under the shade, using a hand-held ceptometer during grain filling indicated that shading reduced incident PAR by exactly 50%. Therefore, incident PAR reduction was reduced by 50% in the calculations of intercepted PAR. Shading reduced PAR interception in both years (P<0.001; Table 5.11). However, there was no shading x genotype interaction for post-anthesis PAR interception. Source size post-anthesis was estimated by multiplying the intercepted radiation post-anthesis by the pre-anthesis RUE, and adding stem WSC reserves measured at GS61 + 5 days (equation 5.1). The same values of pre-anthesis RUE and stem WSC reserves were applied in the shaded and unshaded conditions in the calculation of post-anthesis source. In the unshaded treatment, a trend for differences in source size amongst genotypes was found in 2004 (P=0.068). However, no differences were observed in 2005 (Table 5.12). Cross-year ANOVA indicated a genotypic effect (P=0.023) with values in the range of 1165 (Line 12) to 1567 g m^{-2} (Line 26). The year x genotype interaction was not significant. Differences between genotypes in RUE (P=0.03) and stem WSC (P=0.025) in 2004 accounted for the trend for differences in source size (P=0.068). Source size in 2004 was apparently greater than 2005 (P=0.069). Incident radiation was lower, but RUE and WSC were significantly greater in 2004 than in 2005. There was a positive relationship between source size and combine yield in 2005 (P=0.044; R^2 = 0.68) but not in 2004. However, the genotype means over the two years there was a positive linear correlation between

source size and combine yield (P=0.008; R^2 = 0.86). As expected, shading significantly reduced the post-anthesis source size by 38 and 45% in 2004 and 2005, respectively (P<0.001; Table 5.12). Cross-year ANOVA showed a reduction in source size of 41% (P<0.001). The shading treatment x genotype interaction, however, was not significant in either year.

5.2.8.2 Potential sink size post-anthesis

The estimated sink size was calculated from potential grain weight measured at GS61 + 4 weeks and the number of grains m^{-2} calculated using the individual grain weight for samples from the combine at harvest and the combine grain yield (equation 5.2) for 12 unshaded genotypes in 2004 and 2005 (Table 5.13). Potential sink size was also calculated based on grains m^{-2} calculated using grain weight from hand-harvested samples for the six shaded and unshaded genotypes for comparison. There was a good agreement between the individual grain weight from combine and hand harvested samples in 2004 (6 genotypes) and 2005 (12 genotypes (P<0.05; R^2= 0.90 and 0.92, respectively). For consideration of the 12 genotypes in the unshaded conditions alone, the calculation of potential sink size using combine data was preferred as it relates to larger sample area for grain yield. Regarding the twelve genotypes in unshaded conditions, there were differences in both years (P<0.05), with lines 26 (1049 g m^{-2}) and 13 (1080 g m^{-2}) having a much larger sink size than other genotypes in 2004 and 2005, respectively. There was a positive relationship between sink size and combine yield in 2004 (P = 0.002; R^2= 0.93), but not in 2005. Using mean genotype values over two years, there was a trend for positive linear relationship between sink size and combine yield (P=0.057; R^2 = 0.64). Although there was no relationship between source and sink sizes in either year, averaging the data over years, there was a positive relationship between source and sink sizes amongst the 6 genotypes (P= 0.009; R^2= 0.85). With regard to sink size calculated using hand-harvested estimates of grains m^{-2}, genotypic differences were found in both years (P<0.05) with line 26 having the largest sink size in both years. In both years, shading had no effect on sink size and the interaction between shading and genotype was not significant (Table 5.13).

Table 5.12 Effect of genotype on the potential source size post-anthesis and its components for four DH lines of Rialto x Spark population and the two parents in 2004 and 2005

Genotype	PAR intercepted post-anthesis (MJ m^{-2})						RUE (g MJ^{-1})			Calculated stem WSC (g m^{-2})			Estimated source size (g m^{-2})					
	Unshaded			Shaded			Unshaded			Unshaded			Unshaded			Shaded		
	2004	2005	Mean	2004	2005	Mean	2004	2005	Mean	2004	2005	Mean	2004	2005	Mean	2004	2005	Mean
Line 4	504.7	437 6	471.2	213.3	156.9	185.1	2.17	1.67	1 92	203	324	263	1308	1054	1181	724	589	656
Line 12	450.3	503.1	476.7	245.1	202.6	223.9	1.82	1.87	1 84	258	314	286	1080	1249	1165	708	695	701
Line 15	485.9	538.4	512.2	226 9	239 1	233.0	2.15	1.57	1.86	293	378	335	1337	1243	1290	950	751	851
Line 26	483.2	547.5	515.4	184.4	174.0	179.2	2.65	2.11	2 38	393	297	345	1670	1463	1567	920	652	786
Rialto	488.4	562 4	525.4	127.7	247.1	187.4	1.66	1.62	1 64	554	490	522	1364	1399	1382	860	890	875
Spark	503.0	502.2	502.6	172.5	235.0	203.7	2.58	1.70	2 14	329	217	273	1629	1210	1420	1009	617	813
Mean	485.9	515.2	500.6	195.0	209.1	202.0	2 17	1.76	1.97	338	337	337	1398	1270	1334	862	699	780
SED (df)																		
Year			20.3 (18)			18.33 (2)			0.1010 (22)			31.5 (20)			67.7 (18)			47.1 (2)
Shading				15.65 (21)	20.7 (21)	21.44 (4)										67.9 (14)	52.6 (18)	54.1 (4)
Genotype	28.11 (10)	55.4 (9)	35.1 (18)	27.10 (21)	35.8 (21)	20.55 (38)	0.2843 (10)	0.1817 (10)	0.1749 (22)	80.9 (9)	75.0 (9)	54.6 (20)	177.0 (9)	163.4 (7)	117.3 (18)	117.6 (14)	91.2 (18)	72.2 (28)
Year x shading						30.32 (4)												76.5 (4)
Year x genotype			49.7 (18)			29.07 (38)			0.247 (22)			77.2 (20)			165.9 (18)			104.4 (23.09)
Shading x genotype				38.33 (21)	50.7 (21)	29.07 (38)										166.3 (14)	128.9 (18)	107.8 (27.89)
Year x shading x genotype						41.11 (38)												152.4 (27.89)

Table 5.13 Effect of genotype on the potential sink size for ten DH Lines of Rialto x Spark population and the two parents in 2004 and 2005

	Potential grain weight (mg)					Grains m^{-2}							Estimated sink size (g m^{-2})						
						Calculated using grain weight from combine		Calculated using grain weight from hand-harvested samples					Combine-harvest		Hand-harvest				
	Unshaded		Shaded			Unshaded		Unshaded		Shaded			Unshaded		Unshaded		Shaded		
	2004	2005	2004	2005	Mean	2004	2005	2004	2005	2004	2005	Mean	2004	2005	2004	2005	2004	2005	Mean
Line 1	-	43.4	-	-	-	-	21735	-	16319	-	-	-	-	914	-	715	-	-	-
Line 3	-	43.7	-	-	-	-	22493	-	16900	-	-	-	-	960	-	746	-	-	-
Line 4	45.3	46 7	47.6	47.7	47.7	15285	18557	16780	13219	16464	13746	15105	739	867	761	626	797	594	695
Line 11	-	46.4	-	-	-	-	20760	-	16177	-	-	-	-	1016	-	731	-	-	-
Line 12	44.3	47 3	45.8	48.9	47.3	17636	18013	16540	15803	16724	13472	15098	781	824	733	771	831	665	748
Line 13	-	46.4	-	-	-	-	23240	-	18349	-	-	-	-	1080	-	852	-	-	-
Line 15	46.2	46.7	46.6	44.3	45.5	16009	22090	19708	18327	19110	16905	18008	738	1032	912	853	798	749	773
Line 17	-	46.5	-	-	-	-	17784	-	14354	-	-	-	-	803	-	684	-	-	-
Line 21	-	44.1	-	-	-	-	25226	-	18332	-	-	-	-	878	-	813	-	-	-
Line 26	41.4	43.6	43.3	41.2	42.3	25350	27210	23877	23071	24914	17082	20998	1049	862	990	1003	1118	700	909
Rialto	48.1	47.4	47.2	46.2	46.7	18620	21019	20112	14840	18627	13574	16101	895	998	897	706	879	628	753
Spark	41.2	44.2	43.1	43.7	43.4	20951	24069	21594	17777	18760	15716	17238	829	1062	971	785	958	678	818
Mean	44.4	45.6	45.6	45.3	45.5	18975	21850	19769	16958	19100	15083	17091	834	941	877	774	897	669	783
SED (df)																			
Year					0.431(2)							511.1 (2)							53.4 (2)
Shade			0 313(2)	0.591(2)	0.335(4)					494.0 (10)	484.5 (18)	241.9 (4)					37.6 (14)	30.1 (16)	40.0 (4)
Genotype	1.149 (8)	0.919 (12)	0.97(13)	0.691(9)	0.61(22)	1648.0 (10)	660 5 (21)	1500. 9 (8)	805.4 (14)	855.7 (10)	839.2 (18)	530.4 (24)	68.9 (8)	142.8 (19)	81.2 (7)	40.90 (11)	65.2 (14)	52.2 (16)	38.5 (26)
Year x shade					0 473(4)							565.5 (2.92)							66.8 (4.21)
Year x genotype					0 87(22)							854.5 (12.3)							72.9 (6.58)
Shade x genotype			1.37(13)	0.977(9)	0.87(22)					1210. 1 (10)	1186. 8 (18)	750.1 (24)					92.2 (14)	73.8 (16)	63.8 (18.89)
Year x shade x genotype					1.22(26)							1121 (22.1)							96.9 (15.61)

5.2.8.3 Source- sink balance

If grain yield is taken to be limited by either the source or the sink size, it follows that the lower of the two estimated values would equate to the realised yield. The difference between the estimates of source and sink size is defined here as the source-sink balance: if positive, the crop is sink-limited, whereas if negative, the crop is source-limited. The six genotypes did not differ in source-sink balance in the unshaded treatment in either year (Table 5.14). Cross-year ANOVA showed no differences between genotypes and no interaction between year and genotype was observed. However, an effect of year ($P=0.037$) was found resulting from a larger source-sink balance in 2004 than 2005, that is, crops were more sink-limited in 2004. In general, all genotypes had a positive source-sink balance in both years, suggesting that they were sink-limited. A strong relationship was shown between the realised grain yield from the combine and the predicted yield from the estimates of the source and sink size in both years ($P<0.05$, Figure 5.12). There was no relationship between source and sink size in either year. Because yield is apparently sink-limited in these experiments, the differences between realised yield and potential source size could be used as an index of the extent of sink limitation. There were no genotypic differences in the extent of sink limitation calculated in this way in both years (Table 5.14).

As expected, shading reduced the source-sink balance for all genotypes in both years ($P<0.001$; Table 5.15). For the six genotypes characterized in both the unshaded and shaded treatment, there was no relationship between reduction in grain yield resulting from shading and the source-sink balance in unshaded treatment in either year. There was no relationship between source-sink balance under shading and yield losses due to shading in either year (Figure 5.13). However, there was a relationship between source reduction due to shading and yield losses in 2005 ($P=0.001$; $R^2=0.94$; Figure 5.14), but not in 2004 or for the cross-year mean. The relationship between source reduction under shading and yield loss under shading indicate that Line 26 is closer to source limitation than other genotypes (Figure 5.14).

Table 5.14 The effect of genotype on source-sink balance (calculated as the difference between source and sink sizes based on individual grain weight and grains m^{-2} from combine samples) and extent of sink limitation (calculated as the differences between source size and realised yield) for four lines of Rialto x Spark DH population and the two parents for unshaded control treatment in 2004 and 2005

	Source-sink balance ($g\ m^{-2}$)			Extent of sink limitation ($g\ m^{-2}$)		
	2004	2005	Mean	2004	2005	Mean
Line 4	563	236	400	704	290	497
Line 12	299	338	318	419	536	478
Line 15	599	143	371	769	462	615
Line 26	621	601	611	789	723	756
Rialto	469	402	435	603	539	571
Spark	748	61	404	900	451	676
Mean	550	297	423	697	500	599
SED (df)						
Year			101.1 (14)			67.2 (17)
Genotype	210.6 (7)	136.2 (4)	175.2 (14)	229.3 (7)	149.3 (3)	116.3 (17)
Year x genotype			247.7 (14)			164.5 (17)

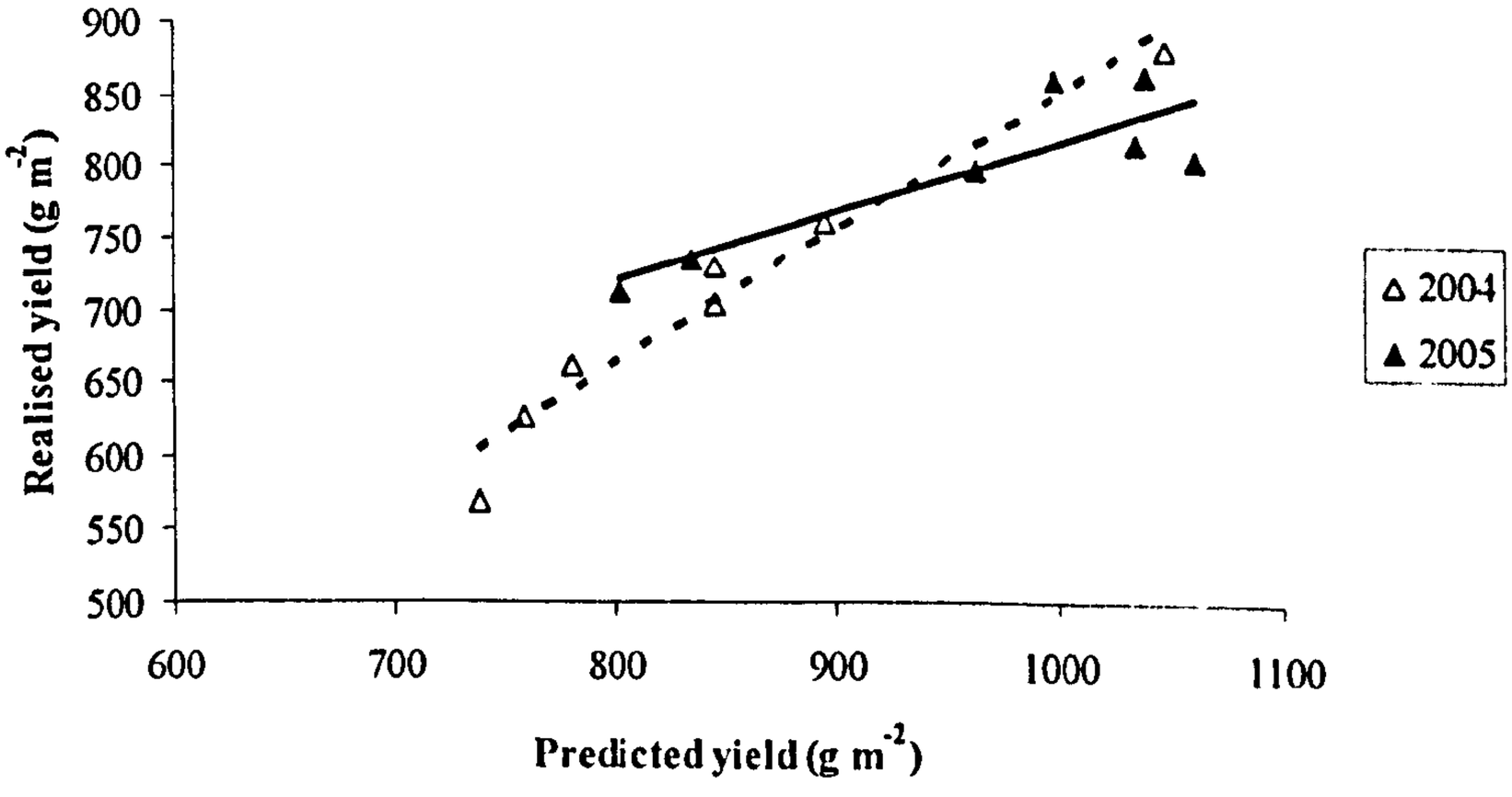


Figure 5.12 Regression of realized yield on predicted yield in 2004 and 2005 for four DH lines of the Rialto x Spark population and the two parents. 2004: $y = 0.9407x - 90.484$; $R^2 = 0.96$; 2005: $y = 0.4801x + 335.98$; $R^2 = 0.75$

Table 5.15 The effect of post-anthesis shading and genotype on source- sink balance (calculated as the difference between source and sink sizes based on individual grain weight and grain m^{-2} from growth analysis) for four Lines of Rialto x Spark DH population and the two parents in 2004 and 2005

	Source-sink balance (g m^{-2} 100% DM)		
	2004	2005	Mean
Unshaded			
Line 4	530	747	638
Line 12	347	620	484
Line 15	425	563	494
Line 26	680	646	663
Rialto	522	736	629
Spark	263	503	383
Mean	461	636	548
Shaded			
Line 4	107	-260	-76
Line 12	-69	-9	-39
Line 15	458	3	230
Line 26	-223	-90	-156
Rialto	30	262	146
Spark	105	-61	22
Mean	68	-26	21
SED (df)			
Year			105.1 (2)
Shading	64.6 (8)	69.5 (12)	65.9 (4)
Genotype	111.8 (8)	120.5 (12)	83.3 (16)
Year x shading			124.0 (3.60)
Year x genotype			150.4 (7.37)
Shading x genotype	158.1 (8)	170.4 (12)	126.1 (19.36)
Year x Shading x Genotype			196.2 (14.96)

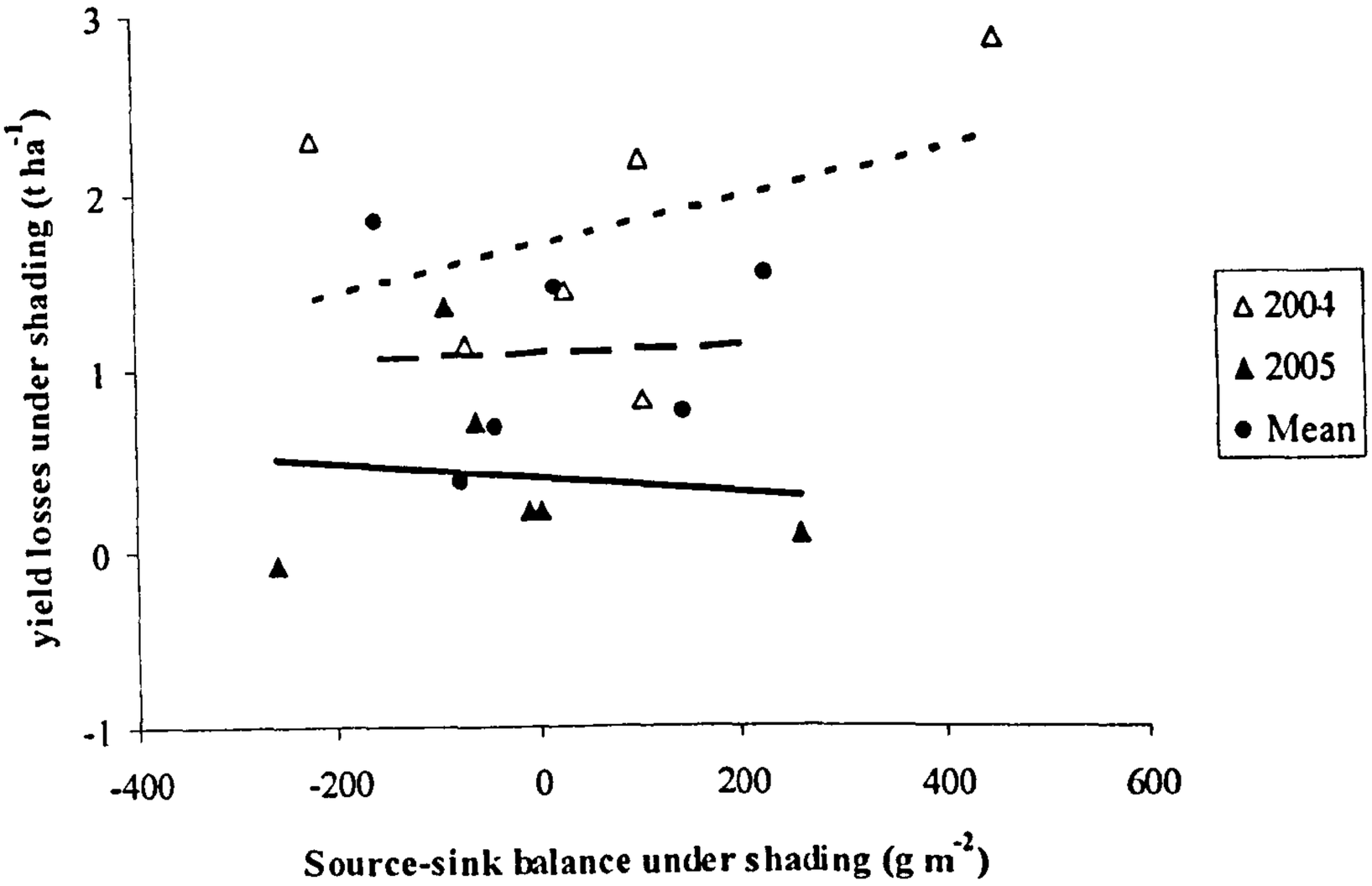


Figure 5.13 Regression of yield losses under shading (85% DM) on source-sink balance under shading in 2004 and 2005 the cross mean. 2004: y = 0.0013x + 1.6906; R^2 = 0.15; 2005: y = -0.0004x + 0.3941; R^2 =0.02; Mean: y = 0.0002x + 1.0935; R^2=0.001

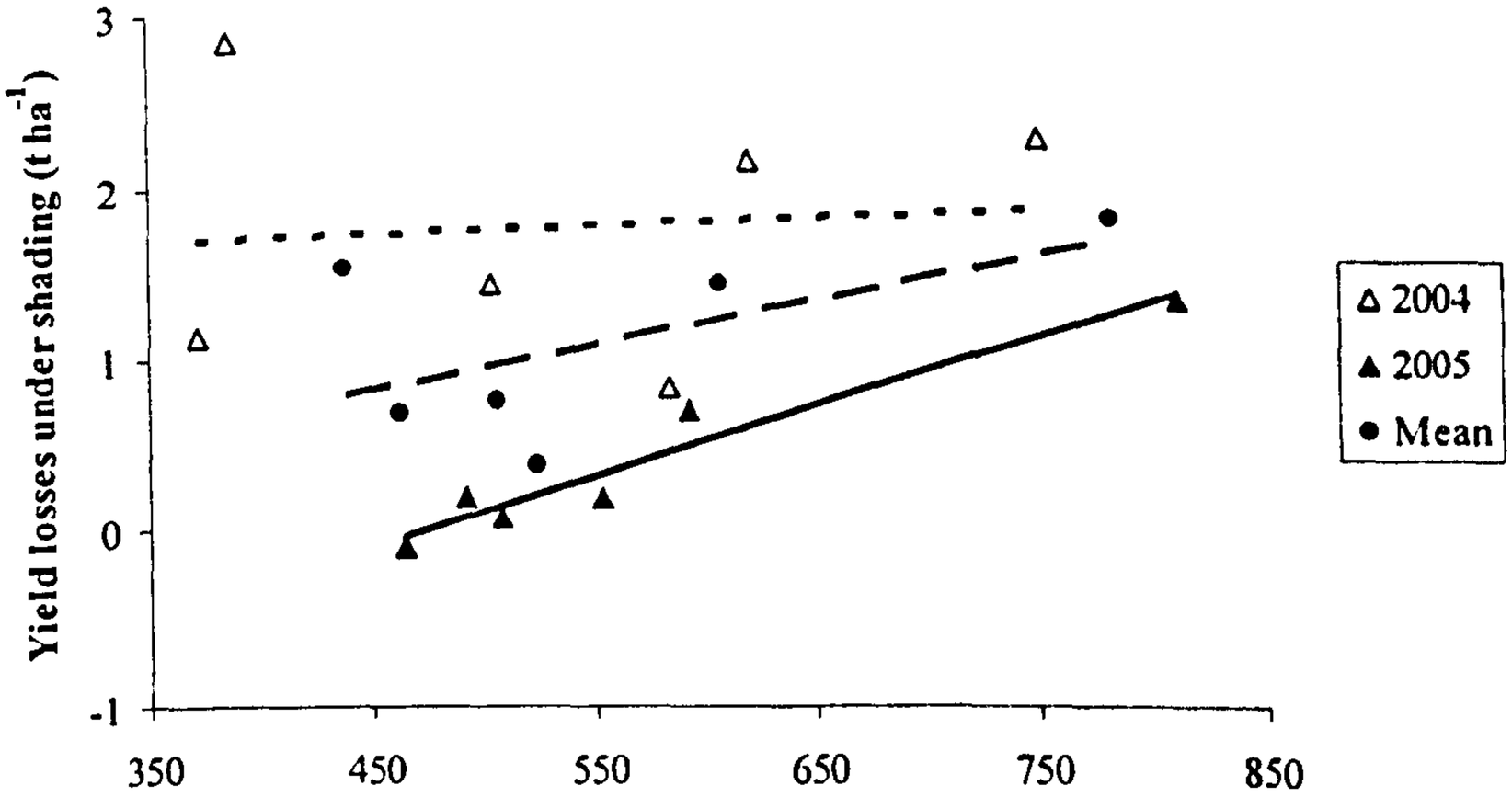


Figure 5.14 Regression of yield losses under shading on source reduction under shading in 2004 and 2005 the cross mean. 2004: y = 0.0005x + 1.5057; R^2 = 0.01; 2005: y = 0.0041x - 1.9309; R^2 =0.94; Mean: y = 0.0026x - 0.3691; R^2=0.33

5.2.8.4 Green area per grain

Green area grain^{-1} (as a possible indicator for source-sink balance) was calculated for the ten DH lines and their parents by dividing grains m^{-2} at harvest by green area per m^2 at GS61 (Table 5.16). There were no differences between genotypes in 2004, but differences were observed in 2005 (P=0.001). Cross-year analysis of variance indicated differences among genotypes in the range 0.285 (line 12) to 0.455 (line 26) (P=0.004). There was no interaction between year and genotype. There was a positive linear relationship between green area per grain and combine yield in 2004 and 2005 (P<0.05; Figure 5.15). Averaging over years, there was a positive relationship between green area per grain and combine yield (P<0.001; R^2= 0.73; Figure 5.15). However, there was no relationship between green area per grain and PGW. Line 26 produced a low PGW from a high green area per grain ratio. When regression was performed for the remaining 11 genotypes without line 26, the relationship was significant (P=0.050; R^2= 0.77)

Table 5.16 The effect of genotype on green area per grain for ten lines of Rialto x Spark DH population and the two parents for unshaded control treatment in 2004 and 2005

Genotype	Green area at GS61 per grain (cm^2)		
	2004	2005	Mean
Line 1	0.39	0.308	0.349
Line 3	0.408	0.319	0.363
Line 4	0.314	0.288	0.301
Line 11	0.344	0.289	0.317
Line 12	0.313	0.257	0.285
Line 13	0.405	0.315	0.360
Line 15	0.374	0.299	0.337
Line 17	0.318	0.262	0.290
Line 21	0.421	0.378	0.400
Line 26	0.484	0.427	0.455
Rialto	0.408	0.322	0.365
Spark	0.300	0.338	0.319
Mean	0.373	0.317	0.345
SED (df)			
Year			0.0155 (32)
Genotype	0.088	0.031	0.0379 (32)
Interaction			0.0536 (32)

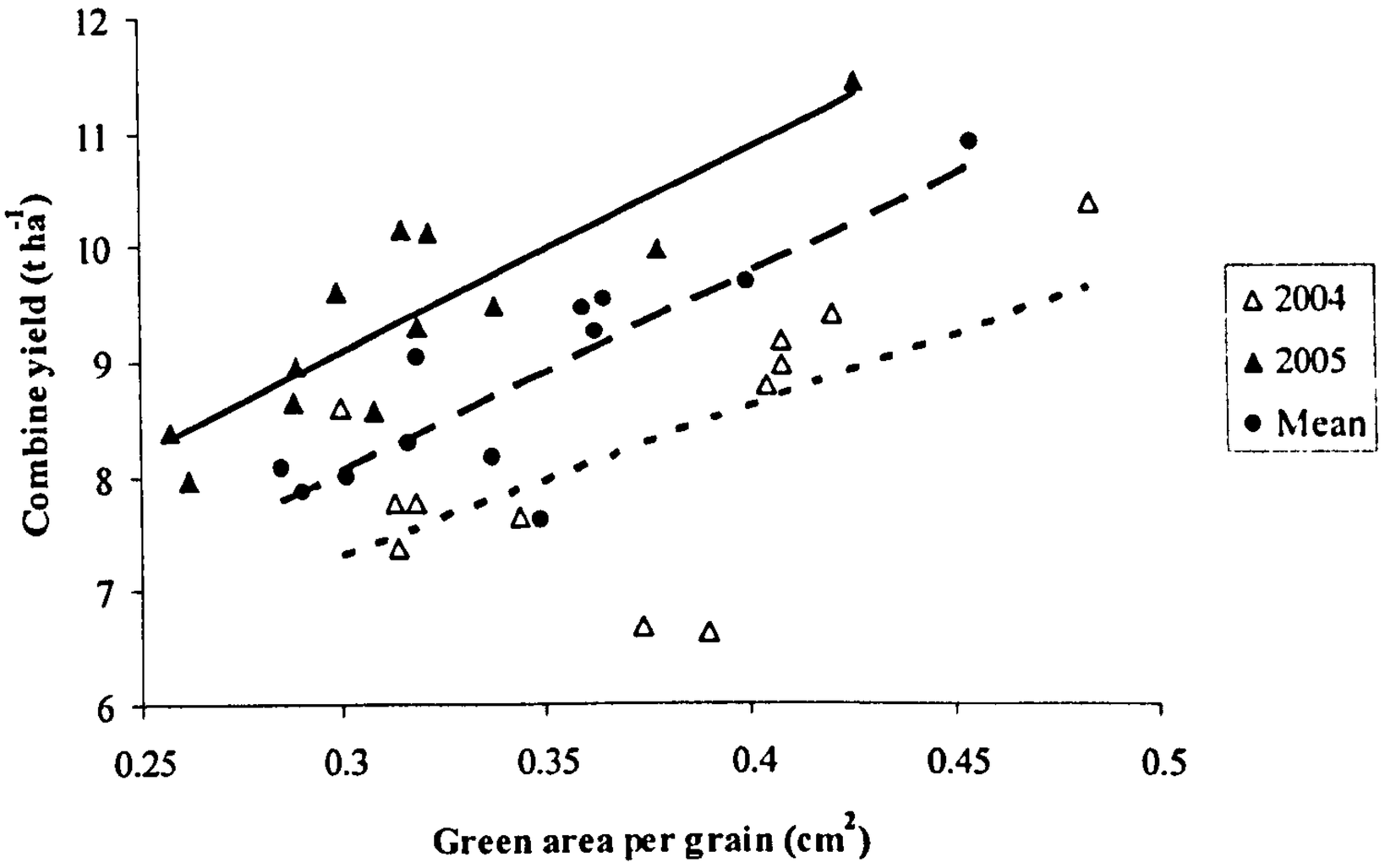


Figure 5.15 Regression of green area per grain on combine grain yield in 2004 and 2005. 2004: y = 12.564x + 3.5621; R^2= 0.38; 2005: y = 17.445x + 3.8444; R^2= 0.76; Cross year mean: y = 17.131x + 2.8983; R^2= 0.7

5.3 Discussion

This discussion aims to provide a physiological analysis of the processes limiting grain source and sink size and hence wheat yield potential during the grain-filling period amongst genotypes in the Rialto x Spark DH population. The discussion will focus on around the differences among genotypes in source and sink type traits, particularly with regard to inter-relationships with mean grain weight. The last part of this discussion will examine a novel model for quantifying genetic differences in sink size, source size and the source-sink balance. An account of the effects of the 1BL/1RS rye translocation and the *Rht-D1b* semi-dwarfing allele on grain yield and associated physiological traits in the UK environment will be presented.

5.3.1 Physiological and numerical components of grain yield

Yield assessed from hand-harvested quadrats was 12% lower than the combine yield, but the ranking among genotypes was similar. Combine yield is, principally, used in the present analysis to discuss processes underpinning yield-formation because the variability of the data is smaller as a result of the larger sampled area (Bloom, 1985; Shearman *et al.*, 2005). Also wheat is normally harvested by combines, and as such values will be more representative of the yields obtained by growers.

Shearman *et al.* (2005) reported the yield of eight historic UK winter wheat varieties to be in the range of 8.75 to 11.40 t ha $^{-1}$, and Foulkes *et al.* (2002) observed a range of 9.05 to 10.61 t ha $^{-1}$ for six modern winter wheat cultivars under irrigation. In the present study, averaged across years, the yields of this DH population showed differences in the range of 7.60 to 10.88 t ha^{-1}. The significant correlation between grain yield and HI amongst genotypes in both 2004 and 2005 and AGDM in 2004 indicated that yield differences amongst lines were mainly related to HI and to a lesser extent AGDM. Differences in HI were expected due to segregation for the semi-dwarf *Rht-D1b* gene. Biomass differences in this population are consistent with previous reports of genetic variation in biomass for modern UK winter wheat (Shearman *et al.*, 2005).

Overall, Rialto (*Rht-D1b*, 1BL/1RS) had greater HI (0.440 vs. 0.427) and a tendency for greater biomass (1854 vs. 1809 g m^{-2}) than Spark (*Rht-D1a*, 1B). This was consistent with Shearman *et al.* (2005) who reported that Rialto had high biomass. With regard to the numerical components of yield, increasing the sink capacity through grains m^{-2} was the main explanation for genetic variation amongst grain yields amongst lines of the population. More grains m^{-2} (2140 grains) were produced by the lines containing *Rht-D1b* allele (see discussion in chapter 4). However, Spark (*Rht-D1a*) breaks the trend for semi-dwarf lines to have more grains m^{-2} which was associated with high tiller production. Previous reports (DEFRA, 2001; 2002) showed large differences in grain number and grain size for Rialto and Spark, in which Spark had more grains m^{-2} and lighter grains. As such, higher-yielding genotypes (e.g. Lines 26 and Rialto), which contained the *Rht-D1b* allele, had better photosynthetic assimilate partitioning to the developing ears, allowing the setting of more grains m^{-2}, indeed present results showed that Rialto (*Rht-D1b*) had greater floret fertility than Spark (*Rht-D1a*) which was an outcome of having heavier spikelets. Thus, more assimilate was allocated to the ear during the rapid ear growth phase, the higher the number of fertile florets, hence, more grains m^{-2}. Grain yield for this modern DH population was phenotypically correlated with the grains m^{-2} in 2004 ($R^2 = 0.71$, $P < 0.001$) and in 2005 ($R^2 = 0.84$, $P < 0.001$). This suggests that the capacity of the grains to accommodate assimilates (i.e. sink strength) was of greater importance for determining yield than the supply of assimilates to growing grains (i.e. source strength) as found by Thorne and Wood (1987). Present results showed genetic differences in PGW. However, no correlation was detected between yield and either PGW or MGW in either year.

Recent investigations showed that genetic gains in winter wheat yields are primarily due to improvement in grains m^{-2} in the UK (Shearman *et al.*, 2005), France (Brancourt-Hulmel *et al.*, 2003) and the Great Plains (Donmez *et al.*, 2001). This is consistent with the relationship between yield and yield components in the present study. The differences in grains ear^{-1} were the main reason for the differences amongst lines of the Rialto x Spark population in grains m^{-2}. Indeed, present results

showed that *Rht-D1b*, on average, accounted for 69% of the variation in grains m^{-2}. Shearman *et al.* (2005) reported that the ears m^{-2} increased linearly during the period from1972 to 1995 in UK winter wheat. Genotypic differences in ears m^{-2} existed in the present study ($P<0.001$). However, there was no relationship between ears m^{-2} and grains m^{-2}.

Similar to previous studies on spring and winter wheat worldwide (Carver and Rayburn, 1994; Villareal *et al.*, 1995, 1998; Ehdaie *et al.*, 2003), the present results showed a positive grain yield response to 1BL/1RS in optimum conditions. Furthermore, the increase in AGDM accumulation pre and post-anthesis (section 4.6.1) with 1BL/1RS was associated with an increase in grains m^{-2} of 1156 m^{-2}. Thus, the increase in grain number (5.5%) conferred by 1BL/1RS was realized in grain yield. In UK winter wheat, there is evidence that 1BL/1RS confers greater accumulation of stem soluble carbohydrate reserves (SSC), a trait associated with yield potential in the population and in the UK. In general, analysis of yield components supported sink limitation. A more detailed analysis of differences in source-sink balance amongst the population between grain number versus grain weight is presented later in this discussion.

5.3.2 Grain number versus grain weight

The negative relationship between grain number and weight is a common phenomenon (Acreche and Slafer, 2006; Reynolds *et al.*, 2005; Shearman *et al.*, 2005; Slafer *et al.*, 1994). Most results show compensatory processes between grain number and weight. This entails that the success of wheat breeders in constantly increasing the number of grains m^{-2} was slightly compensated by associated (but relatively smaller) reduction in the average weight of grains. It is thus very important to understand the causes of such a negative relationship as this understanding will affect future wheat breeding progress. Present study showed a negative relationship between grains m^{-2} and MGW. The increase in the competition for assimilate between the grains with increasing grain number is unlikely to explain this relationship. The source-sink balance model showed the characterized genotypes to be sink-limited by numbers of grains during post-anthesis growth. Thus, the

availability of assimilates would not likely alter this negative relationship (further details from de-graining experiments will be presented in Chapter 6). So, the reduction in grain weight with increased grain number was apparently independent of the level of competition; and the negative relationship could occur because of more grains being placed in positions with less grain weight potential (e.g. distal positions) and thus reducing average individual grain weight (Miralles and Slafer, 1995b). This effect of grain position within the ear will be discussed in detail in the following chapter.

Averaged over years, some genotypes had heavier grains (e.g. Lines 4, 5, 6, 8, 11, 12; 16, 19, 22, 23, and Rialto) while others had more grains (e.g. Lines 3, 7, 8, 9, 10, 13, 21 and 26). Thus, there was a negative linear relationship between grain number and grain size. However, Line 8 showed a higher grain number as well as grain weight. Therefore, further study may be justified to investigate the uncoupling between greater grain weight and higher number of grains. Line 8 was not one of the 12 genotypes in this study for intensive analysis.

5.3.3 Harvest index and above-ground biomass

A theoretical upper limit to HI of 0.62 for winter wheat grown in UK conditions was suggested by Austin (1980) based on the dry weight of component organs from the average of four high-yielding cultivars (Austin *et al.*, 1980a). It was assumed: (i) constant harvest AGDM; (ii) lamina and chaff dry weight could not be reduced because of physiological and mechanical considerations, respectively; (iii) stem and sheath dry weight could be reduced by 50%; and (iv) chaff weight could be increased pro rata to accommodate the extra grain. Shearman *et al.* (2005) modified this theoretical limit in the UK for future years to be 0.66. They maintain all the assumptions except for increasing harvest AGDM by 20%. In the present study, a maximum HI of 0.50 was observed for Line 3 in 2004. Other recent UK investigations have showed a HI of 0.53 for Riband, released in 1985 (Shearman *et al.*, 2005), and 0.61 for Consort, released in 1995 (Spink *et al.*, 2000). In summary, it seems that further improvement of HI in the UK environment may be possible but that it is likely to be only moderate in magnitude.

As expected, the six semi-dwarf genotypes (*Rht-D1b*) had significantly higher harvest indices than the tall (*Rht-D1a*) genotypes. The *Rht-D1b* allele confers insensitivity to gibberellic acid during the stem-extension phase which leads to increased partitioning of biomass to ears and higher spikelet fertility (Gale and Youssefian, 1985). Both *Rht-D1b* allele and the 1BL/1RS chromosome on average positively affected AGDM by 4 and 7% (P= 0.088 and 0.016), respectively.

Anthesis AGDM differed amongst genotypes and was positively associated with the duration between GS31 and GS61 implying a relationship between light interception during that period and AGDM. No correlation was observed between pre-anthesis RUE and grain yield in 2005. This was strongly affected by both Lines 1 and 21. When pre-anthesis RUE was regressed against grain yield (excluding Lines 1 and 21), the relationship become positive (P=0.045). Moreover, there was a trend (P=0.063; R^2 = 0.37) in 2006 when RUE was regressed against grain yield for the ten DH lines excluding their parents implying that RUE may have been a factor contributing to the increase in grain yield. Pre-anthesis RUE was positively associated with anthesis AGDM in 2005 and showed a trend for positive correlation in 2006 (P=0.085; R^2=0.27), whereas effects of canopy size and K_{PAR} on anthesis biomass were neutral. Similarly, Calderini *et al.* (1999) reported that leaf area index and light extinction coefficient are poorly correlated with yield progress in wheat. Present findings indicated that harvest AGDM did not vary amongst the ten lines and their parents. However, AGDM was positively associated with grain yield amongst the genotypes. These results appears contradictory and it can be concluded that there was probably a genetic effect on harvest biomass but that hand-harvested sample areas were insufficiently large to detect this with statistical confidence.

5.3.4 Grain source size

Under normal growing conditions, about half of photosynthate moved into the grain from current photosynthesis originates from the flag leaf with the remainder contributed by the ear, leaf sheaths and penultimate leaf (Rawson *et al.*, 1983). Therefore, the rate and/or duration of grain growth are, at least potentially, limited

by the rate and /or duration of photosynthate production by the flag leaf during grain fill. Because breeders continue to select for larger ears, this may be at the expense of flag leaf size and modern varieties may be therefore closer to source-limitation. Shearman *et al.* (2005) reported that GAI was not changed with year of introduction but modern UK genotypes had smaller flag leaves. Those authors concluded that the source size had tracked increases in sink size. So there is a need to identify ways of simultaneously increasing grain source and sink sizes. In the present study, there were genotypic differences in both pre-anthesis RUE and stem WSC and these resulted in higher source size. These findings were consistent with Shearman *et al.* (2005) who reported an increase in both RUE and WSC with breeding in the UK.

By anthesis, reserves of water-soluble carbohydrate, mostly as fructans, have accumulated in the stem and leaf sheaths of the crop. Maximal amounts are accumulated shortly after flowering (Foulkes *et al.*, 2002). A significant proportion of these stem soluble carbohydrate reserves can be subsequently retranslocated to grains under stress conditions to buffer effects of accelerated senescence (Schnyder, 1993). The amount of stem soluble carbohydrate accumulated has been shown to vary amongst UK-grown commercial cultivars from 250 – 400 g m^{-2} (Foulkes *et al.*, 1998). Shearman *et al.* (2005) found a significant relationship between stem WSC and grain yield under optimal conditions. This was supported, to some extent, by the present findings in which stem WSC was positively correlated with grain yield in 2006 ($P<0.001$).

In the current study, there were genotypic differences in the amount of soluble stem carbohydrate at GS61 + 75 °Cd in the range 243 (Spark) to 487 (Rialto) g m^{-2}. As expected, Rialto had a high ranking, consistent with the findings of Shearman *et al.* (2005), suggesting consistency of genotype ranking across environments and high heritability for this trait. The high stem reserves for Rialto might be associated with a longer thermal duration from mid-stem elongation to the end of flowering which could enable it to lay down more reserve photosynthate. There was no relationship between stem reserves and either PAR intercepted between GS31 and GS61 or pre-anthesis

RUE in either year. The presence of a positive linear regression (P=0.02; R^2= 0.43) between ear DM and stems WSC reserves at anthesis in 2005, together with a non-significant relationship between them in 2006, suggest that there is no evidence for competition between these sinks in modern UK germplasm. This is encouraging for future breeding progress.

Pre-anthesis RUE was positively associated with source size in 2004 (P=0.047) and accounted for 58.5% of the phenotypic variation in source size amongst the genotypes. However, there was no association between post anthesis source size and stem WSC reserves in either year. This suggests that enhancing pre-anthesis RUE will be important in increasing the size of the source in future years. Another avenue to increase the source without detrimentally affecting the sink size would be by extending the thermal duration between GS31 and GS61 by manipulating the genes controlling the thermal duration of this period, namely photoperiod and vernalization genes (Gonzalez *et al.*, 2003).

Genotypes possessing the 1BL/1RS translocation had higher stem reserves than non-1BL/1RS genotypes. There was also evidence of a trend for higher soluble stem carbohydrate with *Rht-D1b* semi-dwarf varieties. For example, the taller non-dwarfs such as Spark had consistently low stem reserves.

5.3.5 Grain sink size

The observed differences between genotypes for both MGW and PGW in this study are consistent with previous findings in the UK (e.g. Shearman *et al.*, 2005). In contrast to grains m^{-2}, however, results showed no statistically significant relationship between MGW and grain yield in either year. Genetic variation in grain weight generally has less effect on genetic variation in wheat yield than grain number (Slafer, Satorre and Andrade, 1993; Slafer *et al.*, 2005). The lack of association between grain weight and yield is consistent with the observation that there has been little success at the simultaneous increase in both grains m^{-2} and grain weight in wheat (Slafer *et al.*, 1993). Indeed, the present study observed a negative relationship between grains m^{-2} and MGW or PGW.

PGW was consistently lower in 2004 than in 2005. The duration between GS31-GS61 in 2005 was 10 d longer than in 2004 (see chapter 4). Dry matter per ear was greater at anthesis in 2005 hence, positively affecting the carpel weight and the size of the endosperm cells. A significant linear regression of PGW on combine MGW amongst genotypes was found in both years. A slight overestimation of PGW could have resulted from of a bias in the sampling procedure through selecting larger than average ears for PGW determination. Despite this potential bias, the relationship between MGW and PGW is encouraging since it may enable the grower to predict the grain weight from simple measurements of the crop shortly after anthesis, thus potentially allowing modifications to be made to crop husbandry (Bingham *et al.*, 2006b). For example, adjustment of late season fungicide application according to the risk of poor grain filling and high screenings may be possible. Green area per grain at anthesis, which might provide a useful indication to the source supply during endosperm cell division, was not related to MGW in the present study. However, radiation interception per grain was calculated for the period of 10 d after anthesis in 2004 and 2005 differed between the six genotypes ($P<0.05$). Moreover, radiation interception per grain showed a trend for a positive relationship with PGW in 2004 ($P=0.089$; $R^2=0.55$) and 2005 ($P=0.093$; $R^2= 0.54$). There are some avenues to increase grain number without affecting grain weight such as removing the vascular restrictions and improving the vascular traces, the activity of starch synthesas enzyme, extending the thermal duration of cell division and expansion and Selection for long ear phenotype which will offer longer distance between spikelets and enhancing individual glume size.

5.3.6 The source-sink balance model

Photo-assimilates for grain-filling originate from both current assimilation and stored carbohydrates. Calculation of PAR absorption and RUE post-anthesis is difficult owing to the fact that non-photosynthetic senescent tissue absorbs some PAR that could result in an over-estimation of PAR interception. However, as canopy senescence begins from the bottom upwards, i.e. lower leaves senesce first, this might be assumed to be a minor confounding factor. The extinction coefficient

should remain approximately the same as the canopy senesces. The use of grains m^{-2} at harvest in the calculation of sink size seems justified because of the strong relationship between grains m^{-2} at harvest and ear dry weight m^{-2} at anthesis in both years ($P<0.05$, section 3.1.1), which indicated that the final grain number was determined prior to anthesis.

The use of stem reserves to calculate source-size is likely to have overestimated source size to some extent. This is because not all stem reserves will be remobilised to the grain. However, in general, the use of pre-anthesis RUE is probably justified, because a significant decrease in RUE would only be expected towards the end of grain filling (Sinclair and Mochow, 1999; Shearman *et al.*, 2005; Bingham *et al.*, 2006a). Furthermore, Calderini *et al.* (1997) examined a set of historic Argentinean winter wheat cultivars released between 1920 and 1990 and concluded that modern cultivars tended to have a very small reduction, or even to maintain their pre-anthesis RUE, during the grain filling period. Similarly, Green (1989) found that modern wheat cultivars maintained their pre-anthesis levels of RUE during most of the grain filling period. The pre-anthesis RUE is independent of sink size and components, so it will in this respect produce a better estimate of potential source size. These reasons indicated the need to use pre-anthesis RUE in this model for the present study to be justified. However, RUE could be modified according to sink strength. There was a closer relationship between sink size and realised yield than between source size and realised yield for the four lines and their parents in both years. This suggests that these four genotypes and their parents were probably sink-limited. The post-anthesis source size is in practice likely to be affected by the sink size. Indeed, recent evidence was reported that post-anthesis RUE can be increased by sink demand (Reynolds *et al.*, 1999; 2000; 2005). Through feedback mechanisms, photosynthesis may be suppressed where grain number per ear is limited, and a large sink size could increase post-anthesis RUE, and lead to more complete remobilisation of stem reserves (Miralles and Slafer, 1997; Gebbing *et al.*, 1999; Reynolds *et al.*, 2005). So, the model should be amended to take this in account.

Measurements of potential grain weight (taken 4 weeks post-anthesis) and grains m^{-2} can be useful in predicting yield. This is supported in the present study by a significant relationship between the realised yield and the predicted yield from the source-sink balance model. The original hypothesis that the UK's modern wheat germplasm was sink-limited has been confirmed by the present results. So, there is a need to identify strategies to improve grain number (such as lengthening the thermal time of the rapid ear growth phase) and potential grain weight (e.g. increasing the lag phase between anthesis and start of grain filling (cell expansion period)). This will be examined in more detail in the next chapters. But we don't know what happened during the last 10 years since Rialto was released in 1995. The hypothesis that yields of the Rialto x Spark lines were sink-limited was further tested by imposing post-anthesis shading on selected genotypes.

Even though the four lines and their parents apparently had the same extent of sink limitation, as indicated by the insignificant differences among these genotypes, there was a trend (non-significant) for some genotypes to be closer to source limitation (e.g. Line 26). Therefore, there is a need to improve the source size together with the sink in future breeding programmes.

5.3.6.1 Responses to shading

Shading was imposed form GS61 +20 d until canopy senescence on four lines as well as the parents. Shading only affected source size in these experiments. Genotypic differences in response to manipulation treatments were hypothesized to arise from differences in source-sink balances. At maturity, the effect of shading on total stem and sheath dry weight was not significant in both years and shading did not increase the amount of stem dry matter lost per m^{-2} during grain filling. This means that most of stem reserves were used even in absence of shading, and there was little scope to increase utilization under shading. Genotypic differences were found in both years in stem sugars, but with no interaction between shading and genotype. The contribution of stem reserves to yield showed a small (not significant) increase in the contribution of stem reserves to the grains.

Because grain yield of the six shaded genotypes was measured by hand-harvesting, it was compared with the unshaded yield obtained by hand-harvesting yield. Yield from shaded plots (50% radiation restriction) was significantly reduced compared to unshaded plants, due to a reduction in mean grain weight. Many authors, e.g. Willey and Holliday (1971); Bremner (1972); Gifford, Bremner and Jones, (1973); Fischer (1975); Fischer and HilleRisLambers (1978); Jenner (1979); Stockman *et al.* (1983); Grabau *et al.* (1990); Grashoff and d'Antuono (1997) reported that post-anthesis shading mainly reduced MGW. In the present study, shading averaging over years reduced MGW by 7%. This small effect implies that Rialto still showing significant extent of sink limitation. The reason for this reduction was the reduction in post-anthesis PAR interception ($P<0.001$). Stem WSC retranslocation was slightly increased by shading (Fischer and HilleRisLambers, 1978), i.e. compensation by increased translocation from other organs. However, a 40% reduction in post-anthesis interception led to only 14% reduction in grain yield. But source may not have been decreased by 40% due to the increase in RUE.

Many authors have studied the role of pre-anthesis stem reserves on buffering grain yield under conditions that adversely affect photosynthesis (e.g. Gallagher, Biscoe and Hunter, 1976; Bidinger, Mosgrave and Fischer, 1977; Austin *et al.*, 1980b; Savin and Slafer, 1991). They reported that the contribution ranged from 17-44% under dry conditions and from 11-49% under normal conditions. Additionally Savin and Slafer (1991) studied the effect of shading on one Argentinean wheat cultivar and reported that 49% of stem reserves contributed to the ear growth under unshaded condition compared to 38% contributed when the cultivar shaded from anthesis to maturity. With regard to genetic differences, smaller differences were obtained in the present study. In 2005 genotypes ranged from 28.5-48.5% for unshaded treatment and from 30.6-43.8% for shading treatment. However, the ranges were wider in 2004 (7.0-49.7% and 6.1-42.9% for unshaded and shading treatments respectively). The existence of the genotypic differences in both years implied that the contribution of stem reserves is strongly influenced by genetic potential of genotypes rather than the effect of shading treatment itself. This was supported by the non-significant effect of shading in both years ($P>0.10$). Post-anthesis shading

significantly reduced total biomass production in 2004. In general, averaged overall genotypes, the contribution of stem reserves was the same under both shaded and unshaded treatments, however, there is a differences between the individual genotypes in the amount of the stem WSC reserves contributed to the grains. Only 73% of vegetative dry matter lost was contributed to grains as indicated by Austin *et al.* (1977). Thus, applying this correction factor to our data showed that the contribution of the pre-anthesis assimilates to the grains in the control treatments became 17.6% in 2004 and 28.9% in 2005. Similarly it became 20.9% and 29.5% in shading treatment for 2004 and 2005 respectively. These values were close to those obtained by Savin and Slafer (1991). By deduction, post-anthesis RUE under shading must have increased. Indeed, post-anthesis RUE for shaded treatment in 2005 was 20% (increased by 0.21 g MJ^{-1}) higher than that for unshaded treatment implying that post-anthesis RUE responds to increase in sink size.

5.3.6.2 The development of the source-sink balance model:

2005 data was more reliable than 2004, so more emphasis will be placed on the 2005 data. The source-sink balance model predicted a trend for a shift toward source limitation in 2005. The fact that Line 15 was still sink- limited and Rialto become slightly more source-limited compared to other genotypes suggests that this may have been due to their good ability to compensate under stress compared to other genotypes. Indeed these genotypes relocate more than 45% of the pre-anthesis reserves to the grains. The model could be used in breeding and also in agronomy because it enables the researchers to have early assessment of the source and sink and consequently their balance of the crop which may offer opportunity for modifying crop management.

1. Stem reserves

The calculation of source size has used the maximum stem WSC, as this represents the maximum contribution available to the potential source of the crop. However, Gebbing, Schnyder, and Kuhbauch, (1998) reported that there is commonly about 75% efficiency in remobilization of these reserves in winter wheat. Similarly, Austin

et al. (1977) reported that only about 73% of pre-anthesis reserves might contribute to the developing grains. So, the model need to adjust to take in the account that only about 73% and not 100% of reserves were contributed to the grains.

2. RUE

Shearman (2001) reported that shading resulted in a large increase in post-anthesis RUE for four varieties. Similarly Marshall and Willey (1983) reported an increase in RUE by 46% for groundnut when intercropped and consequently shaded by pearl millet. In the present study, post-anthesis RUE was increased by 20% in 2005 in response to shading. This implied that RUE is positively responds to greater sink size. Reynolds *et al.* (2005) reported that sink demand will increase post-anthesis RUE.

In the present study, the pre-anthesis RUE was applied to calculate the source size, by using the assumption that RUE was kept stable or slightly decreased over the grain filling period. So, on one hand the use of pre-anthesis RUE in the calculation of source size for the unmanipulated crops might be justified. On the other hand, it would be underestimation in calculation of the source size for shaded crop. This needs further discussion regarding the effect of shading on both rate of grain growth and final grain weight. The model should take in the account that pre-anthesis RUE did not remained on the same level post-anthesis as it is affected by the strength of the sink. So breeders should try to find a quick method (such as image analysis) to measure the number of grains shortly after anthesis which will enable them to predict the change in RUE during grain filling.

5.3.7 Summary

Under normal field conditions, the potential sink capacity was lower than potential source availability which means that the crop is sink-limited and no trend is available toward source limitation. In a similar vein, Slafer and Savin (1994) compared the results of several source sink balance manipulation experiments, and found no evidence of increasing source limitation with breeding.

The efficiency of the remaining source organs were continuously adjusted by the plant. This allows the plant to produce and use the assimilation after imposing the source manipulation. This behaviour increases the complication of the source-sink manipulation experiments, which may result in feedback to increase stomatal conductance and hence RUE (Richards, 1996). Genotypes may respond to manipulation by increasing the remobilization of stem reserves, increasing post-anthesis RUE or delaying senescence.

6 Effect of genotype and grain position on duration and rate of grain growth

Grain weight is the main yield component together with number of grains per unit area. Potential grain weight is determined shortly before anthesis and during early stages of grain growth (Calderini *et al.*, 1999b). Carpel growth immediately before anthesis is crucial in the determination of an upper limit to grain growth (Calderini *et al.*, 1999b; Calderini and Reynolds, 2000). This means that improvement in carpel growth may lead to greater mean grain weight by raising the final weight of the smaller grains within the ears (Calderini *et al.*, 2001). Genotypic differences in final grain weight at maturity have been related to the number and size of endosperm cells per grain formed during the first 2-3 weeks after anthesis (Brocklehurst, 1977; Nicolas *et al.*, 1985). By 2-3 weeks post-anthesis, endosperm cell division is complete and synchronised with termination of rapid net water deposition in the grain (Nicolas *et al.*, 1985). During the period of endosperm cell division, the volumetric growth of the wheat grain is determined largely by the net deposition of water. Additionally, differences in final grain weight also relate to the number of A-type starch granules (Chojecki, Gale and Bayliss, 1986) that are initiated during the first two weeks of grain development (Baruch *et al.*, 1979). The rate of grain growth depends on grain position within the ear, varies between cultivars (genotypes with larger grains at maturity having a faster rate), and increases with rise in temperature (Sofield *et al.*, 1977). In this chapter, the relationship between potential grain weight and final grain dry weight as well as both rate and duration of grain growth has been investigated in apical, central and basal grain positions within the ears for the parental lines Rialto and Spark. Response to post-anthesis shading on the above-mentioned traits will be studied (see Chapter 5). Many authors have argued that genetic yield potential of wheat is sink limited (e.g. Reynolds *et al.*, 2005). Evidence from the present study in Chapter 5 indicated that yield was sink-limited in the lines of Rialto x Spark population. Post-anthesis de-graining was applied by removing 50% of the spikelets from one side of the ear for a further assessment for sink

limitation in the present chapter. Responses of the grains from apical, central and basal locations to sink manipulation were investigated.

Objectives

1. To quantify the relationship between grain water content and final grain weight in two winter wheat genotypes known to differ in mature grain weight and to quantify effects in different pre-determined grain positions within the ear under both unshaded and post-anthesis shaded conditions.

2. To quantify the effect of sink manipulation (post-anthesis de-graining) on the grain weight of twelve genotypes widely differing in grain number per ear and to identify the major physiological factors responsible for any differences in responses to de-graining.

3. To quantify the effect of individual grain position within the ear on potential grain weight, rate and duration of grain growth and final grain weight for Rialto and Spark.

4. To quantify response of individual grain positions to de-graining and shading indicative of source and/or sink limitations to grain growth.

6.1 Materials and methods:

6.1.1 Post-anthesis source: sink manipulation treatments

Shading was imposed post-anthesis as described previously to the two parents (Chapter 5). Post-anthesis de-graining was applied for the ten DH lines and their parents (lines 1, 3, 4, 11, 12, 13, 15, 17, 21, 26, Rialto and Spark) in 2004 and 2005 (in order to increase source supply relative to sink). Ten shoots per sub-plot in the unshaded treatment were de-grained by removing 50% of the spikelets (i.e. all spikelets down one side of ear) at GS61 + 220°Cd (base temp, 0°C)).

6.1.2 Grain water content

Rialto and Spark represented extremes in grains m^{-2} and mature grain weight within the population and therefore were selected for detailed measurements to investigate mechanisms determining individual grain weight including grain water content in 2004 and 2005. Ten ears per sub-plot from both the unshaded and shaded treatments were sampled at weekly intervals starting from two weeks after anthesis until harvest. On each occasion three central, one apical and one basal grain were excised from ears (see Figure 5.1). The fresh weight and dry weight of the bulked grains from each position were measured. Water content was calculated as the difference between fresh weight and dry weight. Grain water content for the data from individual sub-plots was regressed against thermal time (base temp. 0°C) using a quadratic relationship which gave the overall best fit. A separate curve was fitted to the data for each sub plot. Only when $R^2 \geq 0.70$ was the quadratic curve then used to estimate values of the maximum water content to be used in the analysis of variance. Maximum water content per grain and the thermal time from anthesis required to reach the maximum water content was calculated. The quadratic relationship was expressed in the following equation:

$Y = a + bx + cx^2$.....Equation 6.1

Where

Y: is the grain water content (mg)

x: is the thermal time (°Cd)

The value of x at the time of maximum water content was calculated as: $x = -b/(2c)$ when

$dy/dx = 0$.

Then the maximum water content was calculated by applying the calculated maximum x value in equation 6.1.

6.1.3 Ear/grain dry matter growth

Analysis of ear biomass growth and individual grain growth through grain filling was carried out for Rialto and Spark only in 2004 and 2005. The same samples as used for grain water content were used for grain dry matter determination. Growth of individual ears and grains were analyzed against thermal time from GS61 (base temp. 0 °C) by assuming a linear rate of growth from GS61+ 220 °Cd until the weight reached a plateau (slope = 0). For the data from each sub-plot, a regression model (broken stick model) which fitted two straight lines to the data was used to determine the end of linear ear/grain growth, the rate of the linear increase and the final grain weight. These values were then subjected to analysis of variance.

The broken-stick model equation is:

Y= ones +U * (X-t).....Equation 6.2

Where

't' is the point where the two lines meet (giving the date, in thermal time, of the end of grain filling).

'U' is the gradient of the first line (giving the rate of grain growth).

'Ones' is the value of the grain weight at the point of intersection (giving the final grain weight).

(See Appendices III and IV for details of the relevant Genstat program).

6.2 Results

In this section processes affecting the development and subsequent filling of grain determined between anthesis and harvest are quantified for both Rialto and Spark. It specifically addresses grain growth for five pre-determined grain positions within the ear under normal conditions as well as under 50% radiation reduction restriction. Finally, responses to sink manipulation, by removing all the spikelets from one side of the ear during grain filling, for the ten DH lines and their parents are analysed

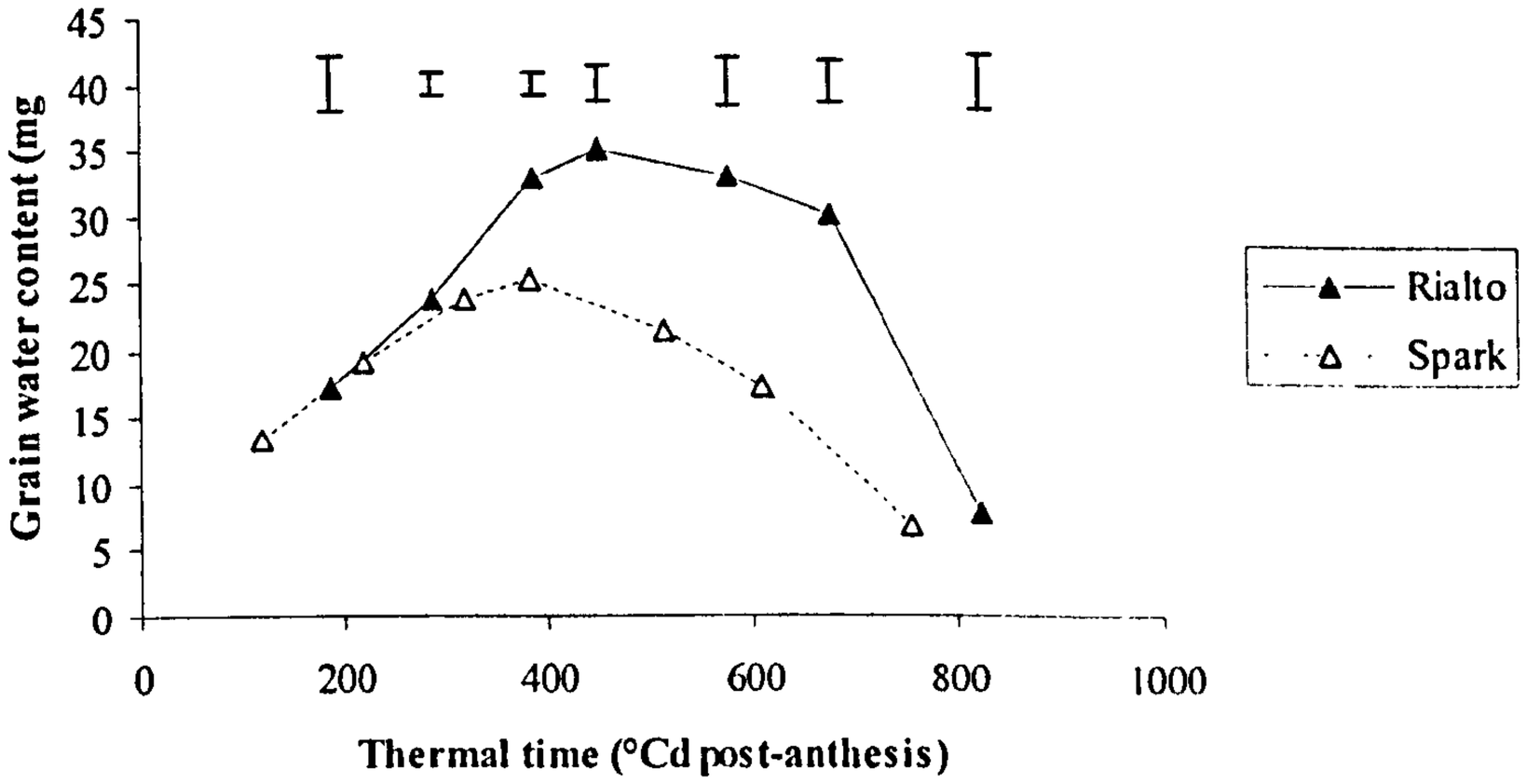
6.2.1 Individual grain moisture content

It is important to measure grain water content because endosperm cells are filled by water early in the grain filling period establishing maximum grain volume, and then water is replaced by assimilate (Schnyder and Baum, 1992). This means that in sink-limited crops final grain weight should be correlated with the grain water content about three weeks after anthesis. The achievement of maximum grain dry matter is approximately coincident with the onset of the rapid decline in water content (Pieta Filho and Ellis, 1991; Schnyder and Baum, 1992).

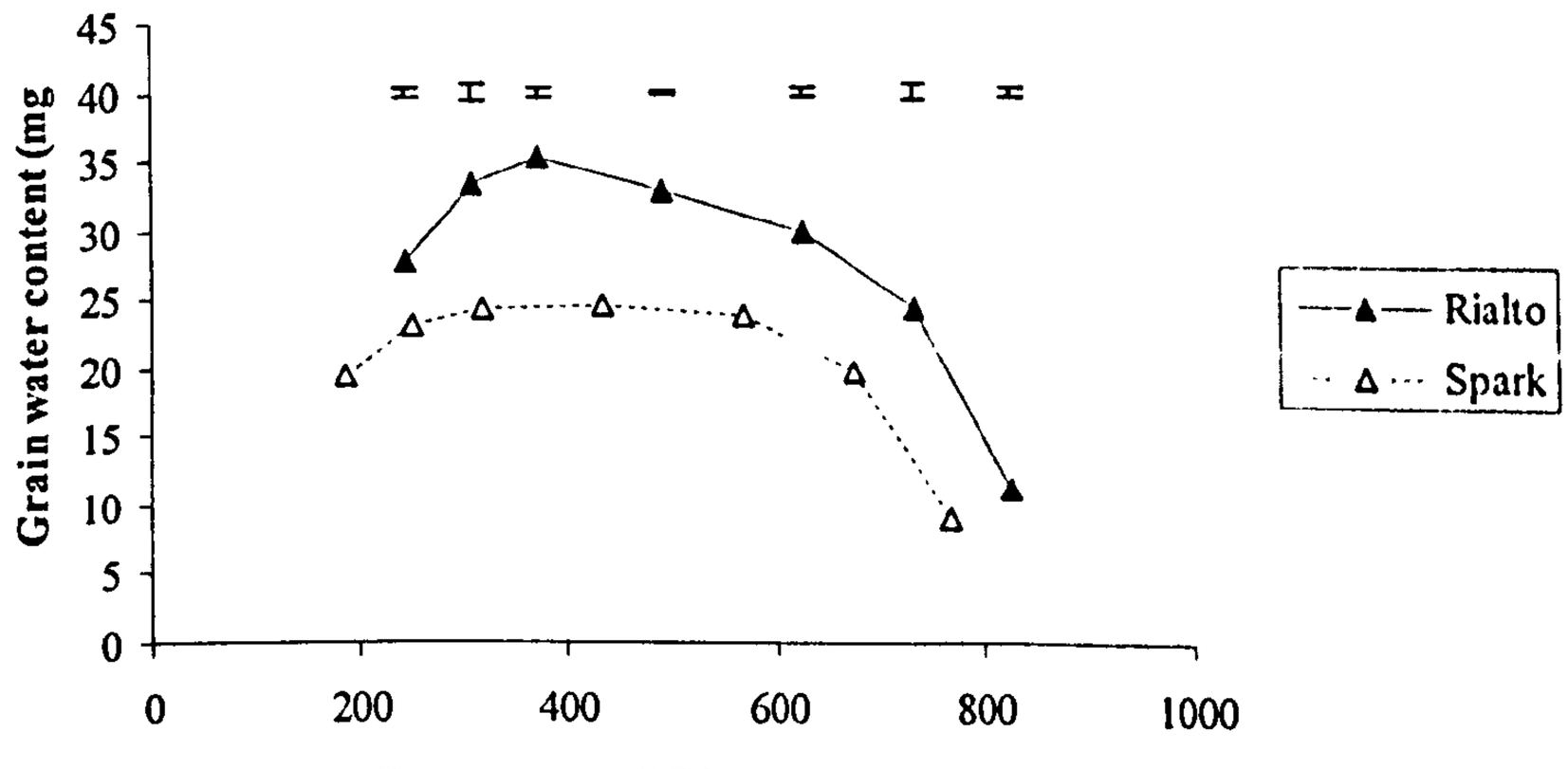
Figure 6.1 shows that grain water content increased initially in the grain filling period (up to 400 °Cd post-anthesis), beyond which it started declining until maturity. Averaging across the five grain positions, Rialto had greater maximum water content (M) than Spark in both years ($P<0.005$; Figure 6.1; Table 6.1). Cross-year analysis of variance showed differences ($P<0.05$) between the parents and among grain positions and indicated their interaction was significant. Grains of Rialto contained 35.8 mg of water while those of Spark contained 26.4 mg. This was consistent with a longer thermal time from GS61 to reach maximum water content for Rialto (460.7 °Cd) compared to Spark (422.3 °Cd) ($P<0.05$). Grain position within the ear affected maximum water content in both years and the thermal time to reach M in 2005 ($P<0.05$). The apical grain (G5) had a smaller maximum water content and a shorter thermal duration to M (22.2 mg and 428.2 °Cd, respectively) than both basal and central grains. However, the central grains (particularly G1 and G2) had the greater M. Grain water content for G2, G3 and G4 and thermal time to

reach M for G1, G2, G3, G4 and G5 was greater for Rialto than Spark in 2004 ($P<0.05$). Shading did not affect maximum grain water content in either year ($P>0.05$; Table 6.1; Figure 6.1c and d), and cross-year analysis of variance showed no effect of shading ($P=0.18$). Similarly, thermal time to reach M was not affected by shading and the interaction between shading and genotypes was not significant. In summary, Rialto grain contained more water and took a longer time to reach M than Spark. Additionally, grains from central spikelets had higher water content and a longer thermal time to M compared to both apical and basal spikelets.

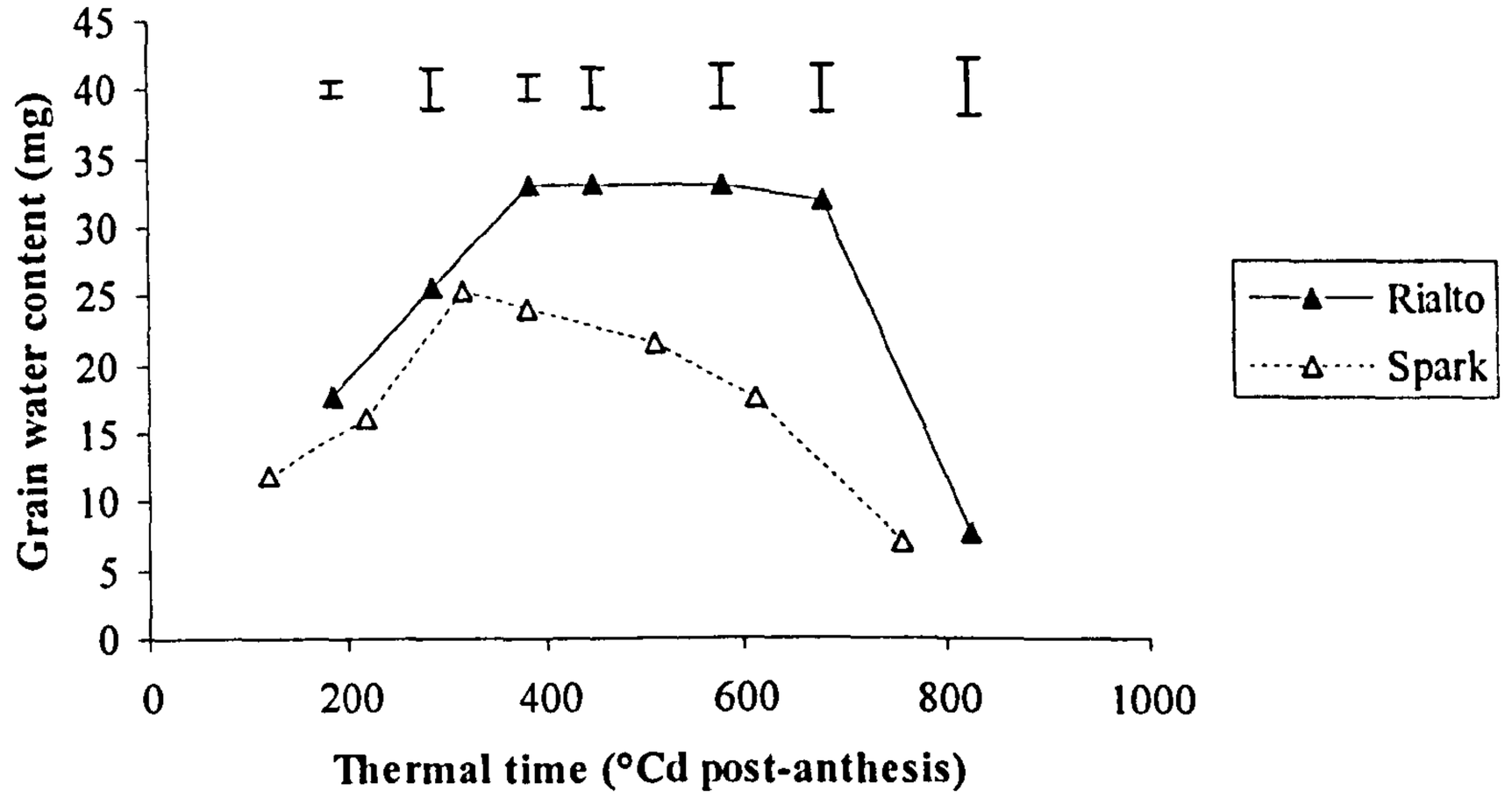
(a) 2004, unshaded



(b) 2005 unshaded



(c) 2004 shaded



(d) 2005 shaded

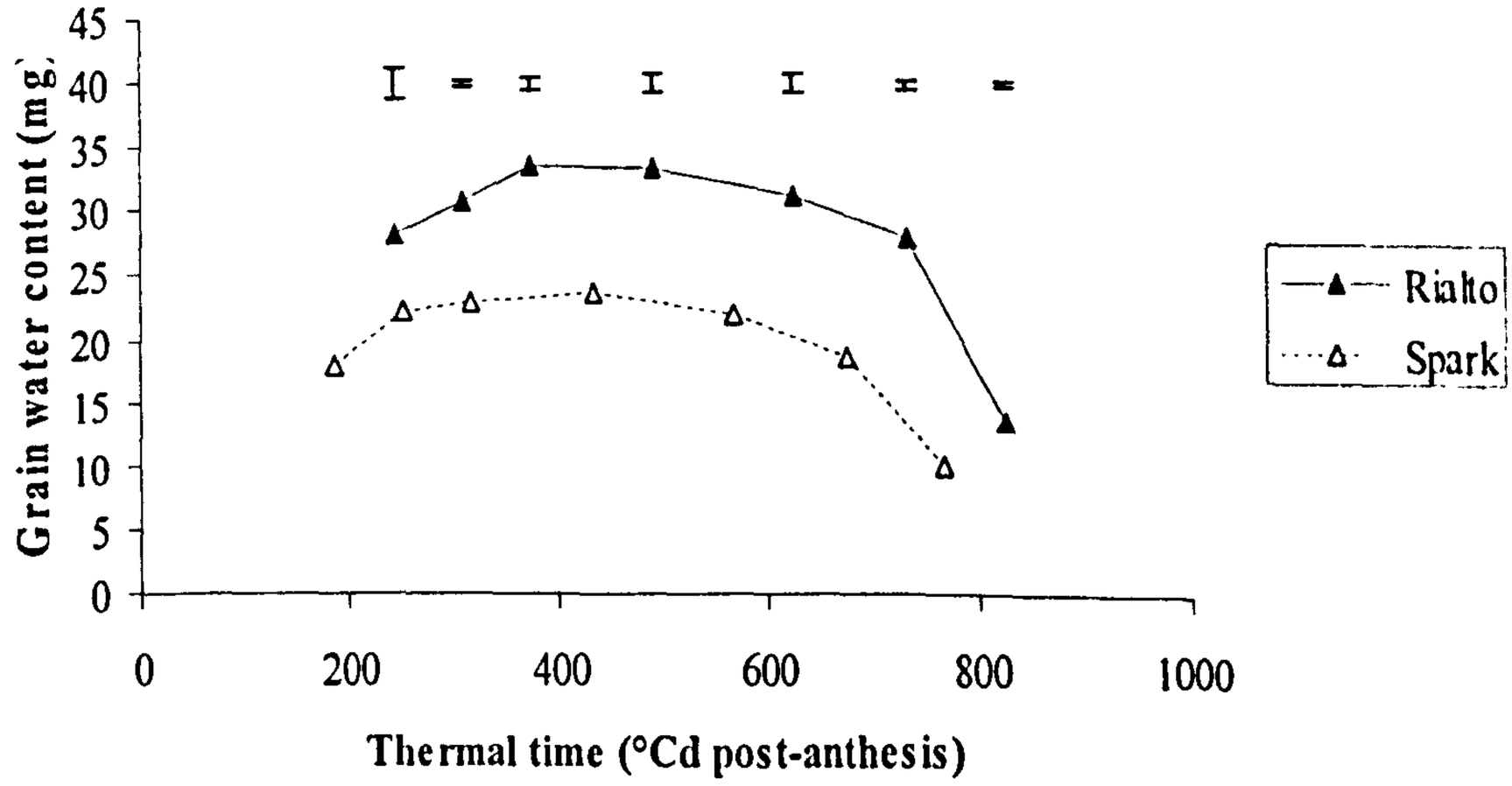


Figure 6.1 Grain water content (mg) for Rialto and Spark for the unshaded (a, b) and shaded (c, d) treatments measured during (a, c) 2004 and (b, d) 2005 (bars show S.E.D, 2df).

Table 6.1 Model-derived parameters for maximum grain water content of grains of Rialto and Spark for unshaded and shaded treatments in 2004 and 2005 based on measurement of individual excised grain positions.

	Position	Time of max water (°Cd)			Maximum water content (mg)		
		2004	2005	Mean	2004	2005	Mean
Unhaded Rialto	GP 1	472.7	443.4	458.1	37.3	39.4	38.3
	GP 2	481.4	438.4	459.9	44.7	41.7	43.2
	GP 3	491 3	432.2	461.7	34.3	34.7	34.5
	GP 4	474.8	489.7	482.2	41.2	37.8	39.5
	GP 5	466.6	416.1	441.3	23.4	23.6	23 5
	Mean	**477.4**	**443.9**	**460.7**	**36.2**	**35.4**	**35.8**
Spark	GP 1	430.2	410.0	420.1	30.2	29.6	29.9
	GP 2	430.6	424.9	427.7	29.8	31.3	30.6
	GP 3	409.9	401.2	405.5	23.2	22.8	23.0
	GP 4	442.8	442.7	442.8	26.9	28.7	27.8
	GP 5	418.4	411.8	415.1	20.3	21.4	20.8
	Mean	**426.4**	**418.1**	**422.3**	**26.1**	**26.8**	**26.4**
GP Mean	GP 1	451.5	426.7	439.1	33.8	34.5	34.1
	GP 2	456.0	431 7	443.8	37.3	36.5	36.9
	GP 3	450 6	416 7	433.6	28.8	28.8	28.8
	GP 4	458.8	466.2	462.5	34.1	33.3	33.7
	GP 5	442.5	414.0	428.2	21.9	22.5	22.2
	Mean	**451.9**	**431.0**	**441.5**	**31.2**	**31.1**	**31.1**
Shaded Rialto	GP 1	470.1	439.9	455.0	33.2	33.5	33.3
	GP 2	457.4	458.5	457.9	21 5	37.7	29.6
	GP 3	499.3	472.2	485.7	42.2	44.4	43.3
	GP 4	504.8	509.4	507.1	47.8	35.3	41.6
	GP 5	495 3	453 0	474.2	17.9	29.5	23.7
	Mean	**485.4**	**466.6**	**476.0**	**32.5**	**36.1**	**34.3**
Spark	GP 1	411 7	429 2	420.4	18 7	28 0	23 4
	GP 2	451 1	426 0	438 6	26 9	33 8	30 4
	GP 3	387 2	422.0	404 6	13.0	29 0	21.0
	GP 4	425 6	454 7	440 2	25.9	29.8	27.8
	GP 5	411 6	422 7	417.2	16.9	19 8	18 3
	Mean	**417.5**	**430.9**	**424.2**	**20.3**	**28.1**	**24.2**
GP Mean	GP 1	440 9	434.6	437.7	26.0	30 8	28.4
	GP 2	454.3	442.3	448.3	24 2	35 8	30.0
	GP 3	443 3	447.1	445 2	27.6	36 7	32.2
	GP 4	465.2	482.1	473.7	36.9	32 6	34.7
	GP 5	453.5	437 9	445.7	17.4	24.7	21.0
	Mean	**451.5**	**448.8**	**450.1**	**26.4**	**32.1**	**29.3**
SED (df)							
Year				6.59 (2)			0 818 (2)
Shading		8.65 (2)	6.08 (2)	5.29 (4)	2.42 (2)	1.39 (2)	1 40 (4)
Genotype		9.99 (4)	9.17 (4)	6.78 (8)	0.813 (4)	4.742 (4)	2.406 (8)
Position		7.88 (28)	9.07 (32)	6.03 (60)	2.87 (28)	2.37 (32)	1.848 (60)
Year x Shade				7.47 (4)			1 973 (4)
Year x Genotype				9.59 (8)			3.40 (8)
Year x position				8.53 (60)			2.61 (60)
Year x shading x Genotype				13 56 (8)			4.81 (8)
Year x shading x position				12.07 (60)			3.70 (60)
Year x genotype x position				12.07 (60)			3 70 (60)
Shading x genotype		14.13 (4)	12.97 (4)	9.59 (8)	1.15 (4)	6.71 (4)	3.40 (8)
Shading x position		11.14 (28)	12.82 (32)	8.53 (60)	4.06 (28)	3.35 (32)	2.613 (60)
Shading x genotype x position		15.75 (28)	18.14 (32)	12.07 (60)	5.74 (28)	4.73 (32)	3.70 (60)
Genotype x position		11.14 (28)	12.82 (32)	8.53 (60)	4.06 (60)	3.35 (32)	2.613 (60)
Interaction of all				17.07 (60)			5.23 (60)

6.2.2 Ear biomass growth

Detailed sequential measurements of individual ear dry matter growth through grain filling were carried out for Rialto and Spark. Growth of individual ears was analyzed against thermal time from GS61 (base temp. 0 °C) by assuming a constant rate of growth from GS61+ 220 °Cd until the weight reached a plateau (slope = 0) at the final grain weight. Thus, for each sub-plot a regression model (broken stick model) which fitted two straight lines to the data (as detailed in section 6.2) was used to determine the end of linear ear growth, the rate of the linear increase and the final ear weight. These values were subjected to analysis of variance.

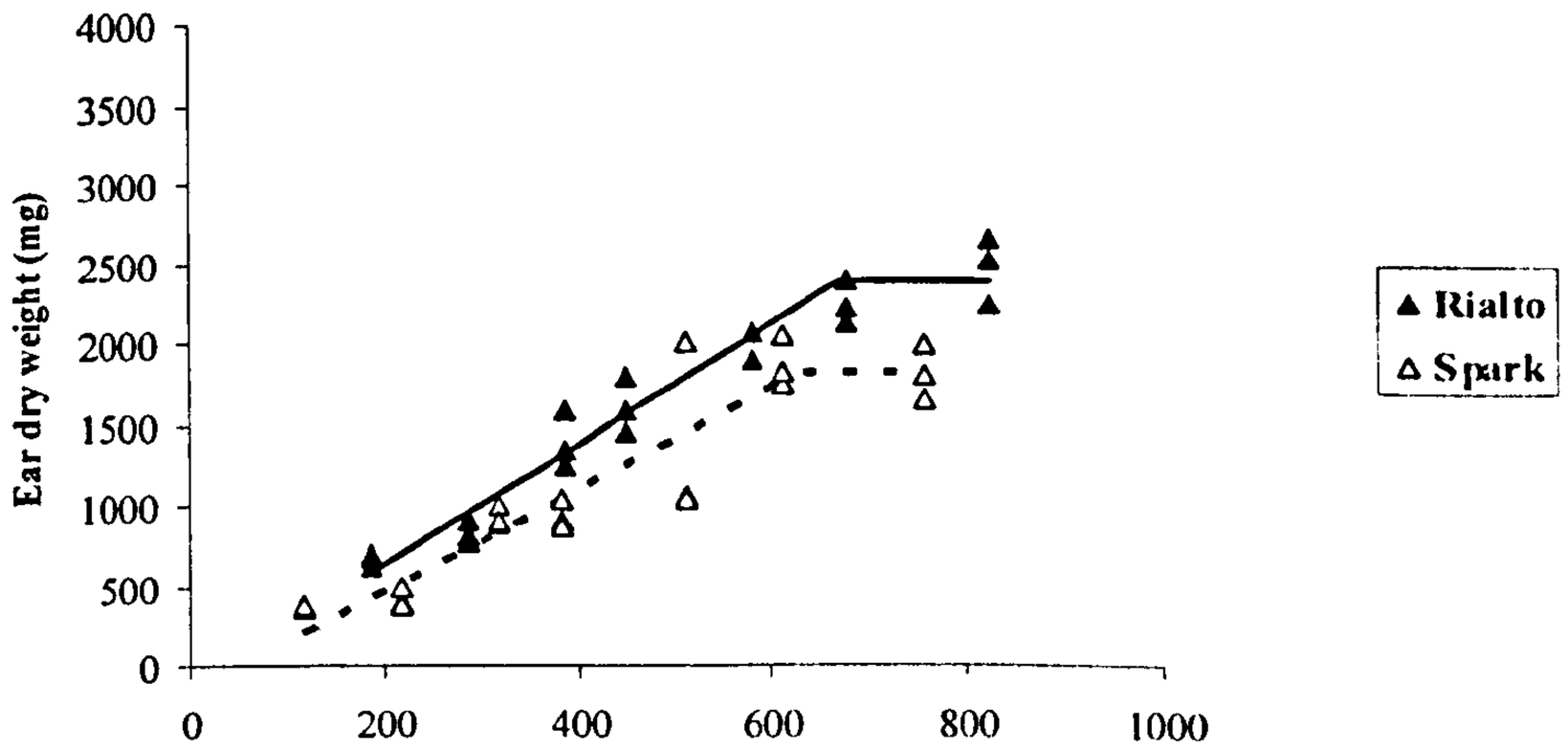
Rialto had greater final ear weight than Spark in both years ($P \leq 0.05$; Figure 6.2a and b; Table 6.2). Differences in rate of ear growth were observed in 2005 ($P<0.001$) but not in 2004 ($P=0.28$). A strong trend for a larger duration of ear DM growth for Rialto than Spark was observed in both years ($P<0.10$). Cross-year analysis of variance indicated that Rialto had a faster rate, a longer duration of ear growth and heavier ears than Spark. ($P<0.05$)

Shading reduced final ear weight in both years ($P<0.05$), consistent with its negative effect on rate of ear growth (Table 6.2; Figure 6.2c and d). Averaging over years and genotypes, final ear weight and ear DM growth rate were reduced by 0.37g and 0.71 mg $°Cd^{-1}$, respectively. However, the end of ear growth was not affected by shading in either year. Furthermore, the interaction between shading and genotype was not significant for ear growth characteristics.

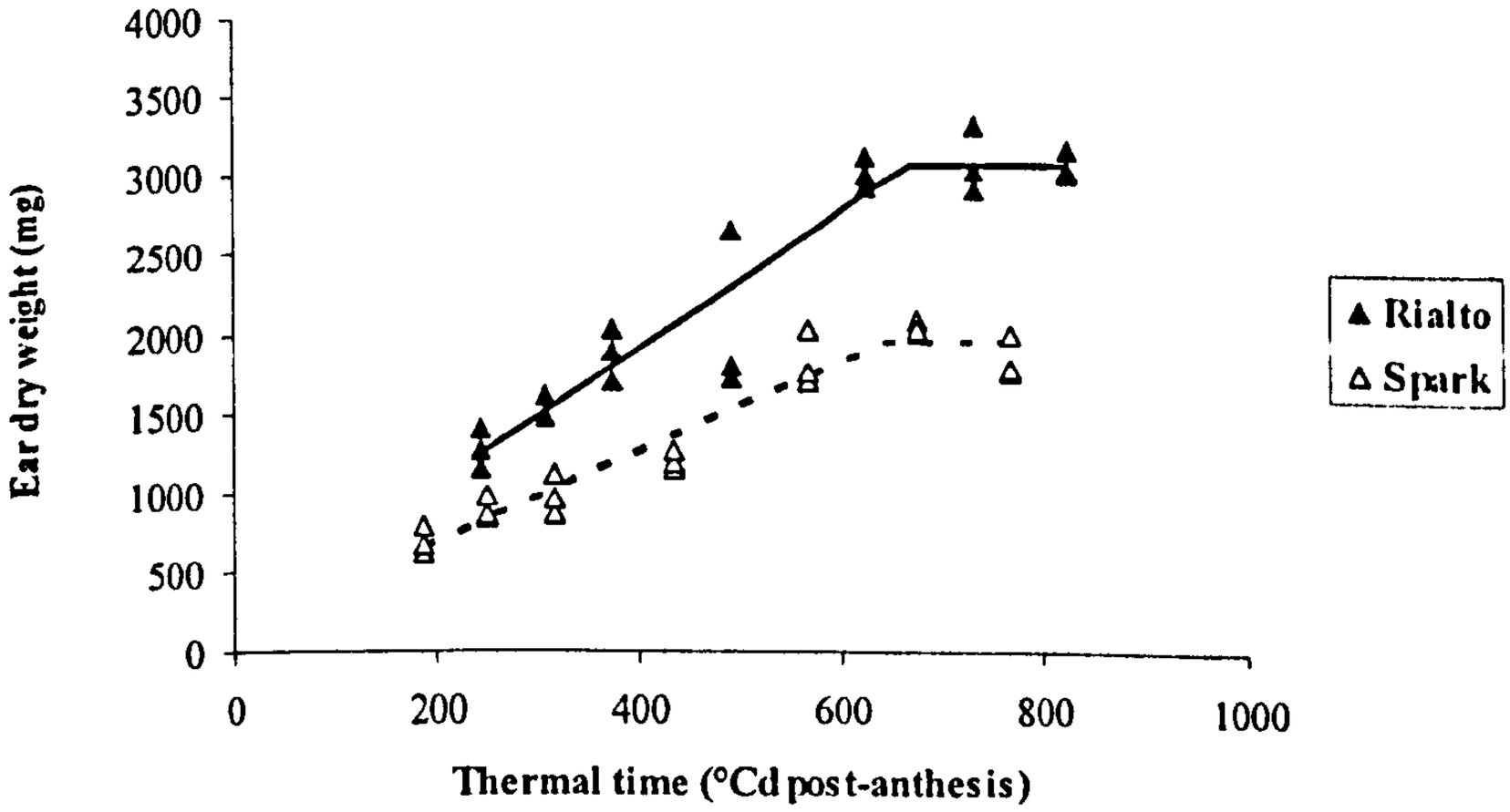
The gain in ear dry weight from the onset of shading was calculated by subtracting the ear dry weight at the time of the onset of shading from the final ear weight derived from the broken stick model (Table 6.2). The calculated source-sink balance predicted that source limitation in shaded plots would have been experienced in 2005 but not 2004. However, the measured effect of shading on ear growth was not as predicted in 2004, as

ear growth was reduced in both parents. This could possibly be due to an over-estimation of source size in 2004 (due to using potential RUE based on pre-anthesis measurements). In addition, stem WSC was estimated from stem and sheath dry weight loss between GS61+ 5d and harvest and was higher than the amount of stem WSC estimated by chemical analysis in 2005. Also GAI at GS61 may have been overestimated in 2004 as discussed previously (Chapter 5). The overestimate of GAI in 2004 seems the most likely reason for the overestimate of source size in 2004.

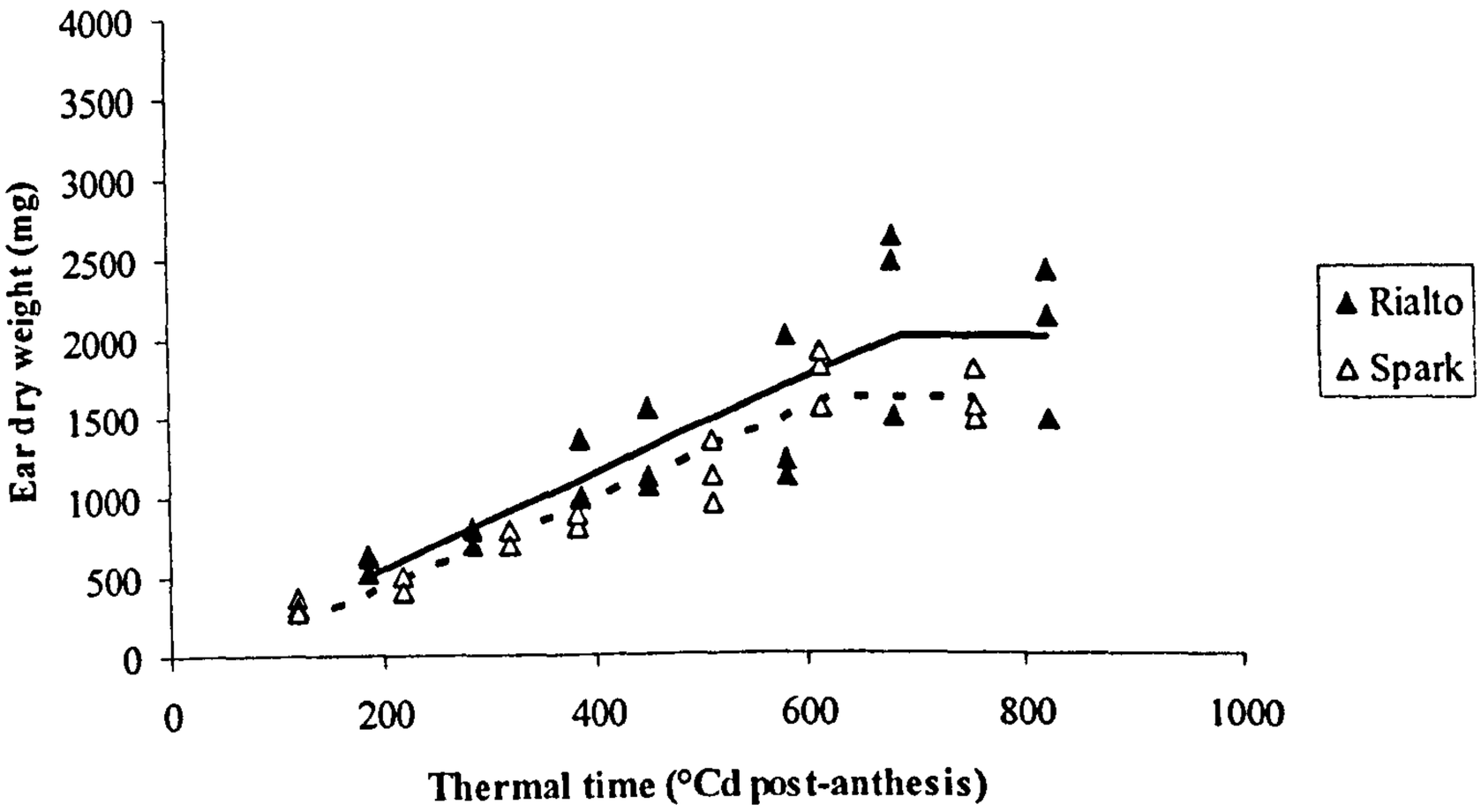
(a) 2004, unshaded



(b) 2005, unshaded



(c) 2004, shaded



(d) 2005, shaded

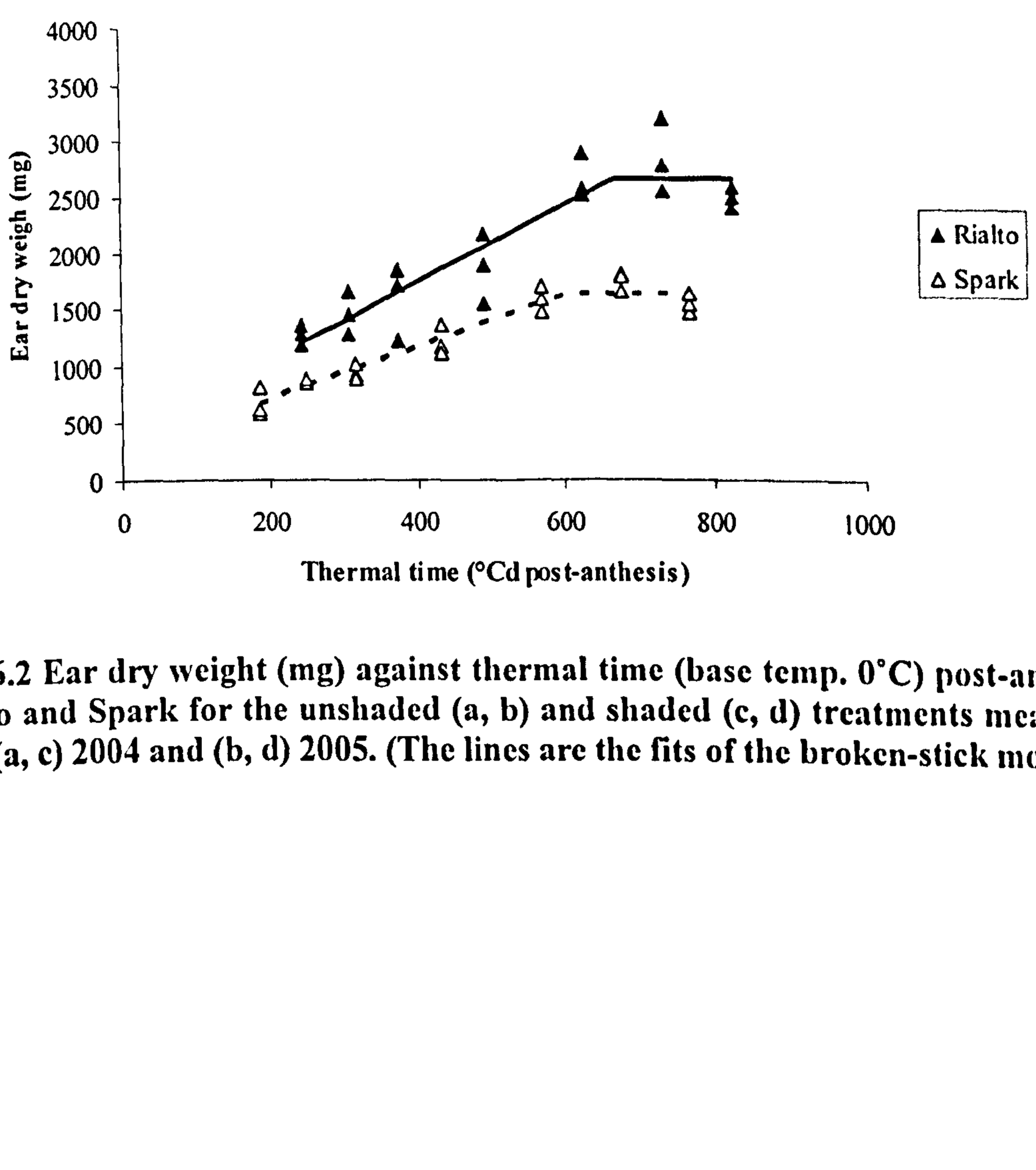


Figure 6.2 Ear dry weight (mg) against thermal time (base temp. 0°C) post-anthesis of Rialto and Spark for the unshaded (a, b) and shaded (c, d) treatments measured during (a, c) 2004 and (b, d) 2005. (The lines are the fits of the broken-stick model)

Table 6.2 Model-derived parameters describing ear growth from GS61 to physiological maturity in shaded and unshaded treatments for Rialto and Spark in 2004 and 2005 based on individual shoot samples

	Rate of ear DM growth mg °Cd^{-1}			Final dry weight (g)			End of ear DM growth °Cd post-anthesis			Ear dry weight at onset of shading (g)			Ear DM growth from onset of shading until maturity (g)		Reduction in ear DM growth under shading (g)	
	2004	2005	Mean	2004	2005	Mean	2004	2005	Mean	2004	2005	Mean	2004	2005	2004	2005
Unshaded																
Rialto	4.01	4.29	4.15	2 48	3 07	2.78	678	670	674	1.62	1.52	1.57	0.86	1.55		
Spark	3.21	2.85	3.03	1 75	1 93	1.84	609	638	623	1.04	0.90	0.97	0.71	1.03		
Mean	3.61	3 57	3.59	2.12	2.50	2 31	643	654	649	1.33	1 21	1.27	0.79	1.29		
Shaded																
Rialto	2.81	3.61	3.21	1 86	2 67	2.26	672	661	667	1.20	1.46	1.33	0.66	1.21	0.20	0.34
Spark	2.78	2.32	2.55	1.57	1.66	1 62	611	617	614	1 10	0.87	0.99	0.47	0.79	0.24	0.24
Mean	2.80	2.97	2.88	1.72	1.66	1.94	642	639	640	1.15	1.16	1.16	0.57	0 50	0.22	0.79
SED (df)													*		*	
Year			0.181 (2)			0 0610 (2)			11.02 (2)						0.212 (2)	
Shading	0 352 (6)	0.170 (6)	0.225 (4)	0.117 (6)	0 0276 (6)	0 0566 (4)	27 3 (6)	17.53 (6)	22.47 (4)	0.257 (6)	0.0624 (6)				0.080 (4)	
Genotype	0.352 (6)	0.170 (6)	0.179 (8)	0.117 (6)	0.0276 (6)	0 0724 (8)	27 3 (6)	17 53 (6)	11.91 (8)	0.257 (6)	0 0624 (6)				0.1520 (8)	
Year x Shading			0.318 (4)			0.0801 (4)			31.78 (4)						0.1125 (4)	
Year x Genotype			0.253 (8)			0 1025 (8)			16.852 (8)						0.2150 (8)	
Shading x genotype	0.498 (6)	0 241 (6)	0.253 (8)	0 1655 (6)	0.0957 (6)	0 1025 (8)	38.6 (6)	24.79 (6)	16.852 (8)	0.364 (6)	0.0883 (6)				0.2150 (8)	
Shading x genotype x year			0 358 (8)			0 1449 (8)			23.83 (8)						0.3041 (8)	

**SED for ear growth is not available as these values are calculated from mean dat*

6.2.3 Individual grain growth dry matter

Grains from five different positions (three from the central spikelet (Grains 1, 2 and 3), one from a basal spikelet (grain 4) and one from an apical spikelet (grain 5)) were monitored on a weekly basis during grain growth (see Figure 5.1). Grain filling was analyzed over thermal time from GS61 (base temp. 0°C) by assuming a linear rate of grain growth from the start of assessments (GS61 + 220 °Cd) until the weight reached a plateau (slope = 0). The end point of grain growth, the final grain dry weight and the rate of linear increase in grain dry matter growth (Figure 6.3) were determined using the two-straight-line model as described in section 6.2. The model was fitted to data from each sub-plot and values for each parameter were subjected to analysis of variance.

6.2.3.1 Final grain weight

Averaging across years, differences among grain positions followed the same pattern in Rialto and Spark. Within the central spikelet, initially grain position 1 was the largest. However, as reported by Bremner and Rawson (1978), the grain weight of grain 2 (G2) eventually exceeded G1 (47.1 compared to 44.5 mg, respectively) (Table 6.3). As anticipated, the central spikelet produced the heaviest grain, within which grain positions 1 and 2 were the largest. The basal spikelet generally produced lighter grain than both G1 and G2 but heavier than G3 of the central spikelet. The apical spikelet was consistently the lightest of the five grains measured. The mean grain weight for both years was higher in Rialto (44.6 mg) than Spark (37.2 mg) ($P<0.005$; Table 6.3). Cross-year analysis of variance showed that there were differences between genotypes and grain positions; and that the grains excised from Rialto were heavier than those of the same position excised from Spark ($P<0.001$). There was no effect of season on final grain weight ($P=0.23$).

Shading reduced final grain weight in 2004 ($P<0.05$), while no effect was observed in 2005 (Figure 6.3c andd; Table 6.3). Cross-year analysis of variance showed a

reduction in final grain weight by 4.1 (Rialto) and 2.7 (Spark) mg. However, the interaction between shading and genotype was not significant in either year. Nevertheless, shading reduced the final grain weight of all grain positions except for

G3 in 2004 ($P<0.05$; Table 6.3). Averaging over years and genotypes, differences among grain positions followed the same pattern in shaded treatment as in the unshaded treatment, in which, within G1 and G2 (within the central spikelet) and G4 produced heavier grains while G5 was consistently the lightest of the grains measured.

6.2.3.2 Rate of grain growth

There was no difference between the parents in the rate of grain growth in either year (Table 6.3). Spark and Rialto had a similar rate of grain growth in the range 65-77 and 72-74 μg $°Cd^{-1}$ in 2004 and 2005, respectively. Cross-year analysis of variance showed neither year nor genotype x grain position had statistically significant effects. Grain position, however, had a significant effect on the rate of grain growth ($P<0.05$). In general, G1, G2 and G4 had faster rates of growth, than the apical grain (G5). Relative differences in the growth rate of the individual grain positions 1-5 were similar in the two parents.

A positive linear relationship was found between final grain weight and the rate of grain growth amongst the grain positions in both years (Figure 6.4), as well as for the average over both years ($P\leq0.05$). However, there was no relationship between final grain weight and the duration of grain growth amongst the five positions ($P>0.05$). Nor was there a linear relationship between rate and duration of grain growth. A linear relationship ($P<0.05$) was observed when maximum water content was regressed against both final grain weight (Figure 6.5) and the rate of grain growth corresponding (Figure 6.6) across the five grain positions in both 2004 and 2005.

Shading reduced the grain growth rate in 2004 ($P<0.05$), while no effect was observed in 2005 (Table 6.3). Cross-year analysis of variance showed a reduction in rate of grain growth by 9 μg $°Cd^{-1}$ in Rialto while Spark was not affected. In 2004, shading had a large effect on the rate of grain growth of G1, G2 and G4 ($P<0.05$) but not in 2005 (Table 6.3). Averaging over years and genotypes, differences among grain positions followed the same pattern as in the unshaded treatment, in which, grain position 1, 2 and 4 had a faster rate of growth than G5.

6.2.3.3 Grain filling duration

The whole period between GS61 and end of grain filling is the grain filling duration. Rialto had a longer grain filling duration than Spark in both years ($P<0.001$; Figure 6.3; Table 6.3). Cross-year ANOVA showed a genotypic difference of 693 compared to 636 °Cd ($P<0.001$). There were differences among grain positions in 2004 ($P=0.029$) but not in 2005. Cross-year ANOVA showed no overall differences among the grain positions. All grains excised from Rialto had a longer filling duration in 2004 compared to the grains excised from the same positions from Spark ($P=0.023$). Averaging across grain positions, shading did not affect the duration of grain filling in either year. There was no shading x genotype interaction and shading increased the filling duration of G4 by 32 °Cd but did not affect the grain filling duration of G1, G3 and G5.

6.2.3.4 Duration of the rapid grain growth phase

The duration of rapid linear grain filling was calculated according to the end of grain filling at the break point of the broken-stick model and intercept on the x axis (Table 6.4). Averaging over years, G1, G2 and G3 had slightly longer durations in the range 558-568 °Cd compared to G4 (504°Cd) and G5 (509°Cd; $P=0.003$). The duration of the rapid grain filling phase differed between Spark and Rialto from 511.1 to 579.9 °Cd in 2004 and from 469.0 to 602.1 °Cd in 2005, respectively. Rialto had a longer duration in both years ($P \leq 0.05$; Figure 6.3; Table 6.4), consistent with its large grain size. In general, parental differences in final grain weight were primarily due to differences in grain growth duration (see Figure 6.3). Figures 6.5 and 6.6 show that both Rialto and Spark had the same rate of grain growth and they differed in the

duration of grain filling. Furthermore, all grains excised from Rialto had longer filling duration in 2004 compared to the grains excised from the same positions from Spark as indicated by a significant genotype effect. Averaging over years and genotypes, G1 and G2 had a longer rapid grain filling duration (569 and 548 °Cd, respectively) and showed the same ranking order as in unshaded treatments, whilst, G3 and G5 had a shorter rapid filling period (515 and 527 °Cd respectively). Shading extended the rapid grain growth period in 2004 by 45.8 °Cd (P= 0.051), but no effect was observed in 2005 (P= 0.63; Table 6.4). The shading x genotype interaction was not significant in either year. Shading increased the filling duration of G4 by 50 °Cd but did not affect the grain filling duration of G1, G2, G3 and G5 (P= 0.021).

(a) 2004, unshaded

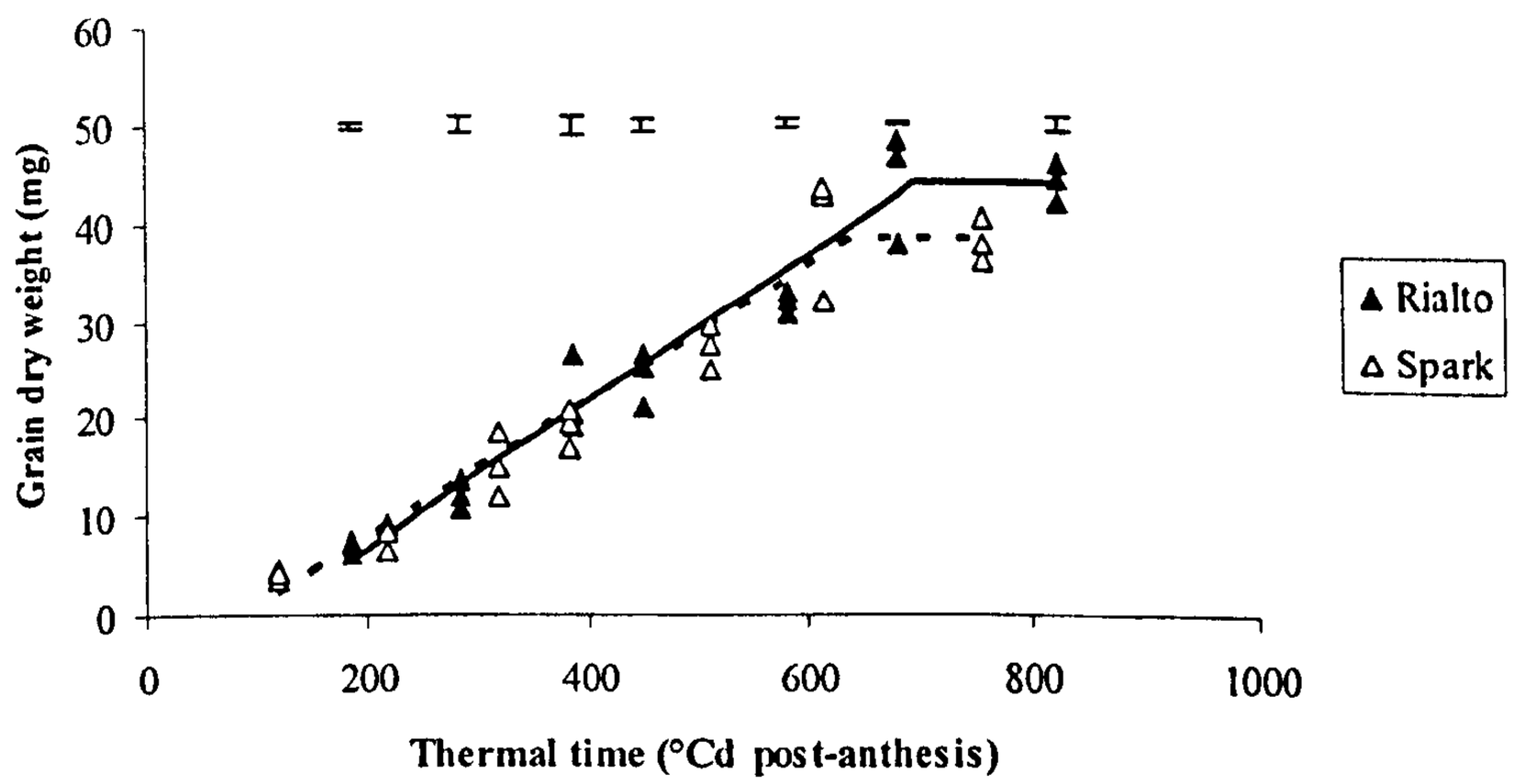
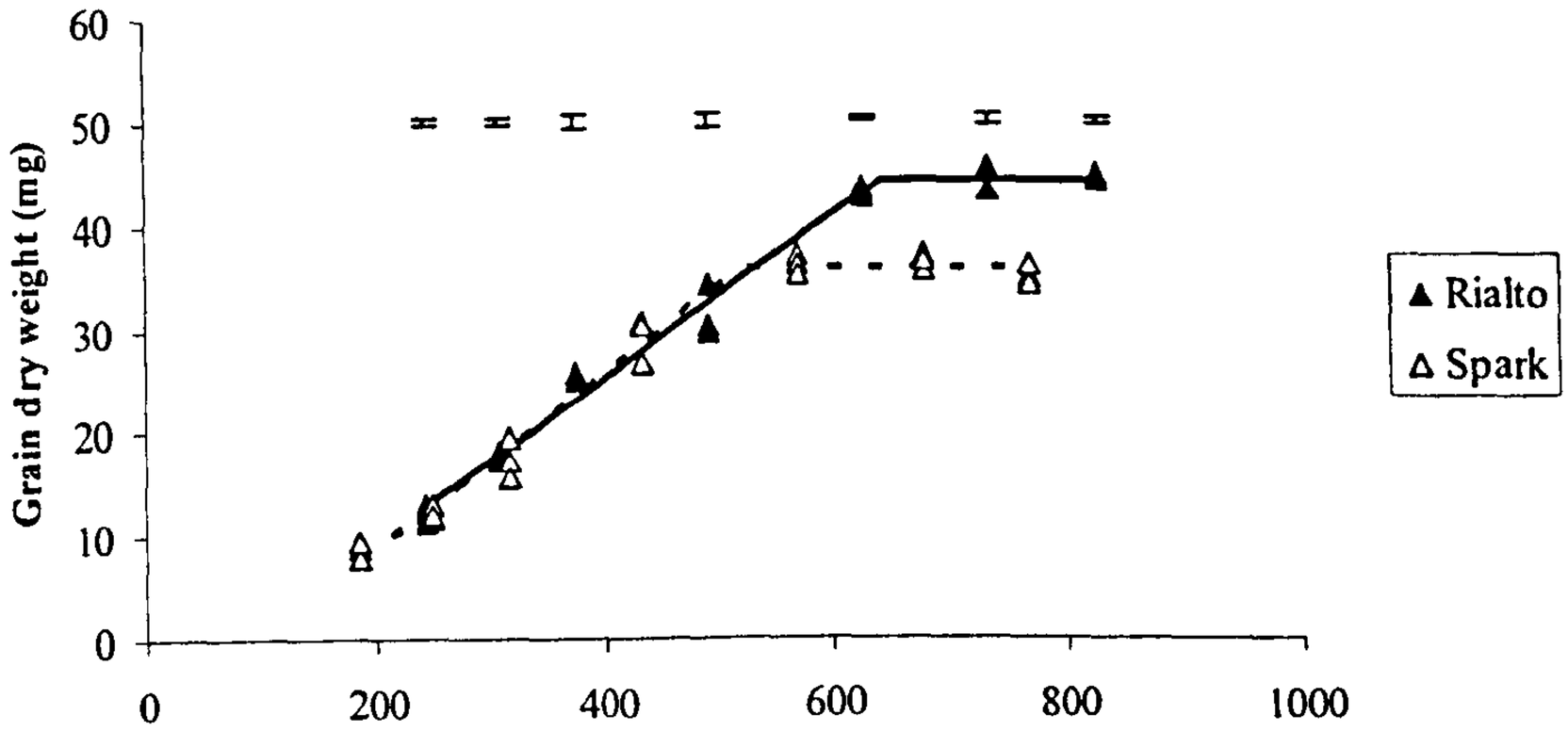


Figure 6.3 Average dry weight of five grain positions (mg) against thermal time (base temp. 0°C) of Rialto and Spark for unshaded (a, b) and shaded (c, d) treatments measured during (a, c) 2004 and (b, d) 2005 (bars show S.E.D, 2df; lines are the fit of broken-stick model).

(b) 2005, unshaded



(c) 2004, shaded

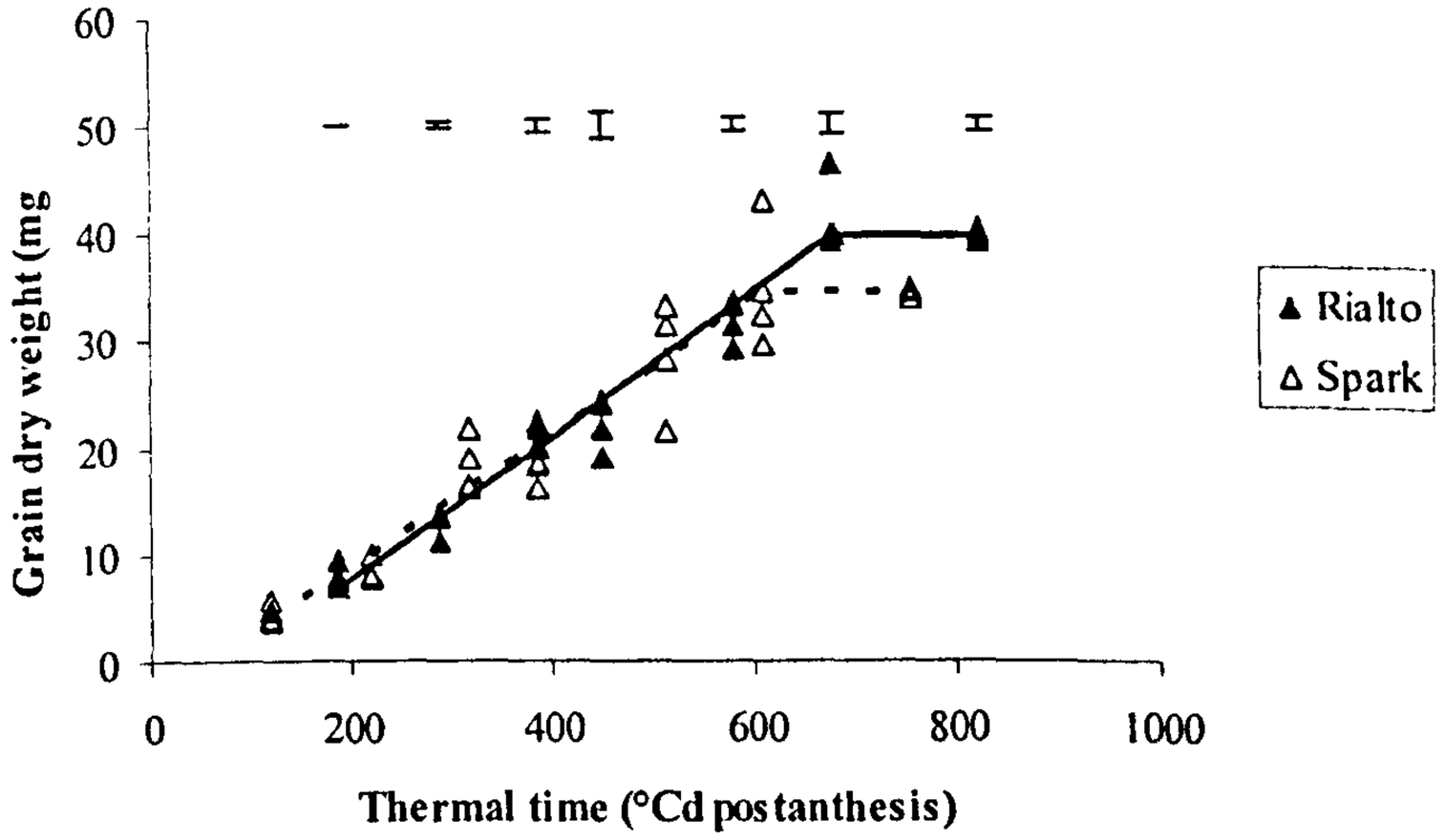


Figure 6.3 Average dry weight of five grain positions (mg) against thermal time (base temp. 0°C) of Rialto and Spark for unshaded (a, b) and shaded (c, d) treatments measured during (a, c) 2004 and (b, d) 2005 (bars show S.E.D, 2df; lines are the fit of broken-stick model).

(d) 2005, shaded

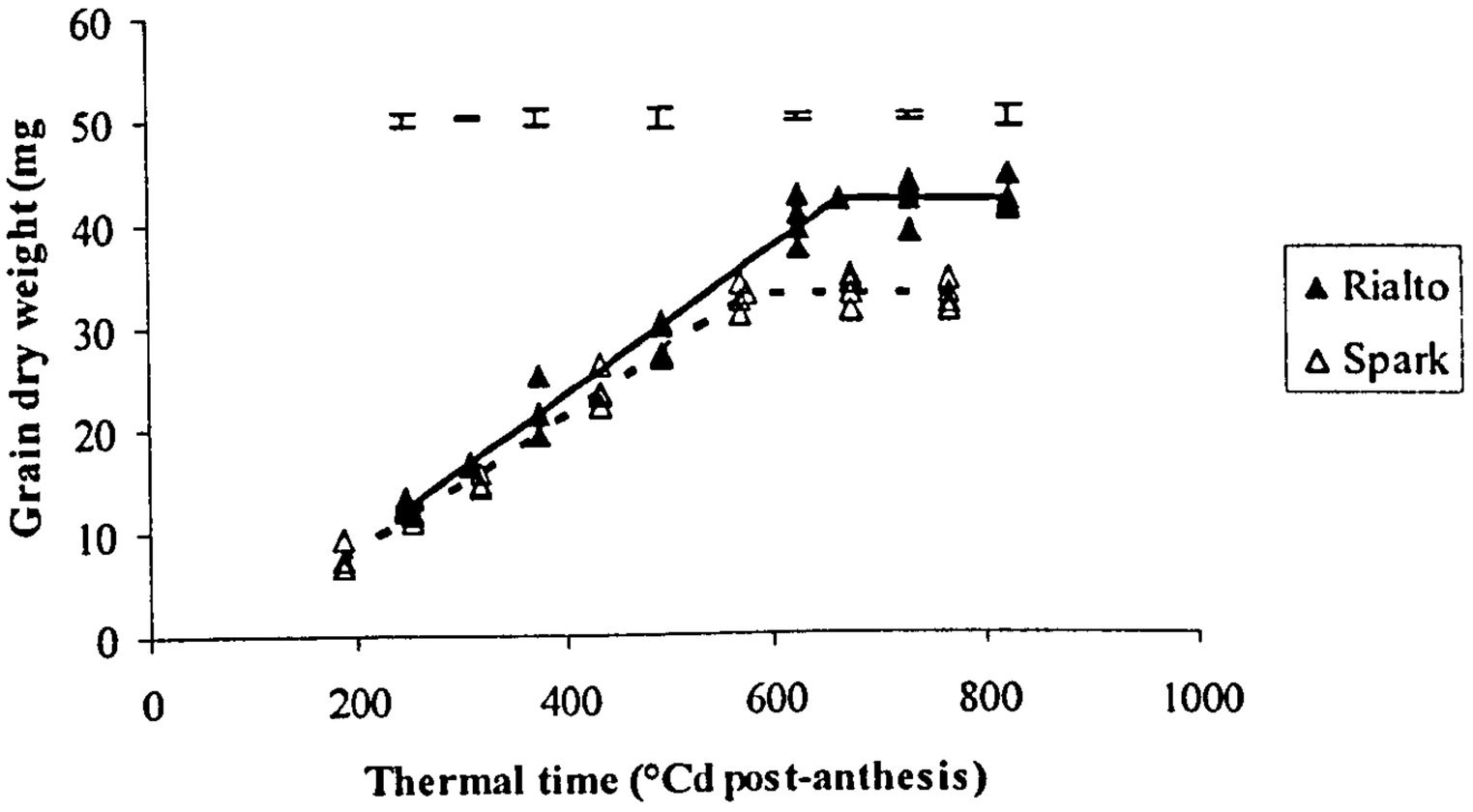


Figure 6.3 Average dry weight of five grain positions (mg) against thermal time (base temp. 0°C) of Rialto and Spark for unshaded (a, b) and shaded (c, d) treatments measured during (a, c) 2004 and (b, d) 2005 (bars show S.E.D, 2df; lines are the fit of broken-stick model).

Table 6.3 Model-derived parameters for rate of grain growth, duration of grain growth and final grain weight of Rialto and Spark for both unshaded and shaded treatments in 2004 and 2005 based on measurement of individual excised grain positions.

		Rate of grain growth (µg °Cd^{-1})			Final weight (mg)			End of grain filling (°Cd)		
	Position	2004	2005	Mean	2004	2005	Mean	2004	2005	Mean
Unshaded Rialto	GP 1	81.4	78.5	80.0	49 0	50.0	49 5	692.3	699 5	695 9
	GP 2	86.0	77.5	81.7	51.8	50.7	51 2	706 9	711 1	709 0
	GP 3	69.2	70.3	69.7	39.8	42.3	41.0	695.7	693 2	694 5
	GP 4	90.1	85.2	88.1	50.3	46.6	48.5	697.7	680 9	689.3
	GP 5	60.3	57.6	58.9	33.8	31.7	32.8	683 4	667 1	675 3
	Mean	**77.5**	**73.8**	**75.7**	**44.9**	**44.2**	**44.6**	**695.2**	**690.4**	**692.8**
Spark	GP 1	71.5	83.0	77.3	39.7	39.4	39.6	617.5	534 0	575 7
	GP 2	85.8	88.5	87.1	42.9	43.3	43.1	627 3	564 5	595 9
	GP 3	67.3	65.5	66.2	35.7	33.1	34.4	633.8	598.9	616 4
	GP 4	82.7	84.5	83.6	37.5	38.1	37.8	563.3	547 3	555.3
	GP 5	65.3	72.3	68.8	33.8	28.0	30.9	620.4	491 6	556 0
	Mean	**74.5**	**78.8**	**76.6**	**37.9**	**36.4**	**37.2**	**612.4**	**547.3**	**579.9**
GP Mean	GP 1	76.5	80.8	78.7	44.3	44.7	44.5	654.9	616.8	635 8
	GP 2	85.9	83.0	84.4	47.3	47.0	47.1	667 1	637 8	652.5
	GP 3	68 3	67.9	68.0	37.7	37.7	37.7	664 8	646.1	655 5
	GP 4	86.4	84.9	85.9	43.9	42.4	43.2	630.5	614.1	622.3
	GP 5	62.8	65.0	63.9	33.8	29.9	31.8	651.9	579 4	615 7
	Mean	**76.0**	**76.3**	**76.2**	**41.4**	**40.3**	**40.9**	**653.8**	**618.9**	**636.4**
Shaded Rialto	GP 1	64.2	70.8	67.5	42.2	46 5	44.4	695 2	680.9	688 1
	GP 2	64.3	75.0	69.6	41.5	48.3	44.9	680 2	712 0	696 1
	GP 3	73.0	70.5	71.7	39.9	41 4	40 7	670 5	682 7	676 6
	GP 4	66.8	81.2	74.0	40.6	47 3	43 9	687 3	707 4	697 3
	GP 5	53.2	48.0	50.6	29.6	27 7	28.7	659 0	662 0	660 5
	Mean	**64.3**	**69.1**	**66.7**	**38.8**	**42.3**	**40.5**	**678.4**	**689.0**	**683.7**
Spark	GP 1	59 4	105.1	82.3	34.9	37.8	36.3	609 2	507 3	558 2
	GP 2	67.0	122.1	94 6	35 0	37 5	36 2	588 0	461 8	524 9
	GP 3	58 0	102.4	80.2	33.0	32 5	32 8	635 1	466 0	550 6
	GP 4	67.8	93.0	80.4	38.3	36.9	37 6	638 4	523 7	581 1
	GP 5	54.9	68.6	61.7	29.2	29.3	29 2	619 6	544 0	581 8
	Mean	**61.4**	**98.20**	**79.8**	**34.1**	**34.8**	**34.4**	**618.1**	**500.5**	**559.3**
GP Mean	GP 1	61.8	88.0	74.9	38.6	42.1	40.4	652 2	594 1	623 2
	GP 2	65.7	98.6	82.1	38.2	42.9	40 6	634 1	586 9	610.5
	GP 3	65.5	86.5	76.0	36.5	36.9	36.7	652 8	574 4	613 6
	GP 4	67.3	87.1	77.2	39.4	42.1	40.8	662 9	615.6	639 2
	GP 5	54.1	58 3	56.2	29.4	28.5	29 0	639 3	603 0	621.2
	Mean	**62.9**	**83.7**	**73.3**	**36.4**	**38.5**	**37.5**	**648.3**	**594.8**	**621.5**
SED (df)				5.638 (2)			0 284 (2)			15 74 (2)
Year		0.45(2)	15 53 (2)	7.765 (4)	0.695 (2)	0.554 (2)	0 445 (4)	11 91 (2)	46 3 (2)	23 9 (4)
Shading		1 34 (4)	9 53 (4)	4 809 (8)	0.987 (4)	0 67 (4)	0 597 (8)	8 78 (4)	27 23 (4)	14 3 (8)
Genotype		2 3 (31)	5 83 (29)	3 092(60)	0 822 (31)	0 771 (29)	0 564 (60)	7 23 (31)	16 7 (29)	8 99 (60)
Position				10.98 (4)			0 528 (5 95)			33 8 (4)
Year x Shading				6.801 (8)			0 844 (8)			20 23 (8)
Year x Genotype				4.373 (60)			0 768 (45 90)			12 71 (60)
Year x position				12.92 (7.13)			1 193 (8)			28 61 (8)
Year x shading x Genotype				12.30 (6.26)			1 189 (35 45)			17 97 (60)
Year x shading x position				9.25 (12.44)			1 315 (37 13)			17 97 (60)
Year x genotype x position		1 89 (4)	13.47 (4)	6.80 (8)	1.396 (4)	0 948 (4)	0 844 (8)	12 41 (4)	38 5 (4)	20 23 (8)
Shading x genotype		3.235 (31)	8.24 (29)	4.373 (60)	1.162 (31)	1 091 (29)	0 798 (60)	10 18 (31)	23 61 (29)	12 71 (60)
Shading x position		4.575 (31)	11.66 (29)	8.77 (20.87)	2.027 (15.35)	1.674 (24.02)	1 315 (37 13)	14 39 (31)	33 4 (29)	17 97 (60)
Shading x genotype x position		3.24 (31)	8.24 (29)	4.373 (60)	1.162 (31)	1 091 (29)	0 798 (60)	10 18 (31)	23 61 (29)	12 71 (60)
Genotype x position				15.10 (13.11)			1.595 (60)			25 42 (60)
Interaction of all										

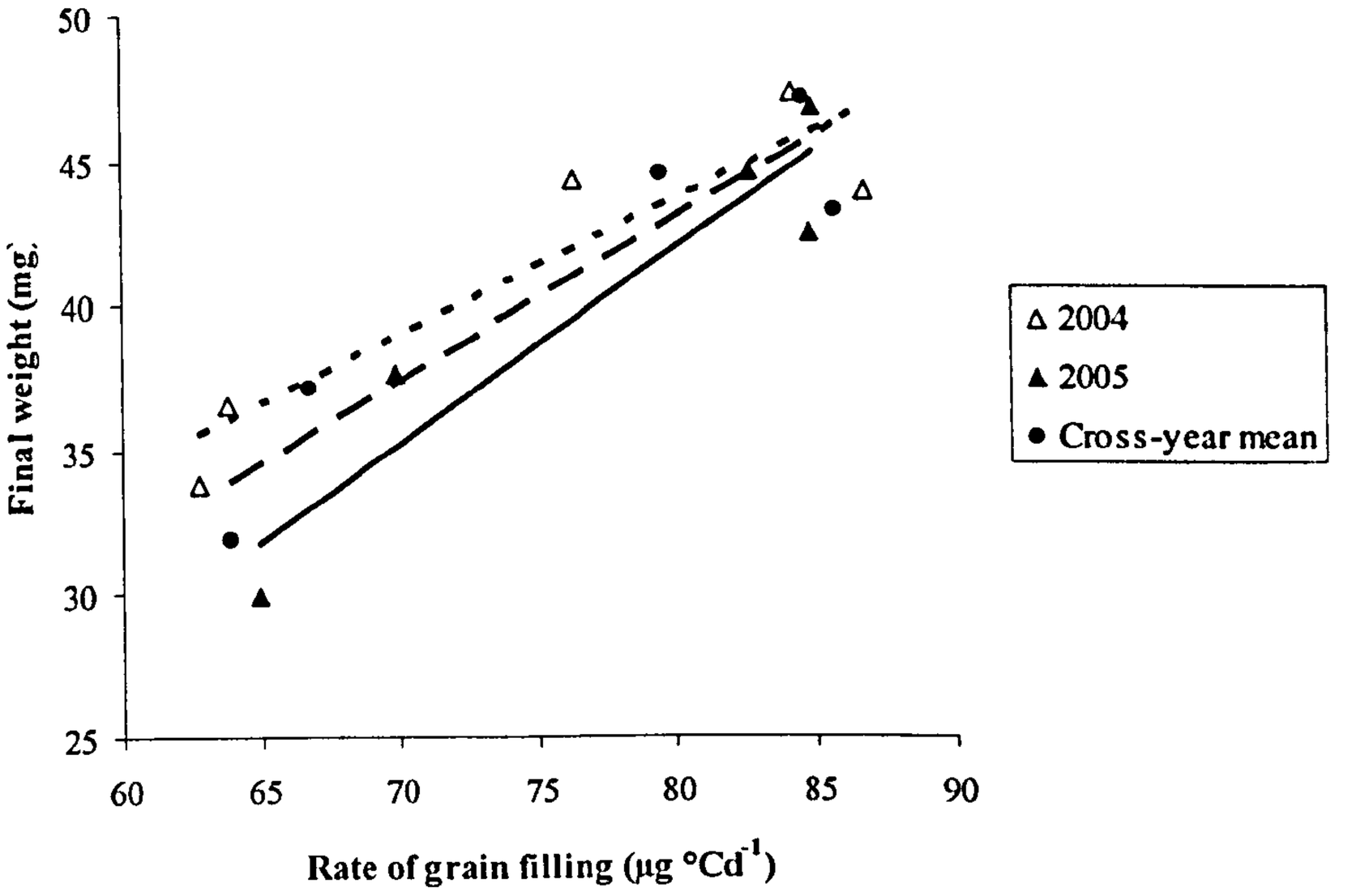


Figure 6.4 Regression of final grain weight on the rate of grain growth for five individual grain positions in 2004 and 2005 and for the cross-year mean in the unshaded treatment. 2004: $y = 0.49x + 4.361$; $R^2 = 0.87$; 2005: $y = 0.6592x - 9.98$; $R^2 = 0.80$; cross-year mean: $y = 0.57x - 2.06$; $R^2 = 0.86$.

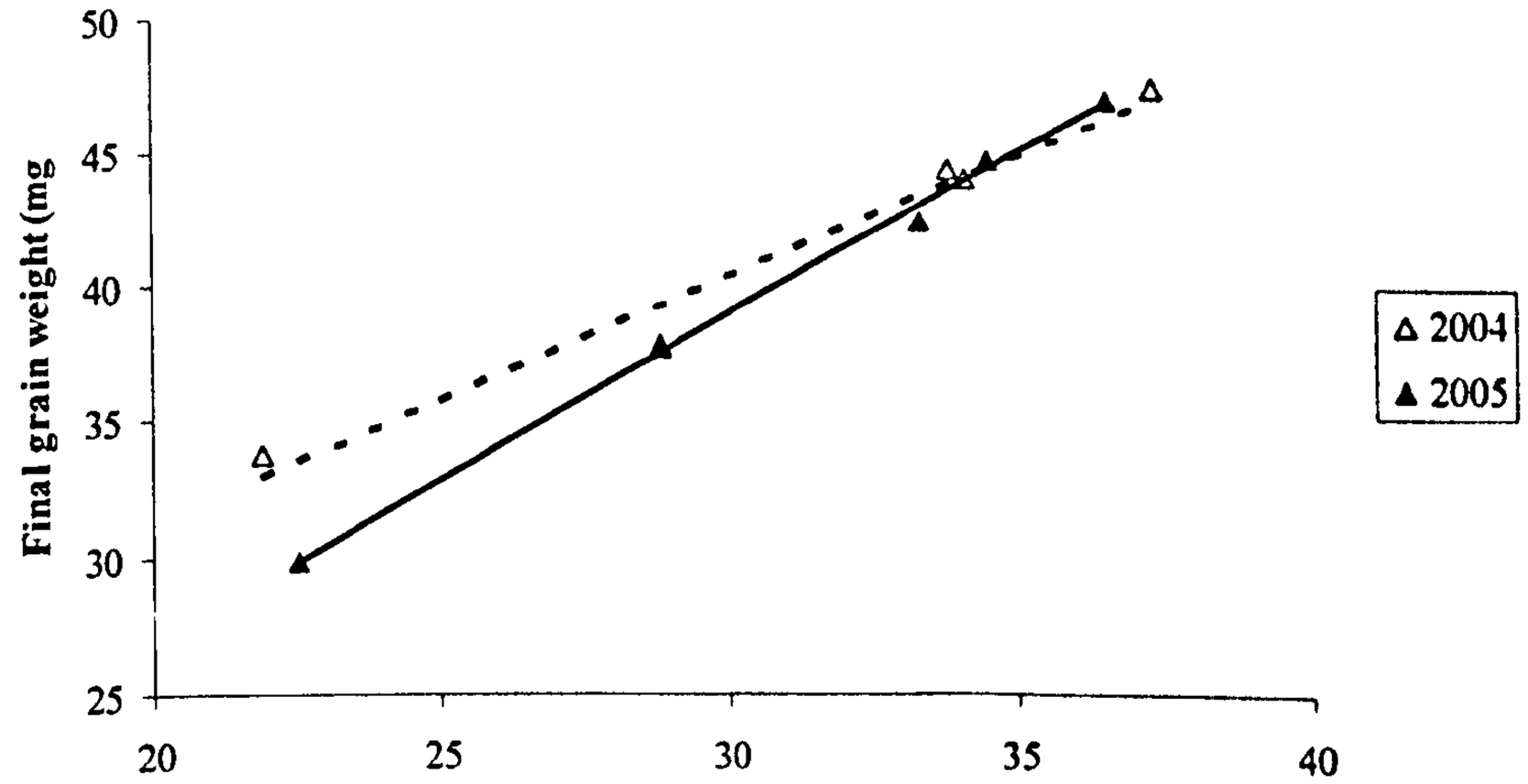


Figure 6.5 Regression of maximum water content on final grain weight averaged across the five grain positions for unshaded treatment in 2004 and 2005. 2004: $y = 0.903x + 13.271$; $R^2 = 0.97$; 2005: $y = 1.2109x + 2.6296$; $R^2 = 0.997$.

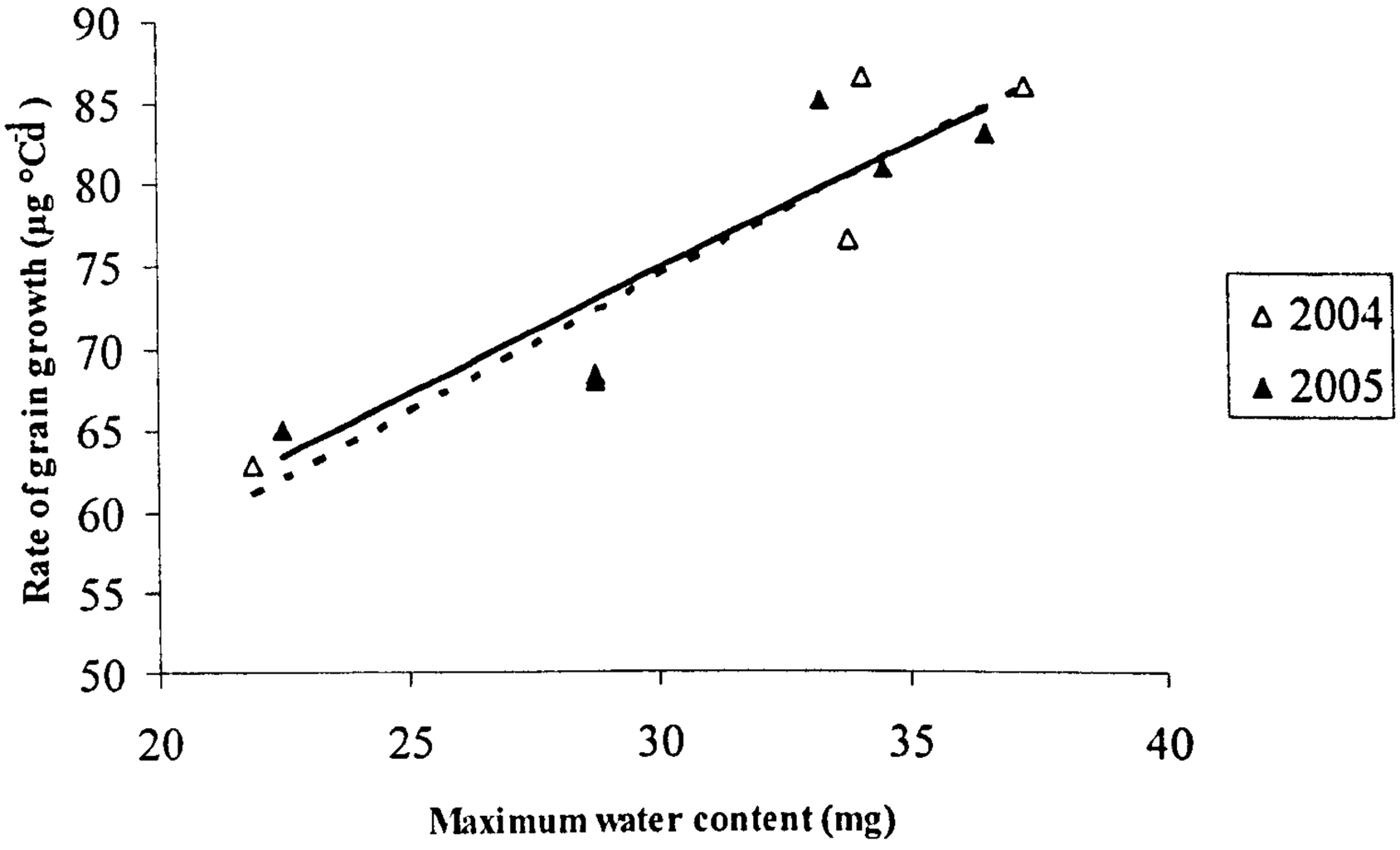


Figure 6.6 Regression of maximum water content on rate grain growth averaged across the five grain positions for shaded treatment in 2004 and 2005. 2004: y = 1.6116x + 25.712; R^2 =0.85; 2005: y = 1.4987x + 29.65; R^2=0.83, respectively

6.2.3.5 Start of the rapid grain filling

The start of rapid grain filling was calculated from the estimates of the end of grain filling and the duration of linear rapid grain filling according to intercept on the x axis. This assumes that the individual grain dry weight is negligible at the onset of rapid grain filling. The start of rapid grain filling occurred considerably earlier than 220 °Cd post-anthesis; on average being closer to 100°Cd in these experiments ($P>0.05$; Table 6.4). Rialto and Spark did not differ in the date of starting rapid grain filling in either year. Averaging over years, there were differences ($P<0.001$) among the grain positions. As expected, the grains in central spikelets began to accumulate dry matter sooner than the grains in apical and basal spikelets. In general, G1, G2 and G3 began to fill before G4 and G5. However, these differences were generally small, and would not have accounted for more than two days. The G1 and G4 excised from Rialto had a longer lag phase compared to the same grain positions (i.e. G1 and G4) excised from Spark in 2004 as indicated by the significant genotype

effect (P= 0.041). No relationship was observed between the duration of grain growth and the maximum water content averaged over both years across grain positions.

With regard to shading, in 2004, shaded plants apparently started grain filling earlier than unshaded ones (P= 0.038). But this is not related to the effect of shading since shading was imposed after the end of the lag phase. However, no difference was observed in 2005 (P= 0.71). The interaction between shade and either genotype and grain position was not significant in either year.

6.2.4 Effects of post-anthesis de-graining

6.2.4.1 Final grain weight in the unshaded control treatment

The response of post-anthesis de-graining was examined over the ten DH lines and their parents for the five grain positions in 2004 and 2005 (Table 6.5). In unshaded sub-plots, the spikelets from one side of the rachis were removed at GS61+ 20 days. Genotypes differed in final grain weight in both years in the control treatment ($P<0.05$). Cross-year analysis of variance showed differences between genotypes ($P<0.001$) in the range of 34.0 (Line 21) to 46.0 mg (Rialto). Grain position affected ($P<0.001$) final grain weight in both years. Cross-year analysis of variance showed that the apical grains (G5) were lighter (30.5 mg) than both basal and central grains ($P<0.001$). There was no interaction between genotype and grain position in either year, but the interaction was significant according to the cross-year ANOVA ($P=0.042$). There was no effect of year, year x genotype and year x genotype x grain position interactions. However, grain weight for G1, G2 and G4 was greater in 2005 compared to 2004 ($P<0.001$).

Table 6.4 Model-derived parameters for start and the duration of rapid grain growth of Rialto and Spark for both shaded and unshaded treatments in 2004 and 2005 for five individual grain positions.

		Start of grain filling (°Cd)			Duration of rapid grain filling		
	Grain	2004	2005	Mean	2004	2005	Mean
Unshaded Rialto	GP 1	90.5	61.8	76.1	601 8	637.8	619 8
	GP 2	104.7	57.5	81 1	602.2	653 6	627.9
	GP 3	119.9	89.1	104 5	575 9	604 1	590 0
	GP 4	141.7	130.6	136 1	556 0	550.3	553 2
	GP 5	120.0	102.5	111.3	563.4	564 6	564 0
	Mean	**115.3**	**88.3**	**101.8**	**579.9**	**602.1**	**591.0**
Spark	GP 1	62.6	56.3	59.4	554 9	477 6	516 3
	GP 2	127.5	70.4	98.9	499 8	494 1	496 9
	GP 3	106.1	75.0	90.5	527 6	523 9	525 8
	GP 4	107.6	91.1	99.4	455.8	456 2	456.0
	GP 5	103.0	98.6	100.8	517.4	393 0	455 2
	Mean	**101.4**	**78.3**	**89.8**	**511.1**	**469.0**	**490.0**
GP Mean	GP 1	76.6	59.1	67.8	578.4	557.7	568 1
	GP 2	116.1	64.0	90.0	551.0	573 9	562 4
	GP 3	113.0	82.1	97.5	551 8	564 0	557 9
	GP 4	124.7	110.9	117.8	505 9	503.3	504 6
	GP 5	111.5	100.6	106.1	540.4	478.8	509 6
	Mean	**108.4**	**83.3**	**95.8**	**545.5**	**535.6**	**540.5**
Shaded Rialto	GP 1	37.0	74.0	55.5	658 2	596 1	627 1
	GP 2	34.5	49.5	42.0	645 8	662.5	654 1
	GP 3	122.5	90.9	106.7	548 0	591 9	569 9
	GP 4	79.9	125.0	102.4	607 4	582 4	594 9
	GP 5	102.4	84.5	93 5	556 6	577 5	567 0
	Mean	**75.3**	**84.8**	**80.0**	**603.2**	**602.0**	**602.6**
Spark	GP 1	22.2	71.4	46 8	587 0	435 9	511 5
	GP 2	61.8	104.6	83.2	526 2	375 2	441 7
	GP 3	63.3	116.5	89 9	571 9	349 5	460 7
	GP 4	73.3	106.4	89 9	565 1	417 3	491 2
	GP 5	85.4	105.8	95.6	534 3	438 2	486 2
	Mean	**61.2**	**100.9**	**81.1**	**556.9**	**399.6**	**478.3**
GP Mean	GP 1	29.6	72.7	51.2	622 6	516 0	569 3
	GP 2	48.2	77.1	62.6	586 0	518 9	547 9
	GP 3	92.9	103.7	98.3	560 0	470 7	515.3
	GP 4	76.6	115.7	96.2	586 3	499 9	543 1
	GP 5	93.9	95.2	94 6	545 5	507 9	526 6
	Mean	**68.3**	**92.9**	**80.6**	**580.1**	**500.8**	**540.5**
SED (df)							
Year				4.86 (2)			20 24 (2)
Shading		8.05 (2)	22.39 (2)	11.9 (4)	8 16 (2)	61 67 (2)	31 1 (4)
Genotype		11.28 (4)	19.03 (4)	11.06 (8)	14.91 (4)	36 09 (4)	19 52 (8)
Position		9.7 (31)	11.75 (27)	7.57 (58)	11 82 (31)	26.77 (27)	14 29 (58)
Year x Shade				16.83 (4)			43.99 (4)
Year x Genotype				15 64 (8)			27 61 (8)
Year x position				10.7 (58)			20.21 (58)
Year x shading x Genotype				22 13 (8)			39 05 (8)
Year x shading x position				15.14 (58)			28 58 (58)
Year x genotype x position				15.14 (58)			28.58 (58)
Shading x genotype		15.96 (4)	26.92 (4)	15.64 (8)	21.09 (4)	51 04 (4)	27 61 (8)
Shading x position		13.72 (31)	16.61 (27)	10.7 (58)	16 72 (31)	37 86 (27)	20.21 (58)
Shading x genotype x position		19.4 (31)	23.5 (27)	15.14 (58)	23.64 (31)	53.54 (27)	28 58 (58)
Genotype x position		13.72 (31)	16.61 (27)	10.7 (58)	16.72 (31)	37 86 (27)	20 21 (58)
Interaction of all				21.4 (58)			40 41 (58)

6.2.4.2 Effect of de-graining on final grain weight

There was a trend for post-anthesis de-graining to reduce grain weight in 2004 (P=0.076), but no effect of de-graining in 2005 (P=0.17; Table 6.5). There was no de-graining x genotype interaction in either year. Cross-year analysis of variance showed a reduction in grain weight as a result of de-graining (P=0.016) but the overall effect was small. However, there was no interaction between de-graining x genotype. Moreover, there was no interaction between year x de-graining x genotype nor between year x genotype. Among the genotypes, there was a non-significant trend for Lines 11, 15 and 21 to positively affect by de-graining in both years perhaps indicating that these genotypes were source-limited. The rest of genotypes showed either negative or neutral responses from the control. However, the genotype x de-graining interaction was not significant.

When analysis of variance was performed for the average of the five grain positions, it showed no effect of de-graining in either year. Lines 15 and 21 gained greater weight than each of Lines 11 and 3 in 2004 (P<0.001). Cross-year analysis showed no effect for de-graining nor de-graining x genotype interaction.

There was an interaction between de-graining and grain position in 2005 (P<0.001) but not in 2004. Cross-year analysis of variance showed a reduction in grain weight of all positions except G4 (increased by 1.49 mg) as a result of de-graining (P=0.058) and an increase in weight of both G4 and G5, consistent with the interaction between year and de-graining and grain position (P=0.04). De-graining reduced final grain weight (4.5 and 2.4 % in 2004 and 2005, respectively) for the average of all genotypes and grain positions (Table 6.5). Among grain positions, dry weights of G1, G2, G3 and G5 in 2004 were negatively affected by de-graining (3.1, 4.0, 6.6 and 15.1%, respectively), while G4 was positively affected (4.34%). However, in 2005 only G4 and G5 were positively affected by de-graining (3.1 and 5.2 % respectively) while dry weight of G1, G2 and G3 were slightly reduced by 7.6, 6.6 and 3.2 %, respectively.

6.2.4.3 Stem and leaf lamina dry weights

In 2004 final dry weight of stem and sheath with 50 % spikelet removal was greater than that of control plants with intact ears (P= 0.007; Table 6.6), while leaf lamina dry weight was unaffected by de-graining. In 2005 de-graining did not affect stem or leaf lamina dry weight. The interaction between the de-graining treatment and genotype showed no significant differences.

Table 6.5 Effect of post-anthesis de-graining on final grain weight for the five grain positions and the average of the five grains positions for ten DH lines of Rialto x Spark population and their parents in 2004 and 2005.

	GP 1 (mg)		GP 2 (mg)		GP 3 (mg)		GP 4 (mg)		GP 5 (mg)		Average 5 GP (mg)		
	2004	2005	2004	2005	2004	2005	2004	2005	2004	2005	2004	2005	Mean
Control													
Line 1	28.1	43.4	35.1	42.9	40.2	33.2	33 5	38 5	31.4	25.3	33 6	36 8	35 2
Line 3	41.1	41.6	37.9	42.7	33 0	37 4	32.8	39.2	26 0	28 6	34.9	38 3	36 6
Line 4	35.3	45.4	37.2	49.9	44.9	41.5	39.0	45 3	30.8	30.3	36 4	43 2	39.8
Line 11	40.7	45.7	52.6	43.4	42.4	39.3	34.9	44.2	43.3	33.0	37.3	41.4	39.4
Line 12	51.3	46.5	41.1	46 6	41.5	41.3	43 1	46 1	33.7	32.2	36.6	42.8	39 7
Line 13	44.8	44.5	31.0	44.2	44.1	44.3	39.5	42 7	33.3	28.7	34 8	41	37.9
Line 15	35.6	44.4	47.3	40.7	45.0	38.9	37.5	40.4	30 7	32.0	35.2	39 4	37 3
Line 17	49 8	46.5	36.8	43.6	44.6	42.0	41.9	45.3	32.3	30.9	49.4	42.1	45.8
Line 21	39.2	38.4	40.0	39.9	40 0	33.1	34 2	38 7	35 6	28.5	31.9	36	34.0
Line 26	40.7	51.0	37.1	39.4	35.4	32.3	39.7	37.5	21.6	19.4	36 4	36.4	36 4
Rialto	42.0	47.6	33.7	44.6	52.6	40.5	50.5	47 2	37.5	33 7	44 7	47 2	46 0
Spark	41.8	40 9	43.0	41.5	37.3	33 1	37.5	40.7	25.9	26 5	37 3	37.1	37.2
Mean	40.9	44.7	39.4	43.3	41.7	38.1	38.7	42.1	31.8	29 1	37.4	40 1	38 8
De-graining													
Line 1	28.7	34.2	32.1	33.5	31 2	30 6	36.3	34 5	30 4	27 9	31 8	33 5	32 7
Line 3	42.6	39.5	40.3	42.0	39.6	36.9	44 9	41 3	28 3	25.5	39 3	37 1	38.2
Line 4	39.3	45.2	43.0	45.4	34.9	38 8	33 0	45.9	29 9	36 4	34 6	42 4	38 5
Line 11	44.5	44.7	51 1	43.9	46 1	39 4	45 6	46 9	20 9	32.4	42 3	41 5	41 9
Line 12	38.5	44.0	34.1	45 5	42 9	38 4	37 0	46 5	22 6	32 6	36 2	41 6	38 9
Line 13	40.0	42.9	28.4	42.3	38 3	39 0	39 6	45 8	23 6	30.2	34 6	40	37 3
Line 15	41.9	39.6	42.9	45.9	42 5	43 3	36 7	43 4	34 5	34 5	41 4	41 6	41 5
Line 17	35.1	41.1	34.2	43.3	42 0	38 2	48 7	43 1	25.8	30 7	37 2	39 4	38 3
Line 21	40.0	43.5	36.9	38.9	35 3	35 0	40 7	42 1	33 5	30 2	37 6	38.2	37 9
Line 26	44.0	36.3	38.0	39 8	39 2	31.8	38 6	42 2	25 3	24.5	37 3	35 1	36 2
Rialto	42.9	46.7	41.5	44.0	45 1	41 3	46 8	48 4	26 2	34 1	40 4	43 1	41 8
Spark	37.5	37.3	31.6	39.4	30.8	29.7	36 5	41 4	23 5	28 6	31 9	35 7	33 8
Mean	39.6	41.3	38 7	42.0	39 0	36.9	40.4	43.5	27.0	30.6	37 1	39 1	38.1

SED (df)	2004	2005	Mean	2004	2005	Mean
Year			0.974 (2)			0.650 (2)
De-graining	0.507 (2)	0.448 (2)	0.338 (4)	0 667 (29)	0 575 (44)	0 831 (4)
Genotype	2.496 (44)	1.419 (43)	1 44 (87)	1 633 (29)	1.408 (44)	1 028 (69)
Position	1.346 (171)	0 708 (188)	0.75 (359)			
Year x De-graining			1.031 (2.49)			1 175 (4)
Year x Genotype			2.179			1 536 (38.77)
Year x position			1.36 (7.56)			
Year x de-graining x genotype			2.944 (67 2)			
Year x de-graining x position			1.692 (17.74)			2 292 (39 79)
Year x genotype x position			3 866 (427.5)			
De-graining x genotype	3.53 (44)	2 007 (43)	2.036 (87)	2.310 (29)	1 992 (44)	1 620 (39 79)
De-graining x position	1.903 (171)	1.001 (188)	1.061 (359)			
De-graining x genotype x GP	6.872 (210.5)	3.69 (214.3)	3.866 (427.5)			
Genotype x position	4.86 (210 5)	2.612 (214.3)	2.734 (427.5)			
Interaction of all			5 501 (379.0)			

Table 6.6 Stem and lamina dry weights (g fertile shoot^{-1}) in the control treatment and the post-anthesis de-graining treatment for ten DH lines and their parents in 2004 and 2005

	Stem DW (g)			Lamina DW (g)		
	2004	2005	Mean	2004	2005	Mean
Control						
Line 1	1.21	1.12	1.17	0.20	0.28	0.24
Line 3	1.17	1.43	1.30	0.20	0.35	0.27
Line 4	1.20	1.34	1.27	0.18	0.37	0.28
Line 11	1.26	1.18	1.22	0.21	0.34	0.27
Line 12	1.53	1.45	1.49	0.24	0.37	0.31
Line 13	1.21	1.03	1.12	0.19	0.32	0.25
Line 15	1.22	1.22	1.22	0.19	0.37	0 28
Line 17	1.65	1.30	1.48	0.27	0.30	0.29
Line 21	1.09	1.07	1.08	0.22	0.34	0.28
Line 26	1.18	1.14	1.16	0.26	0.36	0 31
Rialto	1.38	1.39	1.38	0.32	0.45	0 39
Spark	1.13	1.12	1.13	0 18	0 28	0 23
Mean	1.27	1 23	1.25	0.22	0 34	0 28
De-graining						
Line 1	1.32	1.08	1.20	0 18	0.24	0 21
Line 3	1.44	1.47	1.45	0 24	0 35	0 29
Line 4	1.37	1 34	1.36	0.20	0 36	0 28
Line 11	1 37	1.12	1.25	0.22	0 34	0 28
Line 12	1.59	1.45	1.52	0.20	0.34	0.27
Line 13	1.31	1.08	1.19	0 22	0.32	0 27
Line 15	1.18	1.19	1.18	0.17	0.36	0.26
Line 17	1.73	1.33	1.53	0.27	0 31	0 29
Line 21	1.22	1.10	1.16	0.22	0.34	0.28
Line 26	1.18	1.22	1.20	0.27	0.36	0 31
Rialto	1.41	1.65	1.53	0.29	0.49	0 39
Spark	1.22	1.06	1.14	0.20	0.26	0.23
Mean	1.36	1.26	1.31	0.22	0.34	0 28
SED (df)						
Year			0.0141 (2)			0 0131(2)
De-graining	0 0323 (46)	0.029 (46)	0.0189 (4)	0.011 (46)	0.007 (46)	0 008 (4)
Genotype	0.0791 (46)	0.0704 (46)	0.0532 (88)	0.0259 (46)	0.0181(46)	0 0158 (88)
Year x de-graining			0.027 (4)			0 010 (4)
Year x genotype				0.0222 (88)		
De-graining x genotype	0.112 (46)	0.1 (46)	0.075 (88)	0.037 (46)	0.026 (46)	0.022 (88)
Interaction of all			0.106 (88)			0.031 (88)

6.3 Discussion

This discussion focuses on the physiological determinants of grain weight by examining grain water content, and the rate and duration of grain growth in Rialto and Spark for five different grain positions within unshaded and shaded ears. In addition, the last part of this discussion will address the effect of post-anthesis de-graining on individual grain weight for the ten DH lines and their parents.

6.3.1 Grain water content

Many investigations (Miralles and Slafer, 1995b; Bindraban, 1996; Kruk *et al.*, 1997; Calderini *et al.*, 1999b) have considered grains of the central spikelet with respect to proximal (G1 and G2) and distal (G3, G4 and more) positions. This division is assumed to distinguish between grains with high (proximal) and low (distal) weight potential. In this study, proximal grains (G1 and G2) together with a distal grain (G3) were excised from the central spikelet. Additionally, G4 and G5 were excised from distal spikelets, i.e. basal and apical spikelets, respectively.

The time of maximum water content was 33 and 27 (Rialto) and 31 and 25 (Spark) days after anthesis (GS61) in 2004 and 2005 respectively. On average, it occurred 1 day later than the anticipated GS61 +28 d. These findings agree with previous findings that maximum potential grain weight occurred at GS61 + 28 d (e.g. Macbeth, 1996; Schnyder and Baum, 1992). Present results showed that Rialto had higher grain water content than Spark. Variations in final grain weight among individual grain positions were closely related to the maximum grain water content achieved during grain filling. This implied that the maximum water content was a good predictor of final grain weight. These results were consistent with previous reports (Schnyder and Baum, 1992; Chanda and Singh, 1998; Pepler *et al.*, 2006) of a significant correlation between maximum water content and final grain weight. Schnyder and Baum (1992) reported that poor precision will be obtained by predicting final grain dry weight at earlier developmental stages (stages when grain water content is increasing rapidly), indicating that the capacity of grains was not

established, and concluded that volumetric restriction 'as indicated by the grain water content' was involved in the determination of the sink capacity of the grain.

6.3.2 Grain growth

Grain growth rate and duration can be estimated via several models. The model adopted here is the broken-stick model, which describes changes in dry matter of wheat throughout grain filling. It was applied to growth of five individual grain positions, as well as the mean of these positions. Pepler *et al.* (2006), Miralles, Domingues and Slafer, (1996) and Loss *et al.* (1989) argued that although a logistic model described grain growth accurately, a linear model produced narrower confidence limits for estimating the duration of grain growth. This linear model was used in the present study using a base temperature of 0°C. Although it is possible that a different temperature may have improved the fit (Porter *et al.*, 1987; Chalabi *et al.*, 1988; Slafer and Savin, 1991), a base temperature of 0°C has been widely used in previous work (Hay and Kirby, 1991; Slafer and Rawson , 1995; Shearman, 2001).

6.3.2.1 Grain growth rate and duration

The rate and duration of grain growth determine the grain size and are principally controlled by the availability of assimilates and the capacity of developing grains to use those assimilates. The rapid phase of grain filling started earlier (at 98 and 102 °Cd for both Rialto and Spark, respectively) than the anticipated 220 °Cd post-anthesis in both years. Larger main shoots could have been preferentially sampled and this possibly might have been associated with an earlier start to grain filling earlier than for average shoots, shifting the calculated onset earlier (Shearman, 2001). The parental genotypes did not differ in the time from GS61 to the starting of grain growth (lag phase). Grain filling started in G1, G2 and G3 earlier than in G4 and G5 ($P<0.001$).

Since only two genotypes were monitored, regression could not be performed between final weight and mean grain weight (MGW) from combined harvest samples. However, the mean of the grain weight from the five positions from hand-harvested samples was larger than the MGW from combined samples. This may have resulted from a bias in sampling bigger shoots. The mean grain weight from hand-harvested samples may also have been greater due to different grain weight potentials of the five individual grain

positions compared to the average grain when combined. Not only environmental factors influence grain growth and development of wheat, but also genetic factors have a major contribution (Metzger, Czaplewski, and Rasmusson, 1984; Bruckner and Forhberg, 1987). As expected, present results showed variation in final grain weight was observed between Rialto and Spark under both control and source-reduced conditions. Such differences were associated with the observed genotypic differences in the thermal duration of grain growth. Shearman (2001) reported genetic differences in the duration of rapid grain filling in the range 525 to 759 °Cd for ten cultivars released between 1964 and 1994. Furthermore, she reported the duration for Rialto to be 610 °Cd averaged over two years. This was consistent with the present finding in which Rialto and Spark differed in the length of the grain filling period (591 and 490 °Cd over both years, respectively), but they had similar rate of grain growth.

With regard to individual grain position, distal grains (particularly G3 and G5) which achieved smaller final weights also had slower growth rates during rapid grain filling compared with heavier proximal grains in central spikelets (G1 and G2) but both distal and proximal grains had similar grain filling periods. Therefore, an increase in grain number per ear through more grains in distal spikelets of the ear could explain the negative relationship between the grain number per ear and weight in Rialto versus Spark in the present study. Present results showed that G4 had a faster growth rate and heavier grains than both G3 and G5. Grains in the apical and basal spikelets had longer lag phase compared to those in the central spikelet. This because flowering started by about 1d earlier in the central spikelets and then go further out (i.e. then go toward apical and basal spikelets), so such differences is expected to be small.The weight of central grains (G1 and G2) did not differ from each other. In general, there were differences in grain weight among the grain positions and between Rialto and Spark. This was consistent with Stoddard (1999) who found that grain weight from distal florets within the spikelet (i.e. G3) was lighter than those from the two proximal florets (i.e. G1 and G2) which in turn have almost the same weight. Our results indicated that the accumulation of dry matter in wheat grains follows a predictable pattern associated with internal changes in grain water relations. Similar findings were reported in wheat developing grains (Calderini and

Reynolds, 2000), soybean embryo (Swank, Egli and Pfeiffer, 1987) and maize kernels (Borras, westgate and Otegui, 2003). Moreover, the duration of the lag phase was not associated with differences in potential grain weight. Although the literature (e.g. Shearman, 2001) showed a compensation effect between rate and duration of grain growth, the present results showed that rate and duration were partially independent as indicated by a non-significant association between them. Thus, it may be possible for breeders to select for a longer duration and greater rate of grain growth (at least for G3 and G5) which may be an additive genetic effect.

Grain filling duration could be extended in source-limited environments by introduction of the stay-green genes in wheat (Reynolds *et al.*, 1999). Moreover, not only genetic treatments can enhance green area persistence but also other factors (non-genetic) such as application of strobilurin fungicide have been reported to increase green area duration (Jones, Shearman and Sylvester-Bradley, 2001). To date, breeders have not targeted the grain filling duration as a mechanism to increase grain weight, and individual grain weight has changed little with breeding. This is apparently related to increases in grains in distal positions with lower potential weight resulting in increasing the number of grains but with almost the same average grain weight (as discussed in the previous chapter). A further mechanism to explain the conservation of grain size with breeding is that breeders may have selected for stability in grain size, i.e. they select cultivars that can conserve their grain size under unfavourable condition rather than cultivars with large plump grains under favourable condition but with shrinkage grains under harsh conditions. With regard to the rate of grain growth, this trait can be increased genetically via improving potential grain size, removing the restrictions of vascular connection and /or increasing the rate of sucrose downloading into the grain via enhancing the activity of the synthesaze enzyme. These factors will be discussed further in Chapter 7.

The rate of grain growth for the five grain positions was strongly associated with final grain weight. Similar results have been found previously when duration of grain growth was measured in thermal time units (Simmons *et al.*, 1982; Loss *et al.*, 1989; Miralles and Slafer, 1995b; Calderini and Reynolds, 2000; Santiveri *et al.*, 2002). Although the

endosperm cell number is determined at the early grain growth period (Brocklehurst, 1977; Gleadow, Dalling and Halloran, 1982), the duration of the lag phase was not associated with potential grain weight in the present study. A bias may be existed because start date of GS61 was the same for all grain positions. Calderini *et al.* (1999b) and Calderini and Reynolds (2000) suggested that the weight of carpels at anthesis affected the growth of grain after anthesis and it may be that pre-anthesis effects were important in determining grain size. However, carpel weight was not measured in present study. The positive linear relationship between final weight of individual grains and grain growth rate for all the five grain positions in both years (R^2 =0.87 and 0.80; P<0.05 in 2004 and 2005 respectively) indicated that final grain weight was dependent on changes on grain growth rate rather than duration of grain filling. Ma, MacKown, and Van Sanford, (1996) and Simmons *et al.* (1982) observed a strong relationship between rate of grain growth and final grain weight in wheat cultivars but not for grain positions. Furthermore, in the present study no relationship existed between final grain weight and the duration of grain filling for all the five grain positions in both years. With regard to synthetic wheat cultivars Calderini and Reynolds (2000) showed that the duration of grain filling for cultivars was not linearly associated with final grain weight. Present results suggest a large dependence of grain weight on duration of grain growth in UK context. Similar results were observed in barley by Voltas *et al.* (1998). As discussed previously grain filling duration can be increased by inserting the stay green gene or by applying some fungicide that lead to increase green area duration. Moreover, variation in grain growth rate was closely correlated with maximum volume of the grain (Figure 6.8). Numerous studies in maize (Shannon, 1974; Reddy and Daynard, 1983; Ober *et al.*, 1991; Jones, Schreiber, and Roessler, 1996; Borras *et al.*, 2003) have shown that the rate of grain dry matter accumulation depends upon the number of starch granules available for assimilate deposition. Maximum granule number is determined at about the same time that maximum water content is achieved (Jones *et al.*, 1996). As such, it is possible that the variation between grain positions in grain weight observed in the present study reflects genotypic effect on starch granule number (Jones *et al.*, 1996).

Present results showed that breeders should attempt to increase number of grains in the basal spikelets rather than in the apical or in central ones. This was because this study showed that G4 had a faster rate of growth and heavier final weight than both G3 and G5 and had the ability to respond to extra assimilate late in the season as reported by Stockman *et al.* (1983). The probability of setting more grains in the basal spikelet could be improved by increasing the duration of the rapid ear growth period and / or the period between GS31 to GS61. Higher assimilate availability in the period before anthesis (about 20d) should increase number of fertile florets and hence grains per ear (see discussion in chapter 4). Calderini and Reynolds (2000) studied the possibility of setting extra grain (G4) in the central spiklets and found that cultivars with heavier carpels were associated with setting of distal grains and that the greater assimilate availability immediately before anthesis increases carpel weight at anthesis. This hypothesis could also apply for basal positions in the present work, but care should be taken as their work was on synthetic hexaploid wheat lines. Moreover, novel CIMMYT large ear lines with more spikelets can be used as a way of increasing basal proximal grains.

Maximum grain weights were reviewed by Egli (1981) to fall within 23–55 mg grain^{-1}. However, Pepler *et al.* (2006) reported a smaller, but higher range of 39– 59 mg central grain^{-1} with a mean of 54 mg central grain^{-1}. Stoddard (1999) reported a range of 23.3 to 49.3 mg for eight Australian wheat cultivars. Present results showed a range in final grain weight among the five-grain positions of 32-47 mg associated with a comparatively long grain filling period, typical of the UK (Dimmock and Gooding, 2002). The present study is consistent with previous work demonstrating that differences in final weight between grain positions are more a function of filling rate than filling duration in wheat (Sofield *et al.*, 1977; Miralles and Slafer, 1995b; Dimmock and Gooding, 2002) and barley (Voltas *et al.*, 1998).

6.3.3 Response of grain growth to shading

Altered source-sink ratio (by post-anthesis shading) reduced final weight of individual grains in 2004 because of alteration in grain growth rate. Thus, shading caused an imbalance between source and sink activity that led to smaller grains. However, shading

did not affect the duration of grain growth. Some authors (e.g. Fischer and HilleRisLambers, 1978; Simmons *et al.*, 1982; Borghi *et al.*, 1986) have shown that reduction of source by defoliation leads to smaller amount of assimilate supply to grains, hence, smaller grains. In 2004, shading decreased the rate of grain growth of both Rialto and Spark by 17%. Also, final grain weight was decreased by 14% (Rialto) and 10% (Spark) compared with control plants. This generally small response suggests that control crops were probably sink-limited, but Spark (10%) may be slightly more sink-limited than Rialto (14%). The source supply of both parents apparently met most of their sink demand even when incident radiation was reduced by 50% shading. Increased mobilization of assimilate reserves from the stem to grains could partially counter decreased assimilate supply due to shading. In this study, increased mobilization of assimilate reserves probably compensated for a part of the photosynthate loss due to shading. Pre-anthesis carbohydrate contributed almost the same amount of reserves over both years (39 % of final grain weight) of shaded Rialto and Spark (see section 5.4.13). In addition, RUE post-anthesis for shaded treatment was 20% higher than that for unshaded treatment, and partly contributed in compensation for photosynthate loss by shading (See section 5.4.5). These results implied that post-anthesis RUE was up regulated in relation to a relative increase in grain sink strength. So, breeders might increase RUE through increased sink strength.

6.3.4 Response of grain growth to de-graining

Leaf lamina, ears and stem tissues are the major sources of assimilates during grain filling (Rawson *et al.*, 1983). During early stages after anthesis, when the grain sink strength is low, extra assimilates are often stored in the vegetative tissues (stem reserves) (Blacklow, Darbyshire and Pheloung, 1984). The stored assimilates are later re-translocated to grains as a result of the reduction of source strength and an increase in sink strength (Calderini and Reynolds, 2000). So, post-anthesis de-graining should result in an increase in grain size if grain growth is source-limited

Grain weight of all tested genotypes except Lines 11, 15 and 21 were either unaffected or negatively affected by post-anthesis de-graining. Our findings were consistent with those of Slafer and Savin (1994), Ma *et al.* (1995; 1996) and Calderini and Reynolds (2000), who found no positive response of individual grain size to source-sink manipulation by post-anthesis de-graining of 50% spikelets. This general lack of a positive response would imply that assimilate availability in control plants in this study was sufficient to satisfy fully their grain growth requirements and that grains reached their potential maximum in the control plants. Thus, grain yield in these genotypes was sink-limited during the grain filling period. This is in general agreement with early work of Evans and Rawson (1970), who reported that photosynthesis of both flag leaf and ear alone could satisfy the demand of grains at all times during grain filling. Similarly, Savin and Slafer (1991) reported evidence of sink limitation to grain growth as a result of shading. In general, present results confirmed the hypothesis of sink limitation of grain growth during the grain filling period. This agrees with previous work on wheat and barley (Borghi *et al.*, 1986; Slafer and Savin, 1994; Mirrales and Slafer, 1995b; Dreccer, Grashoff and Rabbinge, 1997; Kruk *et al.*, 1997; Reynolds *et al.*, 2005; Calderini, Reynolds and Slafer, 2006).

Grain growth of Lines 11, 15 and 21 showing a non-significant trend for increases, so, these lines may be close to source-limitation (lines 11 and 15 have high PGW while line 21 had high values of source-type traits), because an increase in source level by 50% spikelet removal enhanced final grain weight. Because the final grain weight of these genotypes was increased as a result of de-graining, it seems likely that the grain growth of these genotypes was close to limitation by the availability of source material rather than by reproductive sink strength. Thus, after grain number is fixed, adverse post-anthesis conditions may affect grain yield of such responsive genotypes more than yields of non-responsive genotypes to de-graining.

With regard to the effect of de-graining on individual grain position within the ear, there was a neutral response to de-graining on individual grain weight for positions G1, G2 and G3. In general, the growth of these grains was sink-limited. However, final weight of

grain 4, and to a lesser degree grain 5, was positively affected by de-graining. This would imply that the growth of these grains was source-limited. This indicates that the physiological basis to final grain weight for G4 and G5 in this population was related to source supply. However, de-graining did not increase the final weight of G3 indicating that this distal grain in the central spikelet is limited by the size of the sink. It is possible that physical restriction on growth by the lemma and palea of distal florets may have limited final grain weight in G3 (Voltas *et al.*, 1998). Also it could be source limitation, but need to change vascular traces since altering the gradient of assimilation has no effect. Furthermore, the potential size of the grain is correlated with carpel size (Scott *et al.*, 1983; Calderini *et al.*, 1999b) and the limitation in weight for G1, G2 and G3 may have been established earlier before imposing the de-graining treatment (i.e. limitation resulted from lower source supply during the lag phase) or might be related to the lower levels of cytokinins that affected endosperm cell division.

The lack of relationship between relative change in grain weight and individual grain weight of controls for genotypes indicated evidence of sink limitation to grain weight in these genotypes. For the sink-limited crops under optimum condition, final grain weight should correlate with potential grain weight as seen in 2005.

6.3.4.1 Stem dry weight

Genotypes that received de-graining treatments had substantially greater stem dry weight at maturity than control plants with intact ears (Table 6.6). This difference gives indirect evidence that de-grained plants might have retained more stem reserves than the controls at harvest. However, these genotypes failed to have a significant compensatory increase in grain weight. Substantial accumulation of reserves in stem tissues implied a limited grain capacity to use assimilates after anthesis consistent with sink-limitation.

6.3.5 Summary

Results showed that breeders should select for longer grain filling period for all grain positions and for faster filling rate for G4 and G5. Because grain 4 is heavier, than G3 and G5 it is recommended to select for extra grains in the proximal positions within basal spikelets within the ear rather than distal positions in central spikelets. The hypothesis that wheat is sink-limited was further tested and confirmed in this chapter. However, a trend for positive response to de-graining in some lines indicated that there may be a shift toward source-limitation in the tested DH population. So, there is likely a requirement for increasing source strength simultaneously with the sink in UK winter wheat breeding programmes in future years.

7 General discussion

Breeders worldwide have successfully improved wheat yields through the introduction of semi-dwarfing alleles during the 1960s and 1970s conferring associated increases in HI (Richards, 2000; Reynolds *et al.*, 2005). More recently in the 1980s in the UK the 1BL/1RS translocation was introduced into feed wheat cultivars associated with a range of disease resistances but also apparently with increased yield potential *per se*. The world demand for wheat is increasing at approximately 2% per year (Rosegrant, Agcaoili-sombill and Perez, 1995; Calderini *et al.*, 1999a; Reynolds *et al.*, 2005), while genetic gain in yield potential of irrigated wheat stands at approximately 1% per year (Sayre *et al.*, 1997; Brancourt-Hulmel *et al.*, 2003; Shearman *et al.*, 2005), so there is a need to increase yield potential to meet the world demand. Most recent investigations indicated that genetic gain in wheat yield is limited by the size of the grain sink (e.g. Reynolds *et al.*, 2005; Shearman *et al.*, 2005). Understanding source or sink limitations on crop yield is critical for the rational design of breeding strategies as well as agricultural practices (Gambin and Borras, 2006). The present research studied the determinants of yield potential in a Rialto x Spark doubled haploid population and their parents. This population was chosen because parents were known to contrast in both source (stem reserves and pre-anthesis RUE) and sink-type traits (e.g. grains m^{-2}, MGW). They also contrasted in the presence/absence of the *Rht-D1b* semi-dwarfing allele and the 1BL/1RS translocation. It was hoped that this study would identify physiological avenues for future genetic yield progress.

7.1 Harvest index and biomass

Present results showed that HI accounted for 55% of the phenotypic variation in grain yield in the DH population. Harvest index has been considered as the main driving force for the improvement in genetic yield potential in wheat both before and after the green revolution and particularly with regard to the introduction of semi-dwarfing genes. Austin (1980) estimated the theoretical upper limit for HI to be *ca.* 0.62 due to certain amount of biomass being required for physical support and assimilate production. Present results showed that 0.50 was the highest value for any line of this modern DH population. This

implies some scope for further yield increases via HI. Shearman *et al.* (2005) studied a series of UK cultivars released between 1972 and 1995 and reported that 0.53 was the highest value for HI, with HI being increased by 0.0045 per year. Those authors suggested that Austin's upper limit of 0.62 may be slightly optimistic the UK and modified this limit to be 0.66 in UK winter wheat. The increase in HI reported by Shearman *et al.* (2005) was associated with increase partitioning to the ears as a direct effect of the introduction of the semi-dwarfing allele. In the present study, in addition to effects of the semi-dwarf allele, genotypic differences in the amount of stem soluble carbohydrate accumulated ($P<0.05$) might have contributed to the observed differences in HI. The more the stem WSC, the greater the potential post-anthesis partitioning to the grains so selecting for higher stem reserves may be an avenue for future progress in HI.

Stem reserves was positively correlated with yield in 2006 in agreement with findings elsewhere in the literature that the contribution of stem reserves may be important under optimal conditions in addition to under post-anthesis stress conditions. For example, Gallagher *et al.* (1975) showed that under conditions of stress, such as drought, re-translocation of stem reserves can supply up to 70% of the final yield in barley. The present study showed that shading caused a slight increase (3%) in the re-translocation of stem reserves to the grains. Foulkes *et al.* (1998) reported that effectively all stem WSC reserves of modern wheat varieties (as was the case in the present study under optimal conditions) were remobilized by harvest in both droughted and irrigated crops.

In the current study, ANOVA indicated there were no genotypic differences in harvest AGDM (cross-year ANOVA P value of 0.13). However, harvest AGDM correlated phenotypically with grain yield and accounted for 28% of the differences in grain yield amongst the genotypes. This implies that the variation in yield was mainly related to variation in HI, but that there was probably a smaller contribution from AGDM. Our findings with regard to biomass were therefore not conclusive but there was an apparent genetic effect, indicated by a significant increase in biomass with 1BL/1RS, and a significant correlation between AGDM and grain yield amongst the DH lines. Improved AGDM might be associated with a reduction in partitioning to roots, so the total biomass remains similar as AGDM increases. This was indirectly supported by findings of

Foulkes *et al.* (1998) that the newer varieties from 1969 to 1988 were less able to absorb soil N under a nil N treatment possibly due to their smaller root system compared to their predecessors. Other recent investigations (Reynolds *et al.*, 2005; Shearman *et al.*, 2005) reported that harvest biomass was associated with genetic gains in yield. Therefore, a breeding approach is needed that will select for genotypes with higher biomass capacity while maintaining the high partitioning of assimilate to the grain (Babar *et al.*, 2006; Muurinen and Peltonen-Sainio, 2006). An increase in harvest biomass may be achieved by increasing the amount of radiation interception and/or radiation-use efficiency. Potential physiological avenues for increasing these components will now be discussed.

7.2 Radiation interception

The current study showed a positive linear relationship between accumulated PAR interception during the GS31 to GS61 period and the duration of the same period amongst the genotypes. The amount of radiation that is intercepted by a crop is dependent on the amount of incident radiation and the ability of the crop to intercept it (Slafer *et al.*, 1999). Both developmental and canopy size and architecture factors can be genetically manipulated. Thus, the crop might accumulate more intercepted radiation by increasing the length of the growing cycle or increasing total daily interception by the canopy.

Genetic manipulation to increase the relative duration of the stem-elongation phase and hence intercepted radiation and ear biomass has been suggested as a mechanism to improve yield potential in wheat (Slafer *et al.*, 1996; Reynolds *et al.*, 2005). However, delaying flowering significantly in the UK could increase grain losses associated with late harvesting, so advancing GS31 whilst maintaining GS61 is the preferred pattern of development. An extended stem-elongation period should directly favour greater ear biomass and stem WSC reserves. The rapid ear-growth period can be extended through manipulating the photoperiod (Ppd) and vernalization (Vrn) genes (Reynolds *et al.*, 1999; Gonzalez *et al.*, 2003) which could lead to increased radiation intercepted during this important period. This in turn will positively affect the number of fertile florets per spikelet hence, increasing grains m^{-2} and yield. Thus, manipulating photoperiod

sensitivity during stem-elongation period probably offers the best prospect for progress. In the present study, genotypes differed in accumulated intercepted radiation between GS31 and GS61, with differences caused by differences in the length of stem-elongation phases rather than by differences in canopy architecture or leaf traits affecting the extinction coefficient.

Alternatively, increasing the capacity of the crop to intercept radiation may be achieved through increasing the GAI hence fractional interception. However, increasing GAI later in the pre-anthesis period is unlikely to be beneficial because the optimum GAI of about 5-6 (at which point the crop is able to intercept more than 95% of the incident radiation (Sylvester-Bradley *et al.*, 1997; Reynolds *et al.*, 2005)) is already achieved. Any further increase in GAI will be associated with diminishing returns in fractional interception, increased lodging and disease risk and also wasted assimilate in maintenance respiration (Shearman *et al.*, 2005).

Genotypic differences in tiller production and a linear relationship between shoot production and number of non-surviving shoots were found, which means that greater shoot production resulted in greater shoot death. These wasteful tillers may compete for assimilate with fertile tillers and lead to reduction in its ear size. So, a reduction in tiller death might offer another window for increasing partitioning to developing ears pre-anthesis (Shearman *et al.* 2005).

7.3 Radiation-use efficiency

Positive linear relationships between RUE and each of ear biomass at anthesis, grains m^{-2}, and grain yield in the present study indicated the importance of RUE for enhancing grain number and yield potential.

In the present study, the significant correlation with yield and biomass indicated that pre-anthesis RUE was contributing to genetic variation in biomass in this population. Similarly, Shearman *et al.* (2005) reported an increase in pre-anthesis RUE of modern UK cultivars correlated with genetic gains in yield. The period between GS31 to GS61 is

important in determining grains m^{-2} and hence yields (Fischer, 1985; Reynolds *et al.*, 2000; Shearman *et al.*, 2005). During the latter part of this period (i.e. the rapid ear-growth period), more than 95% of radiation is usually intercepted by the winter wheat crop (Sylvester-Bradley *et al.*, 1997). This means that increasing RUE during the time between GS31 and GS61 could increase grains m^{-2}, which could increase yield if grain growth is sink-limited (Fischer, 1983, 1985; Reynolds *et al.*, 2000, 2005). Several authors have reported that modern wheat cultivars were sink-limited during the post-anthesis period (e.g. Shearman *et al.*, 2005; Cartelle *et al.*, 2006). Evidence for increases in post-anthesis photosynthetic rate as a result of increases in grain number in such sink-limited cultivars implies up-regulation photosynthetic rate according to grain sink strength (Sayre *et al.*, 1997; Fischer *et al.*, 1998; Reynolds *et al.*, 2005; Calderini *et al.*, 2006). If potential grain number can be increased, RUE during grain-filling may be increased in response to alleviation of feedback inhibition, permitting synchronized increases in final biomass and yield. Moreover, Fischer (1983) and Fischer and Stockman (1986) studied cultivars contrasting in the presence of the semi-dwarfing genes and reported that cultivars with *Rht-D1b* alleles had greater partitioning to ears at anthesis (as was the case in the present study) and an increased post-anthesis RUE during grain-filling compared to tall cultivars. However, overall RUE is not increased in *Rht-D1b* genotypes, because while post-anthesis RUE was increased in semi-dwarf lines compared to tall lines, the pre-anthesis RUE was reduced. (Reynolds *et al.*, 2005). Present results showed 1BL/1RS translocation improved RUE under favourable conditions which confirmed the hypothesis that 1BL/1RS increases RUE.

Present results showed a positive linear relationship between pre-anthesis RUE and specific leaf weight. Shearman *et al.* (2005) reported a reduction in the flag leaf size with breeding without any reduction in PAR interception. The authors related this to the production of more shoots m^{-2}, but also reported an increased SLW with year of introduction and positive correlation with pre-anthesis RUE. Smaller flag leaves with higher SLW may enable more light penetration deeper in the canopy and hence reduce light saturation of upper leaves (Muurinen and Peltonen-Sainio, 2006). However, some

care should be taken in reducing flag leaf size as the flag leaf size could be presently close to it is optimum.

RUE can also be increased by improving the canopy structure through selection for erect leaves. In the UK, there is evidence for yield to increase in cultivars having erect leaves (Innes and Blackwell, 1983). However, most cultivars already have erect leaves. So, this trait has limited scope for improvement.

Additional potential mechanisms for genetically improving RUE were reviewed by Reynolds *et al.* (2000). At the metabolic level, the Rubisco enzyme might be genetically modified to improve its specificity for CO_2 which could result in increasing the net photosynthetic rate by about 20% and reduced photorespiration (Austin, 1999; Reynolds *et al.*, 2000). This could theoretically be achieved by introduce genes from blue-green algae with higher specificity. At the level of the canopy, a more optimal source and sink balance could improve overall RUE (Slafer *et al.*, 1996; Richards, 1996; Kruk *et al.*, 1997). For example, there may be periods during pre-anthesis growth when RUE is indirectly reduced by sink limitation, and that genetic modification of the source: sink balance could result in a better use of otherwise underutilized photosynthetic capacity (Reynolds *et al.*, 2005).

7.4 Limitation of yield potential in the UK (source or sink)

Grain filling can be limited through either the supply of photosynthate or the capacity of the grain to accumulate the available assimilates (Bingham *et al.*, 2006b). In the present study, the source-sink balance model indicated that each of six genotypes assessed was sink-limited. The model may have overestimated source and hence overestimated the extent of sink limitation. Present findings generally confirmed the original hypothesis of wheat sink limitation by grain number and potential weight. Borras *et al.* (2004), Reynolds *et al.* (2005) and Calderini *et al.* (2006) concluded that grain filling in spring wheat worldwide was mostly sink-limited. The extent of source and sink limitation was also investigated in present study through responses to post-anthesis shading and de-graining. Responses to post-anthesis shading in the current study indicated that the six

genotypes examined were sink-limited, but they differ in the extent of sink limitation (i.e. some of them closer to source limitation than others). Responses to post-anthesis de-graining in the present study indicated that nine of the genotypes examined were sink-limited while the remaining three were marginally source-limited. This added some support to the hypothesis that modern UK cultivars, although sink limited, may be closer to source limitation than their predecessors. If so, this means that there is a need to improve both source and sink strength simultaneously in breeding in future years. Source size can be improved by increasing the seasonal radiation interception (by optimizing canopy structure, leaf angle, SLW and the duration between GS31 and GS61 as well as the rapid ear-growth period), RUE and stem carbohydrate reserves. Sink size is determined by grains m^{-2} and PGW. In the present study, the number of grains m^{-2} at harvest was positively correlated with ear DW m^{-2} at anthesis and ear index. So, targeting both the duration between GS31-GS61 and the critical rapid ear-growth phase should lead to more fertile florets per ear and hence more grains m^{-2}. The extra grains should, in turn, increase the demand for assimilate and lead to improved post-anthesis RUE. The present study showed a positive linear association between RUE and grains m^{-2} and that RUE accounted for 51% of the variation in grain m^{-2}. With regard to potential grain weight a longer lag phase immediately post-anthesis resulting in greater assimilate availability could increase the cell division and expansion rate and the endosperm cell number. In the current study, Rialto had longer lag phase and higher PGW than Spark. In summary, increasing both grain number and potential grain weight in sink-limited crops will enhance the sink size and result in increased yield. The above-mentioned factors for enhancing source and sink sizes will now be further considered.

7.4.1 Determinants of grain sink capacity and avenues for future progress

The knowledge of factors affecting both grain number and mean grain weight is important for underpinning increases in yield potential.

7.4.1.1 Avenues to increase grains m^{-2}

Grains m^{-2} at harvest was positively associated with anthesis ear DM m^{-2}. This indicates that grain number can be further increased through enhancing ear growth during the critical ear growth phase (about 20 d before anthesis), adding to the effect of the semi-dwarfing allele on floret fertility. Present results indicated that this can be achieved by lengthening stem-elongation phase or by altering partitioning between the ear and stem. So, lengthening this period will provide more assimilate to the growing ears and increase the number of fertile florets, hence the number of grains and the potential yield. Moreover, grain m^{-2} was positively correlated with ear index at anthesis, accounting for 23% of the variation in grain m^{-2}.

Grain yield was positively correlated with grain number in this study. However, there was no relationship between grain yield and MGW and a negative relationship between grain number and weight. Moreover, Acreche and Slafer (2006) showed that when grain number increased in wheat, MGW was concurrently reduced, but independently of any competitive relationship between the growing grains. Furthermore, the UK investigation of Shearman *et al.* (2005) showed no change for MGW with breeding. The current population was originally produced in part because the parents were contrasting in stem WSC reserves. Present results showed a positive linear relationship between ear DM at anthesis and stem WSC reserves ($P=0.02$; $R^2= 0.42$). This indicated that stem WSC reserve increases did not occur as a result of decreased partitioning to the ear.

Rialto (*Rht-D1b*, 1BL/1RS) had more fertile florets and heavier spikelets (ears) than Spark (*Rht-D1a*, 1B). This was mainly due to the effect of the semi-dwarfing gene which reduced the competition between stems and ears. This leads to increase assimilate partitioning to ears which in turn increased number of fertile florets and hence, increased number of grains m^{-2} and yield (Fischer and Stockman, 1986; Gonzalez *et al.*, 2003). The effect of the semi-dwarfing genes on partitioning and grains m^{-2} was confirmed in the present study. Additionally, 1BL/1RS increased grains m^{-2} in this study.

This modern DH population possessed lines with plant height generally in the optimum range (of between 70 and 90 cm) (Slafer *et al.*, 2005) and a further reduction with breeding could result in poorer radiation distribution and a decrease in the biomass (Miralles and Slafer, 1995a; Reynolds *et al.*, 2005). However, it is possible that floret fertility (grain number) could potentially be enhanced by further reduction in competition between ear and stem through reducing the length of the peduncle (the most rapidly expanding organ just before anthesis) through selection (Richards, 1996).

The linear relationship between the duration between GS31 and GS61 and PAR interception accumulated over the same period indicated the importance of the length of such period on radiation interception and hence on biomass production and partitioning. However, the best prospects for increasing grains m^{-2} in future years may be through increasing RUE and ear biomass and not radiation interception

7.4.1.2 Avenues for increasing potential MGW

Mean grain weights of the grains (G1 and G2) from the central spikelet within the ear were heavier compared to G4, G3 and G5, respectively. These differences in weight were potentially related to restriction of the grain size by the glume or to poorer vascular connection for G3-G5. Because the weight of G4 and G5 was increased by de-graining, it appears that the grains in these positions may be subjected to some degree of source limitation.

As discussed earlier, grain yield was not correlated with MGW, but was correlated with grains m^{-2}. Grains m^{-2} was negatively correlated with MGW. Since lines of this UK winter wheat population were probably sink-limited during grain filling (see Chapter 5), this implies that MGW may have been negatively correlated with grain number because the extra grains were formed in distal positions with lower weight that reducing the average mean grain weight (see Chapter 6) (Slafer *et al.*, 1996; Shearman *et al.*, 2005; Acreche and Slafer, 2006). Thus, the genotypes were sink-limited although there was some evidence that the grains positions G4 and G5 may have been marginally source-limited.

The observed positive linear relationship between MGW and PGW in this study was encouraging since it may enable growers to predict the final grain weight from simple measurements of the crop shortly after anthesis, thus potentially allowing modifications (such as application of fungicide or fertilizers) to be made to crop husbandry (Bingham *et al.*, 2006b)

Genetic differences in radiation interception per grain for the 10 d period after anthesis (i.e. the period of rapid cell division and expansion) were observed in the current study, and there was trend for a relationship with PGW. As a result of rapid cell division during this period (lag phase), the number of the endosperm cells is determined, which in turn influences potential grain size (Brocklehurst, 1977). The rate of cell division, and hence the endosperm cell number, is affected by the level of assimilates (Brocklehurst, 1977). So, selection for a longer cell division and expansion period (lag phase) could increase assimilate supply and positively affect potential grain size.

There are other factors which might restrict maximum potential grain size other than assimilate availability during the cell division and expansion phase. These factors include physical restrictions by glumes (Millet, 1986; Voltas *et al.*, 1998), insufficient vascular connections within the ear (Natrova and Natra, 1993) and the presence of the semi-dwarfing genes that results in smaller cell dimension because of insensitivity to gibberellic acid and hence lower grain weight (Miralles *et al.*, 1996).

Present results showed that breeders should attempt to increase number of grains in the basal spikelets rather than in the apical or in central ones. This was because G4 had a faster rate of growth and heavier final weight than both G3 and G5 and had the ability to respond to extra assimilate late in the season as reported by Stockman *et al.* (1983). The probability of setting more grains in the basal spikelet could be improved by increasing the duration of the rapid ear growth period and / or the period between GS31 to GS61. Higher assimilate availability in the period before anthesis (about 20d) should increase number of fertile florets and hence grains per ear

Final grain weight for positions G1, G2 and G3 were not affected by de-graining, indicating that the growth of these grains was sink-limited. However, final weight of G 4, and to a lesser degree G 5, was positively affected by de-graining. This would imply that the growth of these grains was source-limited. However, de-graining did not increase the final weight of G3 indicating that this distal grain in the central spikelet is limited by the size of the sink. It is possible that physical restriction on growth by the lemma and palea of distal florets may have limited final grain weight in G3 (Voltas *et al.*, 1998). Also it could be source limitation, but need to change vascular traces since altering the gradient of assimilation has no effect. Furthermore, the potential size of the grain is correlated with carpel size (Scott *et al.*, 1983; Calderini *et al.*, 1999b) and the limitation in weight for G1, G2 and G3 may have been established earlier before imposing the de-graining treatment (i.e. limitation resulted from lower source supply during the lag phase) but this did not appear to be the cause in the present study, or might be related to the lower levels of cytokinins that affected endosperm cell division.

Present results indicated that genotypes differed in the thermal duration of grain filling while grain positions differed in the rate of grain filling. However, there was no relationship between rate and duration of grain growth for the grain positions. Grain-filling duration could be genetically extended by introduction of the stay-green genes in wheat (Reynolds *et al.*, 1999) or non-genetically by e.g. application of strobilurin fungicide have been reported to increase green area duration (Jones *et al.*, 2001). But these may not be relevant in sink-limited crop. Breeders should select for increasing the potential grain size first and then the increase in source size became relevant to satisfy the requirements of the increased grain size.

With regard to rate of grain growth, this trait can be increased genetically via improving potential grain size, reducing any restrictions of poor vascular connections and /or increasing the rate of sucrose downloading into the grain via enhancing the activity of the starch synthase enzyme (Jiang *et al.*, 2003).

Line 8 showed a consistent positive departure from the overall negative relationship between grain number and grain weight. So, there is a need to investigate further the mechanisms potentially responsible for producing higher grain number as well as heavier grains. As previously discussed, there are some avenues to increase grain weight without affecting grain number. These could include removing the vascular restriction and optimising activity of the starch synthase enzyme and extending the thermal duration of cell division and expansion. Selection for a long-ear phenotype may increase distance between spikelets and enhancing individual glume size and hence potential grain size through removing physical restrictions.

Shading decreased the rate of grain growth (by 17%) and final grain weight of both Rialto and Spark (by 14% and 10%, respectively) compared with control crops. Such small responses suggested that control crops were probably sink-limited, and that Spark may be slightly more sink-limited than Rialto. Averaging over genotypes, the amount of stem reserves contributed to grains under shading is similar to the amount contributed under unshaded conditions. So, increased remobilization of assimilate reserves from the stem to grains could in the present study counter decreased assimilate supply due to shading. However, effects were small and pre-anthesis carbohydrate contributed almost the same amount of reserves over both years (39 % of final grain weight) for shaded Rialto and Spark crops (see section 5.4.13). However, there were some differences between individual lines. Post-anthesis RUE for shaded treatment was 20% higher than that for the unshaded treatment, and partly compensated for photosynthate loss by shading (See section 5.4.5). These results show that post-anthesis RUE is up-regulated in relation to grain sink strength and suggested that post-anthesis RUE may respond to further genetic increases in sink size.

The existence of the genotypic differences in stem WSC reserves and high utilization under optimal conditions suggested that selection for stem WSC reserves should consider as priority trait in breeding programmes.

7.4.2 Post-anthesis source supply

In the present study, genotypes did not differ in post-anthesis PAR interception. However, Rialto and Spark differed in the duration of grain growth. Because the yield in the present study principally limited by the strength of the sink and not the assimilate availability, increasing the post-anthesis green area duration may be of limited use since the assimilate produced by the crop was already more than the requirement by the sink. Thus, increasing the grain number and or the grain weight (stronger sink) is the pre-requisite and lengthening the grain filling period (through sink-related effects) becomes a preferred option. The introduction of stay-green genes into wheat could extend grain filling duration (Reynolds *et al.*, 1999). Other factors, such as application of strobilurin fungicides may increase the canopy green area at anthesis or increasing chlorophyll content and hence improving source size (Jones *et al.*, 2001). These effects may be important in the future to increase the post-anthesis source size concomitantly with grain sink size. Genetic differences were found in stem reserves which may be considered as an important source of assimilate during grain growth. This trait has a high heritability (Ruuska *et al.*, 2006) and selection for such trait will help in enhancing the source strength in breeding programmes.

7.4.3 Development of the source-sink balance model

The source-sink balance model used the pre-anthesis RUE and the stem reserves at anthesis + 75 °Cd as components of the post-anthesis source. RUE has been reported to be lower late in the post-anthesis period (Sinclair and Muchow, 1999). However, this reduction may be not significant because senescence starts from the bottom of the canopy on leaves that already have low photosynthetic activity. The model supposed that pre-anthesis RUE did not change during the post-anthesis period. The present study showed that RUE can be increased post-anthesis if relative sink strength was increased as indicated by the 20% increase in post-anthesis RUE as a result of shading. Thus, RUE could be modified in the model according to sink size by multiplying the RUE by a coefficient or correction factor. The current model does not adjust RUE according to sink strength so, this is a possible area for future improvement. Only about 73% of stem WSC reserves accumulated contribute to the grains (Austin *et al.*, 1977). So, the model may need some adjustment since currently 100% of stem WSC reserves are assumed to

contribute to grains. In general, the present study showed that shading resulted in (i) extending the green area duration by about 3 days (ii) enhanced RUE by 20% which implied that genotypes had the ability to enhance their resources utilization and minimize their waste under stress conditions. However, the contribution of stem reserves to the grains was similar under shaded and unshaded conditions which implied that the genotypes in unshaded conditions were using all their available stem reserves, although the efficiency of utilization may have been less than 100%. Some losses may be due to respiration and transport (Gebbing *et al.*, 1999)

Because field evaluations are expensive and time consuming, an easy, rapid, and inexpensive selection tool would help breeders consistently screen large numbers of genotypes in a relatively short time (Reynolds *et al.*, 1999). It would also be very advantageous if such a selection tool has a higher heritability than grain yield, shows a strong correlation with grain yield, and could detect high yielding genotypes rapidly and efficiently from a large number of genotypes (Baber *et al* 2006). For example canopy temperature, which can be measured remotely using infrared thermometry, has been shown to be well associated with the yield of wheat cultivars (Reynolds *et al.*, 1994; Fischer *et al.*, 1998). More recent studies suggest that spectral reflectance indices (SPI) provides another promising remote sensing technique for screening genotypes (Araus, 1996; Araus, Casadesus and Bort, 2001). These indices have been used to predict different vegetation parameters, such as green biomass and green leaf area index (Tucker and Sellers, 1986). Spectral reflectance indices have also been developed to assess radiation-use efficiency by the plants (Penuelas, Filella and Gamon, 1995), and water status of the canopy (Penuelas *et al.*, 1993). Spectral reflectance indices have been widely reported by different authors to assess different physiological conditions of the canopy such as total dry matter, LAI, photosynthetic capacity (Sellers, 1987), as well as green LAI and fraction of photosynthetically active radiation absorption (Wiegand and Richardson, 1990; Baret and Guyot, 1991; Wiegand *et al.*, 1991). Spectral reflectance indices have also proven to be useful in the assessment of early biomass and vigor of different wheat genotypes (Elliott and Regan, 1993; Bellairs *et al.*, 1996).

7.5 Application of physiological traits in plant breeding

Breeders worldwide still largely use empirical selection methods for grain yield *per se* (Loss and Siddique, 1994). However, this selection method is not efficient because of low heritability of yield and a high genotype x environment interaction (Jackson *et al.*, 1996;). Alternatively, understanding yield limiting traits may help breeders to improve yield through indirect selection for these traits. This approach is considered as complementary to the strategies used in conventional breeding programmes to accelerate yield improvement (Araus, 1996; Slafer and Araus, 1998; Reynolds *et al.*, 2000, 2002 and 2005). Before inclusion in a breeding programmes, such physiological traits must: (i) show enough genetic variability, (ii) have a high genetic correlation with yield, (iii) have a higher heritability than yield itself in genetic populations representative of those being evaluated (Jackson *et al.*, 1996) and (iv) have a rapid-screening method that can be applied. Identify the genetic control of target traits, physiologists should choose parents contrasting in potential yield enhancing characteristics (Shearman *et al.*, 2005). These parents should then be crossed and the resultant mapping population characterized for physiological and molecular traits in both the field and the laboratory, respectively. This will lead to identifying and tagging important genes or quantitative trait loci (QTLs). As mentioned earlier, any trait to be targeted by breeders must be directly related to yield. Yield itself can be expressed either via the numerical component model (i.e. ears m^{-2}, spikelets per ear, grains per ear and MGW) or the resource capture model (i.e. light interception, RUE and HI). However, because the yield component model cannot precisely identify the interrelationships between yield forming processes, the resource capture model has been principally used in the present chapter to identify key traits to be used by breeders. In the present study, the period between GS31 and GS61, pre-anthesis RUE, and stem WSC reserves were significantly related to yield. Thus, the thermal time duration between GS31 and GS61, pre-anthesis RUE, and stem WSC reserves are identified as potential traits for yield improvement.

In summary, there is a genetic difference in HI but because it is close to the theoretical upper limit, the scope to improve yield via targeting HI is limited. In the future, it will be important to select for traits underlying higher AGDM since this trait associated with yield gains. The hypothesis that there is genetic variation in pre-anthesis RUE positively

correlated with grains m^{-2} was confirmed in the present study. This means that selection for RUE should have a positive impact on yield. Genetic variation in stem WSC reserves was found in the present study, and was also correlated with yield. The present results showed that selection for stem WSC reserves might be beneficial selection criteria since it was not at the expense of ear DM as indicated by the positive linear relationship between these two traits.

The present study showed that there might be a possibility to break down the overall negative relationship between grain number and weight. So, physiologists should identify mechanisms responsible for breaking the linkage in the future years. These could include removing the vascular restriction and optimising activity of the starch synthase enzyme and extending the thermal duration of cell division and expansion. Selection for a long-ear phenotype may increase distance between spikelets and enhancing individual glume size and hence potential grain size through removing physical restrictions. The population assessed in the present study differed in the length of the period between GS31 and GS61 by 9 days. Longer durations will enable the plant to intercept more radiation and contribute more photosynthate to the developing ear and hence increase grains per ear. Additionally, longer duration between GS31 and GS61 should reduce competition between developing tillers and resulted in more ears m^{-2}.

7.6 Conclusion

1. In general, this study indicates that grain number per unit area and to a lesser degree MGW are the main driving force for enhancing yield potential in UK winter wheat. Enhancing these traits should directly translate into improving yield potential. The original hypothesis that the UK's modern wheat germplasm was sink-limited has been confirmed by the present results. However, there was some evidence for marginal source-limitation in a minority of the lines assessed in the DH population. So, there is a need to identify strategies to improve grain number and potential grain weight, whilst ideally also increasing source-type traits in future breeding programmes.

2. In the present study, genotypes differed in the length of the period between GS31 and GS61 by 9 d. A longer thermal time between GS31 and GS61 allowed the crop to intercept more radiation (Figure 4.4) and positively affected the grains m^{-2}. Manipulating the photoperiod sensitivity during stem-elongation may provide an avenue for increasing grain number in UK wheat. Because anthesis time is approximately optimized, the most realistic option is probably to advance the onset of stem extension. There may be some limit to this in the UK due to the danger of the frost damage (Shearman *et al.*, 2005). Genotypes with a longer stem-elongation period (e.g. Rialto and Line 26) had higher ear dry weight due to more intercepted radiation during that period. This, in turn, resulted in more fertile florets than those with shortened stem elongation period (e.g. Line 3 and Spark). This confirmed the original hypothesis that a longer thermal time between GS31 and GS61 will increase grains m^{-2} through it is effect on ear DM and hence floret fertility.

3. The introduction of 1BL/1RS in the late 1980s into UK wheat varieties may have played a role in raising stem reserve capacity and RUE in modern cultivars. This study has shown that 1BL/1RS increased pre-anthesis RUE. This confirmed the original hypothesis that 1BL/1RS increased pre-anthesis RUE. This effect of higher RUE might be partly related to the positive effect of 1BL/1RS observed on specific leaf weight. The improvement of SLW with 1BL/1RS is consistent with a potential mechanism for improving yield stability.

4. The hypothesis that higher RUE pre-anthesis amongst the genotypes increases above-ground biomass hence grains m^{-2} was tested in the present study. RUE was positively correlated with both ear biomass at anthesis and grains m^{-2} implying that RUE was a factor contributing to the increase in grains m^{-2}. RUE was associated with SLW across genotypes.

5. Historically, genetic gain in yield potential was associated with grain partitioning more than biomass production (Araus *et al.*, 2002). However, recently in the UK, Shearman *et al.* (2005) reported that yield progress since about 1985 was more associated with biomass production. Present results showed a positive linear association between harvest biomass and grain yield amongst the lines in the DH population. It can be concluded that there was probably a genetic effect on harvest biomass amongst the DH lines that positively associated with grain yield.

6. Genotypes differed in the amount of PAR intercepted during the 10 d after anthesis; and the amount of radiation intercepted during the period of 10 d after anthesis (the lag phase) showed a trend for positive linear relationship with potential grain weight. Present results showed some indication that longer lag phase of Rialto than Spark was associated with higher PGW. Overall it seems that increasing the amount of PAR intercepted during the lag phase may be more important than the length of the lag phase itself; breeders in the UK might nevertheless consider selection for a longer lag phase

7. In the present study, the weight for grain positions G1, G2 and G3 was not increased by increasing the relative assimilate supply by 100% through de-graining. Physical restriction by the glumes or poor vascular connections serving the grain may have limited the potential size of the grain. There is a need to understand further the causes of such effects. G4 and G5 may be source-limited since their weight was increased by de-graining. Potential grain size can be increased by manipulating ear morphology (Reynolds *et al.*, 2000). For example, entering the CIMMYT spring wheat with a long ear rachis (about 15 cm) into the UK winter wheat material (Rialto) is the most recent work aimed to increase yield (John Foulkes, *Personal communication*).

8. Present results showed that breeders should attempt to increase the number of proximal grains in the basal spikelets rather than proximal grains in the apical

spikelets or distal grain in central spikelets. This was because G4 had a faster rate of growth and heavier final weight than both G3 and G5 and had possibly the ability to respond to extra assimilate late in the season as reported by Stockman *et al.* (1983). Increased spikelets number and the possibility of selecting more grains in proximal positions of the basal spikelets might be favoured by crosses with the novel CIMMYT large-ear phenotype lines mentioned above.

9. The present results showed genetic differences in stem WSC reserves. There is a possibility to increase stem WSC reserves without lowering ear DM since regression analysis showed a positive linear relationship between these two traits which suggests that they are not competing. Moreover, Shearman *et al.* (2005) reported an increase in stem WSC reserves with breeding. So, it is may be possible to select for this trait to improve yield potential. Foulkes *et al.* (2001) identifyed two chromosomes as carrying QTL of significant effect on WSC, 1B and 2A. These QTLs may help in selection and developing markers.

10. A breeder could select for longer grain filling duration for the genotypes and for faster filling rate for the grain positions. With regard to rate of grain growth, this trait can be increased genetically via improving potential grain size, reducing any restrictions of poor vascular connections and /or increasing the rate of sucrose downloading into the grain during the later stages of grain growth via enhancing the activity of the starch synthase enzyme (Jiang *et al.*, 2003). Duration of grain filling could also potentially be increased by introducing the stay-green trait, but because the crop is currently sink-limited, the first priority is to increase potential grain size.

11. Although the source-sink balance model confirmed the original hypothesis of wheat sink limitation by grain number and potential grain weight, the model needs further improvement since it consider 100% and not 73% (as reported by Austin *et al.*, 1977) of reserves were contributed to the grains. The model

supposed the pre-anthesis RUE will stay the same without change during the post-anthesis period. However present results as well as other results (e.g. Reynolds *et al.*, 2000; 2005) found that RUE was affected by changes in the sink strength. So, the model needs future developments.

12. Present data indicated that grain filling rate and duration were partially independent in UK winter wheat germplasm which might enable the breeders to search for variation between these two traits and to improve one of them without affecting the other.

7.7 Future work

1. 1BL/1RS improved the RUE. This means that some genes coming from rye increased RUE. QTL analysis could be carried out for a population recombining for the 1BL/1RS fragment which might be developed using the PH locus gene controlling pairing of homologous chromosomes during meiosis.

2. Line 8 showed a departure from the overall negative relationship between grain number and grain weight. So there is a requirement to study the causes of this genetically and physiologically. Is it, for example, related to higher grains to ear DM ratio or to sub-traits influencing potential grain weight.

3. The relationship between each of RUE and stem reserves and grain yield could be tested in other mapping populations and in other environment to confirm these effects in UK winter wheat.

4. An extended stem elongation between GS31-GS61 may be achieved by selecting either for higher sensitivity to photoperiod or for differences in intrinsic earliness during the stem elongation phase. It has been reported that the stem elongation phase of cereals is sensitive to photoperiod and that extending this phase (by exposure to short photoperiod) results in an increased number of fertile florets (Slafer *et al.*, 2001) and yield. This sensitivity of stem elongation to photoperiod

seems to be independent of that of previous phases (Slafer and Rawson, 1994; Gonzalez *et al.*, 2002). Future research is required on the genetic bases determining sensitivity to photoperiod during stem elongation before this information may be useful for practical breeding. Valuable approaches might include: (i) determining what genes are down- or up-regulated when responses to photoperiod take place during different phases (before and after the onset of stem elongation), and (ii) identifying genes/QTLs for differences in length of different phases (and/or for responsiveness to photoperiod in these phases) within mapping populations (Slafer *et al.*, 2005).

5. Recently *Gn 1a*, a gene that determines grain set in rice via regulation of cytokinin levels, has been shown correspond to a major QTL for yield potential in rice (Ashikari *et al.*, 2005). Geneticists, physiologists and breeders should therefore investigate the effect of orthologues of this gene on floret fertility in wheat.

6. Genetic analysis to identify QTLs for RUE and stem WSC reserves could be progressed using the Rialto x Spark population as a genetic map has already been developed for this population by the Nickerson-Advanta breeding company. There would be requirement to screen all the available lines from this population (100+) for stem WSC reserves and indicators of RUE and then to do QTL analysis to identify candidate markers.

7. Most major changes in UK wheats, such as the introduction of semi-dwarfing genes (which reduced the height of the plant, but increased the yield), have been introduced from wide crosses. Crosses with synthetic wheat derivatives can introduce new genes into the UK gene pool useful for improving grain number and potential grain weight which may allow further improvements in UK yield potential.

8. Improvements in yield potential by screening for RUE will require the integration of new tools/ sub-traits as selection criteria to complement traditional breeding approaches. These tools should be high-throughput and inexpensive. An example which may offer some immediate promise is canopy temperature depression measured via an infra-red thermometer as an indicator of canopy photosynthesis hence RUE, and investigations to examine the correlation between canopy temperature depression and yield potential in the UK environment may be justified.

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Appendices

Appendix I

Experiment	2003/04
Field Name	S24
Field altitude	50 m
Soil texture & series	Dunnington Heath Series, Medium stony loam to 80 cm over clay
Drainage	good
Previous crop	Winter oat
Soil Indices	P: 4, K: 3, Mg: 4, PH: 7.4
Cultivations	Ploughed 16/09/03 Cambridge rolled 16/09/03 Power-harrow 24/09/03 Cambridge rolled 27/09/03
Sowing	24/09/03
Seed rate	375 seeds m^{-2}
Drill type	Oyjard
Row width	13.45 cm
Plot length	12 m
Plot width	1.61m Drilled / 2.00 m centre to centre
Fertiliser	1.0 l ha^{-1} manganese 10/11/03 138 kg ha^{-1} N29%, SO_4 7.5% (40 kg/ha N all plots 17/03/04 345 kg ha^{-1} N29%, SO_4 7.5% (100 kg ha^{-1} N) all plots 08/04/04 276 kg ha^{-1} N29%, SO_4 7.5% (80 kg ha^{-1} N) all plots 19/04/04 2.0 l manganese 23/04/04
Herbicide	3.0 l ha^{-1} Isoproturon & 2.0 l ha^{-1} Trifluralin 10/11/03 25 g ha^{-1} Ally 23/04/04 0.75 l ha^{-1} Starane 23/04/04 0.125 l ha^{-1} Topik 23/04/04
Plant growth ragulators	2.5 l ha^{-1} New 5C Cycocel 23/04/04 0.5 l ha^{-1} Terpal + 0.04 l ha^{-1} Libsorb (agj) 25/05/04
Fungicide	0.5 l ha^{-1} Landmark 23/04/04 1.0 l ha^{-1} Bravo 23/04/04 0.75 l ha^{-1} Opus + 0.5 l ha^{-1} Acanto 25/05/04 0.35 l ha^{-1} Folicur + 0.5 l ha^{-1} Tern 14/06/04
Insecticide	0.25 l ha^{-1} Cypermethrin 10/11/03 0.7 l ha^{-1} Dursban 14/06/04
Miscellaneous	2.01 ha^{-1} Cropspray (oil) 23/04/04
Harvest date	31/08/05

Experiment	2004/05
Field Name	S24
Field altitude	50 m
Soil texture & series	Dunnington Heath Series, Medium stony loam to 80 cm over clay
Drainage	good
Previous crop	Winter oat
Soil Indices	P: 5, K: 3, Mg: 5, PH: 7.5
Cultivations	Ploughed 25/09/04 Power-harrow 26/09/04 Roll-after drilling 28/09/04
Sowing	27/09/04
Seed rate	250 seeds m^{-2}
Drill type	Oyjard
Row width	12.5 cm
Plot length	18 m
Plot width	1.625 m
Fertiliser	2.5 l ha^{-1} manganese 10/12/04 138 kg ha^{-1} N29%, SO_4 7.5% (40 kg/ha N all plots 16/03/05
Herbicide	2.3l ha^{-1} Teflan 28/09/04 (pre-emergence) 4 l ha^{-1} Isotron 500 + 0.8 l ha^{-1} Panther 14/11/04
Plant growth ragulators	
Fungicide	
Insecticide	0.250 l ha^{-1} Toppel 10 14/11/04
Miscellaneous	
Harvest date	16/08/05

Experiment	2005/06
Field Name	S04
Field altitude	50 m
Soil texture & series	Dunnington Heath Series, Medium stony loam to 80 cm over clay
Drainage	good
Previous crop	Winter oat
Available N	72.9 kg ha^{-1}
Soil Indices	P: 4, K: 3, Mg: 4, PH: 6.8
Cultivations	Ploughed 01/09/05 Power-harrow 02/09/05
Sowing	07/01/05
Seed rate	300 seeds m^{-2}
Drill type	Oyjard
Row width	13.45 cm
Plot length	12 m
Plot width	1.65m
Fertiliser	2.0 l ha^{-1} manganese 14/12/05 148 kg ha^{-1} N27%, SO_3 9% (40 kg ha^{-1} N) all plots 23/03/06 296 kg ha^{-1} N27%, SO_3 9% (80 kg ha^{-1} N) all plots 10/04/06 2.0 l manganese 22/04/06 296 kg ha^{-1} N27%, SO_3 9% (80 kg ha^{-1} N) all plots 04/05/06
Herbicide	2.3 l ha^{-1} Trifluralin 11/10/05 3.2 l ha^{-1} IPU + 0.8 l ha^{-1} Panther 14/12/05 1.5 l ha^{-1} Starane 28/04/06 0.45 l ha^{-1} Axial 10/05/06
Plant growth ragulators	2.25 l ha^{-1} Chlormequat 22/04/06 0.75 l ha^{-1} Terpal 17/05/06
Fungicide	0.5 l ha^{-1} Opus 28/04/06 1.0 l ha^{-1} Bravo 28/04/06 0.5 l ha^{-1} Corbel 28/04/06 0.75 l ha^{-1} Opus + 1.0 l ha^{-1} Bravo + 0.33 l ha^{-1} Corbel 25/05/06 0.1 l ha^{-1} Swing Gold + 0.3 l ha^{-1} Corbel 27/06/06
Insecticide	0.25 l ha^{-1} Toppel (cypermethrin) 12/12/05 0.7 l ha^{-1} Cyren 15/06/06
Miscellaneous	1.0 l ha^{-1} Adigor 10/05/06 0.2 l ha^{-1} Activator (adj) 17/05/06
Harvest date	09/08/06

Experimental plans 2003/04

REPLICATE 1

SHADING TRT	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
LINE No	D	10	2	19	21	26	8	14	9	28	20	1	24	11	4	18	17	28	7	13	27	15	5	22	12	23	3	16	6	27	D
PLOT No	D	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	D

REPLICATE 2

SHADING TRT	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
LINE No	D	28	21	4	19	1	24	13	23	27	8	10	14	18	7	20	15	2	26	11	17	6	27	9	3	22	12	16	18	5	D
PLOT No	D	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	D

REPLICATE 3

SHADING TRT	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
LINE No	D	12	26	28	7	23	5	17	27	3	13	24	8	16	6	11	18	20	9	4	28	2	21	15	10	27	1	22	14	19	D
PLOT No	D	117	118	119	120	121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144	145	D

EXPT 1
RIALTO X SPARK

1	68B	15	63C
2	116D	16	30C
3	83 B	17	47A
4	93D	18	13A
5	107B	19	94F
6	No ID 1	20	30D
7	65B	21	69C
8	152A	22	69B
9	72C	23	94A
10	229A	24	31A
11	117A	25	N/A
12	No ID 2	26	81A
13	109A	27	Rialto
14	50A	28	Spark

1 = UNSHADED
2 = SHADED

N

Experimental plans 2003/04 (contd)

REPLICATE 1

	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	D
	5	22	16	2	23	9	17	3	15	27	21	10	24	28	8	12	18	7	19	28	11	14	13	20	6	26	4	27	1	
D	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	

REPLICATE 2

	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	D
	13	27	18	11	26	8	22	12	7	28	4	15	27	5	21	9	28	16	23	6	20	2	14	19	10	17	3	1	24	
D	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116	

REPLICATE 3

	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	D
	18	8	13	20	1	24	28	6	27	12	7	2	19	28	14	11	5	15	22	3	26	9	21	16	4	23	27	10	17	
D	146	147	148	149	150	151	152	153	154	155	156	157	158	159	160	161	162	163	164	165	166	167	168	169	170	171	172	173	174	

Experimental plans 2004/05

REPLICATE 1

SHADING TRT	Discard	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Discard
LINE No		10	2	19	21	26	8	14	9	28	20	1	24	11	4	18	17	28	7	13	27	15	5	22	12	23	3	16	6	27
PLOT No		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29

REPLICATE 2

SHADING TRT	Discard	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	Discard
LINE No		10	24	15	5	28	17	21	9	27	7	1	3	23	16	6	28	13	20	22	11	19	8	14	2	27	4	12	26	18
PLOT No		59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87

REPLICATE 3

SHADING TRT	Discard	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Discard
LINE No		12	26	28	7	23	5	17	27	3	13	24	8	16	6	11	18	20	9	4	28	2	21	15	10	27	1	22	14	19
PLOT No		117	118	119	120	121	122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137	138	139	140	141	142	143	144	145

RIALTO X SPARK

1	68B	15	63C
2	116D	16	30C
3	83 B	17	47A
4	93D	18	13A
5	107B	19	94F
6	No ID 1	20	30D
7	65B	21	69C
8	152A	22	69B
9	72C	23	94A
10	229A	24	31A
11	117A	25	N/A
12	No ID 2	26	81A
13	109A	27	Rialto
14	50A	28	Spark

1 = UNSHADED
2 = SHADED

Experimental plans 2004/05 (contd)

REPLICATE 1

SHADING TRT	Discard	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	Discard
LINE No		7	24	4	16	2	28	1	27	15	26	21	23	10	13	6	28	8	27	20	9	22	14	18	11	5	17	19	3	12
PLOT No		30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58

REPLICATE 2

SHADING TRT	Discard	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Discard
LINE No		21	26	4	22	12	6	14	23	3	20	7	27	13	18	1	28	15	11	19	8	27	16	2	24	10	5	28	9	17
PLOT No		88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	116

REPLICATE 3

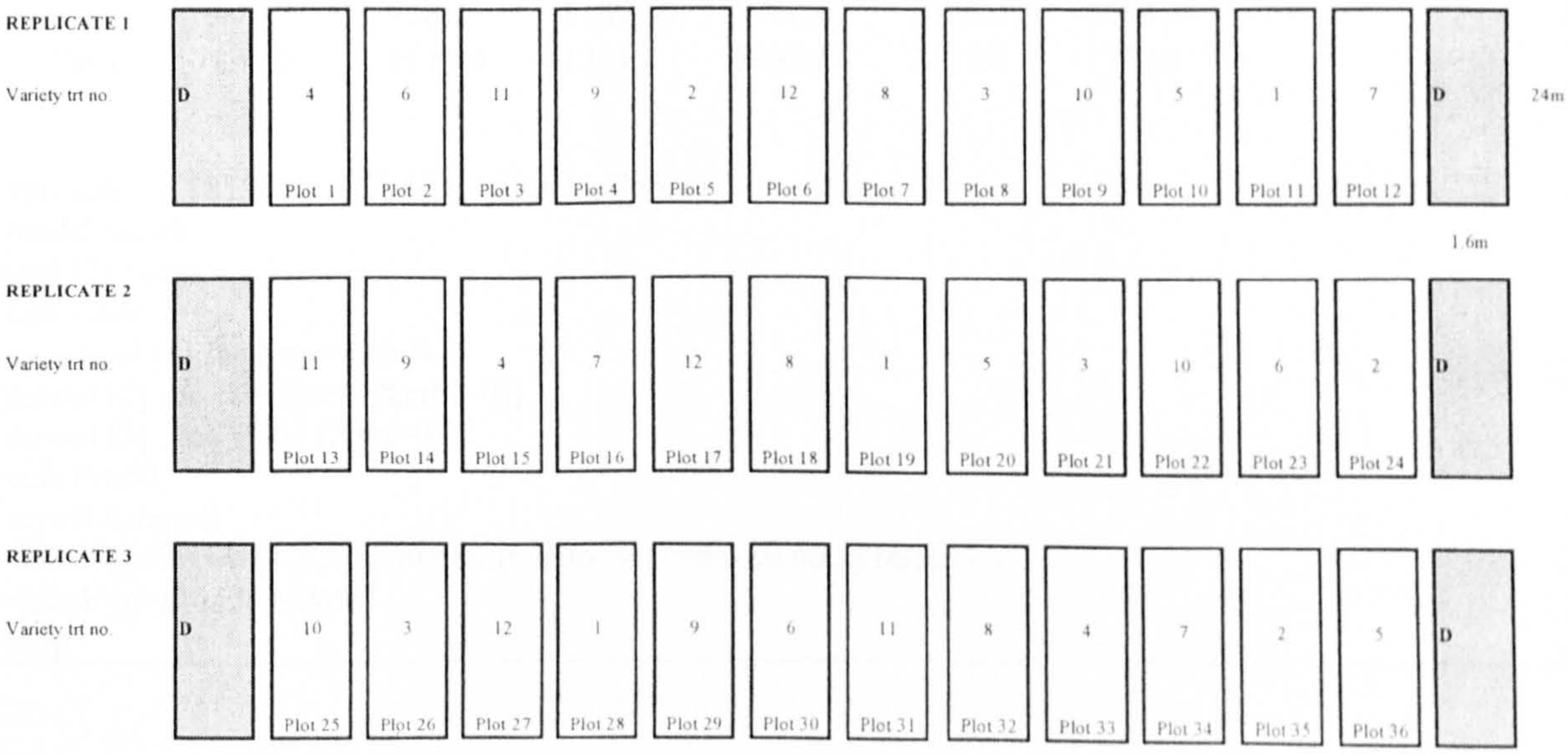
SHADING TRT	Discard	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	Discard
LINE No		18	8	13	20	1	24	28	6	27	12	7	2	19	28	14	11	5	15	22	3	26	9	21	16	4	23	27	10	17
PLOT No		146	147	148	149	150	151	152	153	154	155	156	157	158	159	160	161	162	163	164	165	166	167	168	169	170	171	172	173	174

RIALTO X SPARK

1	68B	15	63C
2	116D	16	30C
3	83 B	17	47A
4	93D	18	13A
5	107B	19	94F
6	No ID 1	20	30D
7	65B	21	69C
8	152A	22	69B
9	72C	23	94A
10	229A	24	31A
11	117A	25	N/A
12	No ID 2	26	81A
13	109A	27	Rialto
14	50A	28	Spark

1 = UNSHADED
2 = SHADED

Experimantal plan 2005/06



Line trt no.

1 Line 1
2 Line 3
3 Line 4
4 Line 11
5 Line 12
6 Line 13
7 Line 15
8 Line 17
9 Line 21
10 Line 26
11 Line 27 (Rialto)
12 Line 28 (Spark)

Appendix III The program used in broken stick model to calculate rate and end of grain filling, in addition to the final grain weight (example for ear growth of Spark unmanipulated in 2005)

```
read Spark

  615.6    849.9    861.5    1156.0   1710.4   2017.7   1756.6
  666.5    970.7    958.7    1189.3   1760.6   2088.4   1983.5
  780.1    863.2    1113.0   1261.4   2035.6   2037.8   1771.5
:

vari date;! ((187, 251, 317, 434, 569,676, 768)3)
model Spark
vari [21] ones
calc ones=1
expr twol [1];!e (Xmt=date-t)
&twol [2] ; !e (U=Xmt* (Xmt<=0))
&twol [3] ; !e (V=0* (Xmt>0))
calc t=650
rcycle t;step=2
fitnonl[calc=twol[1,2,3];cons=omit;selin=y;pr=mo,su,est,f] ones,U,V
rgraph [g=h] index=date
stop
```

The program selects the two lines and the point of intersection by calculating the overall fit that gives the smallest residual sum of squares. This is done iteratively within the program. An initial estimate for the point t, where the 2 lines meet. is put in the program to speed up the procedure. In this case; the initial value is 650.

Appendix IV Example of the output derived from broken stick model (example for ear growth of Spark unmanipulated in 2005).

Response variate: *Spark*
Nonlinear parameters: t
Model calculations: twol[1], twol[2], twol[3]

Summary of analysis

Source	d.f.	s.s.	m.s.	v.r.
Regression	4	43653507.	10913377.	542.43
Residual	17	342027.	20119.	
Total	21	43995534.	2095025.	

Percentage variance accounted for 92.6
Standard error of observations is estimated to be 142..

Message: the following units have large standardized residuals.

Unit	Response	Residual
19	2036.	2.13

Estimates of parameters

Parameter	estimate	s.e.
t	643.0	36.9
* Linear		
ones	1942.6	57.9
U	2.824	0.269
V	0	*

Message: some standard errors are unavailable due to singularity.

Fitted values and residuals

Unit	Response	Fitted value	Standardized residual
1	616.	655.	-0.28
2	850.	835.	0.10
3	862.	1022.	-1.13
4	1156.	1352.	-1.38
5	1710.	1733.	-0.16
6	2018.	1943.	0.53
7	1757.	1943.	-1.31
8	666.	655.	0.08
9	971.	835.	0.95
10	959.	1022.	-0.44
11	1189.	1352.	-1.15
12	1761.	1733.	0.19
13	2088.	1943.	1.03
14	1984.	1943.	0.29
15	780.	655.	0.88
16	863.	835.	0.20
17	1113.	1022.	0.64
18	1261.	1352.	-0.64
19	2036.	1733.	2.13
20	2038.	1943.	0.67
21	1772.	1943.	-1.21
Mean	**1355.**	**1355.**	**0.00**

The equations of the two lines can be produced from the output. The first line is estimated over the period from first date measured up to time t. the value at the initial date can be found in the fitted values for unit 1. i.e. 655. The slope of the line is 2.824 over the period 187.0 (the first data) up to 643.0, so the equation of the first line is:

Wt = 655 + 2.824 * (Date – 187.0).

For 187.0 <Date ≤ 643.0

For Date > 643.0, Wt = 1942.6