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1 **Grain number and grain filling of two-row malting barley in response to variation in**
2 **post-anthesis radiation: Analysis by grain position on the ear and its implications for**
3 **yield improvement and quality.**

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1 **Abstract**

2 Grain weight is reported to be a relatively well conserved characteristic across spring barley
3 (*Hordeum vulgare* L.) crops that vary in grain number m^{-2} . Understanding the mechanisms
4 that promote stability in grain weight is important to ensure that efforts to increase grain
5 number beyond current high levels successfully increase yield without compromising grain
6 quality. The aims of this study were to establish 1) whether post-anthesis grain abortion
7 contributes to the stability of grain weight by helping match grain numbers to post-anthesis
8 assimilate supply and 2) whether variations in post-anthesis assimilation per unit grain
9 number affect the heterogeneity of grain weight. Field experiments were conducted in a high-
10 yield potential environment for spring barley in 2011 and 2012. Crops were either shaded
11 post anthesis (a 59% reduction in radiation incident on the crop) to reduce net carbon
12 assimilation or grown unshaded. Grain growth was measured at different spikelet locations
13 on the ear and on different shoots (main shoot and tillers) of the same plant. Shading crops
14 from 14 days after anthesis until the harvest maturity reduced yield by 19-20%, mean grain
15 weight (MGW) by 12-16% and harvest index by 5-6%, but did not significantly affect grain
16 number in either year. The magnitudes of these effects were considerably lower than the
17 reduction in radiation imposed by shading suggesting some compensatory adjustment in
18 radiation use efficiency or dry matter partitioning to grain after shading. The rate of grain
19 filling was higher for grains in central spikelets than grains at distal or basal locations on the
20 ear. Shading reduced the rate of grain filling to a similar extent (23-27%) at most locations
21 evaluated on the ear, but had no effect on the duration of grain filling. In spite of the
22 comparable effects of shading on grain growth across different spikelet positions and
23 hierarchy of shoots, crops harvested after shading tended to have a more variable individual
24 grain weight (larger interquartile range and coefficient of variation) than crops that were
25 unshaded. The results show that post-anthesis grain abortion does not contribute to the

1 stability of MGW in spring barley. Moreover, low levels of post-anthesis radiation in crops of
2 large grain number m^{-2} (sink capacity) can increase heterogeneity of grain weight, which may
3 have negative consequences for grain quality.

4 **Keywords:** barley, grain number, grain weight, heterogeneity, radiation, spikelet location

5

6 **1. Introduction**

7 Understanding the relationships between grain number formation, grain development and
8 grain filling is fundamental to our efforts to increase cereal yields through plant breeding and
9 improved crop management. Grain yield of barley, as with other cereals, is the product of two
10 components, the number of grains produced per unit ground area and the mean grain weight
11 (MGW; Gallagher et al., 1975). While grain number in barley varies widely with location and
12 season and typically accounts for the majority of the variation in yield across environments
13 (Gallagher et al., 1975; Baethgen et al., 1995; Abeledo et al., 2003; del Moral et al., 2003;
14 Bingham et al., 2007; Peltonen-Sainio et al., 2007; Serrago et al., 2013; Kennedy et al.,
15 2017), grain weight tends to be less variable and is poorly correlated with yield (Gallagher et
16 al. 1975; Bulman et al. 1993; Baethgen et al., 1995; Abeledo et al., 2003; Sadras and Slafer,
17 2012). The smaller variability in MGW may be a consequence of evolutionary and/or
18 breeding selection for increased grain size, as larger seed with larger embryos and storage
19 reserves have a greater chance of producing seedlings that establish successfully, are able to
20 compete with neighbouring plants and tolerate damage from herbivores (Sadras, 2007). At
21 present, the physiological mechanisms that underlie this apparent conservation of MGW are
22 not fully understood, especially in the context of the large variations in grain number. A
23 relatively stable MGW implies that the number of grains set is in some way matched to the
24 potential of the crop to supply assimilates for grain filling (i.e. the sink capacity is set lower

1 than the source capacity or that the sink and source capacities are maintained in relatively
2 close balance) and that only in circumstances where post-anthesis assimilation is reduced
3 significantly, for example by drought or disease, will grains fail to fill adequately.

4 There are several possible mechanisms through which this might be achieved. Firstly,
5 grain numbers and grain storage capacity (potential grain size) may be determined
6 concomitantly prior to fertilization according to some common measure of overall assimilate
7 availability (Sinclair and Jamieson, 2006; Sadras and Denison, 2009). In this way an upper
8 limit may be set on grain size and the numbers of grains adjusted in concert. There is ample
9 evidence to suggest that the number of tillers and florets that survive to produce ears and
10 grains respectively is regulated by assimilate availability or organ and crop growth rate
11 during late stem extension (Gallagher et al., 1976; Hay and Kirby, 1991; Prystupa et al.,
12 2004; Slafer et al., 2009; Sadras and Slafer, 2012). Similarly, potential grain size has been
13 correlated with carpel size at anthesis which, in turn, is sensitive to treatments that vary
14 carbon assimilation and crop growth prior to ear emergence (Calderini et al., 2001, 2006).

15 Secondly, the same outcome (a relatively conserved MGW) might be achieved if there
16 was some mechanism for reducing the number of grains after anthesis if conditions during
17 grain development and filling restrict the assimilation capacity. Adjustments in the grain
18 number of barley and other small grain crops have been observed in studies evaluating post-
19 anthesis treatments such as shading, temperature modification, and drought (Habgood and
20 Uddin, 1983; Nicolas et al., 1985; Grashoff and d'Antuono, 1997; Zinselmeier et al., 1999;
21 Boyer and Westgate, 2004; Boyer and McLaughlin, 2007; Estrada-Campuzano et al., 2008;
22 Sanchez-Bragado et al., 2016). In many cases the mechanisms responsible for these losses
23 have not been elucidated, although grain abortion has been reported for several species
24 (Zinselmeier et al., 1999; Estrada-Campuzano et al., 2008). In maize, the abortion of grains
25 induced by crop water stress can be prevented by the exogenous supply of sugars, suggesting

1 that a limited carbohydrate supply may be responsible for the abortion (Zinselmeier et al.,
2 1999). Not only are grain numbers sensitive to reductions in assimilation, but the growth of
3 surviving grains can also be affected differentially depending on their location on the ear. In
4 wheat grain growth was reduced by post-anthesis shading to a smaller extent in florets
5 located closest to the base of the rachilla compared to those further away, thereby increasing
6 the variation in individual grain weight (Bremner and Rawson, 1978). Similarly grains in
7 florets closest to the rachilla were least sensitive to increases in assimilate availability
8 induced by partial degrading (Xie et al., 2015). Comparable data on the response of grains at
9 different positions on barley ears are lacking.

10 The mechanisms by which grain numbers are matched to the potential supply of
11 assimilate during grain filling and the way in which assimilate is allocated between grains,
12 both within and between ears, has implications for how plant breeding might increase yield
13 without compromising grain quality. Currently yield of barley is generally considered to be
14 sink limited (Bingham et al. 2007a; Kennedy et al., 2017). A route to increase the yield of
15 sink limited crops is, therefore, to increase grain numbers (Pedro et al., 2012; Reynolds et al.,
16 2012; Miralles et al., 2000; Gonzales et al., 2003). However, if the sink capacity is expanded
17 so that source and sink are brought into closer balance at the start of grain filling, the crop
18 may be at greater risk of source limitation should environmental conditions subsequently
19 deteriorate. Significant grain abortion in the face of increased source limitation could help
20 maintain grain quality, but restrict yield improvement. Alternatively, if post-anthesis grain
21 abortion is not an important mechanism in barley, the result of increased source limitation
22 might be a reduction in grain quality associated with lower mean grain weight and possibly
23 greater heterogeneity of individual grain weight. In spring barley heterogeneity of grain
24 weight is undesirable for maltsters, because variable grain are more difficult to process
25 (Passarella et al., 2003).

1 Questions about the regulation of grain numbers in response to post-anthesis assimilation
2 and its potential consequences for yield and quality are best answered through a detailed
3 analysis of grain formation and growth at specific spikelet positions on the ear as this
4 provides a greater resolution than standard yield component analysis. The aim of the research
5 reported here was to investigate the effects of varying post-anthesis assimilation, through
6 shading, on grain growth of spring barley at discrete positions on ears of main shoots and
7 primary tillers. The specific objectives were to 1) establish whether there is any evidence of
8 grain abortion in response to a reduction in post-anthesis incident radiation and hence
9 assimilation and 2) determine the effects of variations in radiation per unit grain number on
10 heterogeneity of grain weight.

11

12 **2. Materials and Methods**

13 2.1 Site characteristics and experimental design

14 Field experiments were conducted on spring barley (*Hordeum vulgare* L., cv Quench) at
15 Teagasc, Oak Park, Carlow, Ireland in 2011 and 2012. Quench is a two-row malting variety
16 selected because of its popularity amongst growers at the time of the study. Its yield and grain
17 quality characteristics were representative of other recommended varieties. In each year the
18 fields were sheltered, relatively flat and located 52° 51' N, 6° 54' W at an altitude of 57 m.
19 The top soil texture (determined by hand texture analysis) was loam (USDA, Rowell, 1994)
20 with a moderate moisture holding capacity. The site was characterised by continuous arable
21 production and the experiments occupied a position in the rotation that is standard practice
22 for commercial spring barley production in the region. In 2011 the previous crop was winter
23 barley and in 2012 it was winter wheat.

1 Crops were sown on 10th March 2011 and 14th March 2012 at a seed rate of 330 viable
2 seeds m⁻². Shading and unshaded control treatments were allocated at random to plots to give
3 a randomised block design with six replicates in 2011 and four replicates in 2012. Plot size in
4 2011 was 6 m² (2 m wide x 3 m long) with 2 m wide discard plots between shaded and
5 control plots to avoid overshadowing. Shading treatments were applied to entire plots in
6 2011. Plot size in 2012 was 96 m² (4 m wide x 24 m long) and shades were erected over sub-
7 plots of 2 x 3 m; here the shades were located alongside discard areas *within* plots to avoid
8 overshadowing. Shaded and unshaded plots were further sub-divided into two sampling
9 areas; one for destructive sampling of ears for grain growth assessment during the treatment
10 period and one for final grain number, biomass and yield determination at harvest. These are
11 referred to as the frequent and final sampling areas respectively.

12 2.2 General husbandry and imposition of shading

13 Crops were managed for high yield potential with the aim of keeping the crop well supplied
14 with mineral nutrients and free of pests and disease. Nitrogen applications of 132 (2011) and
15 154 kg N ha⁻¹ (2012) were split (50:50) between early post-emergence when tramlines
16 became visible and during tillering. Maintenance applications of P and K were made after
17 sowing based on soil chemical analysis. Fungicides were applied shortly before stem
18 extension and at ear emergence. Applications of aphicide and herbicide were as required.

19 Shading treatments were imposed 14 days after Zadoks growth stage (GS) 55 (50% ear
20 emergence; Tottman and Broad, 1987). As anthesis in spring barley tends to occur before the
21 ear is fully emerged this timing also corresponded to approximately 14 days after anthesis
22 (GS 61). The timing of shading was selected to avoid potential interference with the
23 fertilization of grains, particularly on later developing tiller ears. Shades were left in place
24 until after grain physiological maturity (GS 87). The shading material used was an open

1 weave black polystyrene shade-netting (Tildenet Ltd., Bristol, UK) erected on a frame of
2 fencing posts and rope at a height of 1.1 m above ground level. Simultaneous measurements
3 of photosynthetically active radiation (PAR) above the crop canopy under the shades and in
4 adjacent unshaded areas were made using a Sunscan Canopy Analysis System (Delta T
5 Devices, Cambridge, UK). Shading reduced incident PAR by 59% averaged across replicate
6 plots.

7 2.2 Microclimate and non-destructive measurements of crop growth

8 A pyranometer (SPLite2, Kipp and Zonen B. V., Delft, Netherlands) and a relative
9 humidity/temperature probe (MP100A, Rotronic Instruments (UK) Ltd., Crawley, UK)
10 connected to a data logger (CR1000, Campbell Scientific Ltd., Loughborough, UK) were
11 installed in shaded and unshaded treatment areas in 2011 to monitor environments hourly for
12 solar radiation, relative humidity and temperature. In both 2011 and 2012, soil was sampled
13 to 30 cm using a Dutch style auger in shaded and unshaded plots at the end of the shading
14 period to determine the gravimetric soil moisture content of the upper profile (Rowell, 1994).
15 Crop height was measured throughout the shading period in the undisturbed final sampling
16 areas of treated and untreated plots or sub plots by measuring the height of five randomly
17 selected shoots from ground level to the uppermost leaf ligule or ear collar (if present). The
18 percentage green area of whole treatment areas was estimated by visual assessment at
19 approximately weekly intervals during the latter stages of canopy senescence in shaded and
20 unshaded treatments. On each occasion a single plot score was estimated based on the combined
21 area of leaf laminae, stems and ears, including awns. Plots were inspected for leaning and lodging at
22 each visit to the site and its occurrence recorded if observed. The severity was assessed just
23 prior to harvest by estimating the % area affected in each of the following five categories:
24 shoots upright; shoots leaning slightly ($0-5^\circ$ from the vertical); shoots leaning ($5^\circ-45^\circ$ from

1 the vertical); shoots lodged (45°-90° from the vertical); brackled (stem failure a 1/4 or more
2 up its length).

3 2.3 Destructive sampling and measurements

4 Grain weight was assessed at individual grain locations, or zones, on ears of control and
5 shaded plants at the beginning of the treatment period and again at harvest in both seasons.
6 Additional weekly assessments were carried out during the treatment period in 2011. These
7 detailed grain weight assessments were carried out on the main stem ear (MS) only in 2011,
8 but also on two subsequent tiller ears (T1 and T2) in 2012. In 2011, ten main stem ears per
9 plot were sampled at random from the designated frequent sampling area on each sampling
10 occasion (main stems had been tagged with a small wire ring prior to the onset of tillering so
11 that they could be identified from the primary tillers). In 2012, ten plants were sampled from
12 the designated sampling area and the MS, T1 and T2 were identified based on their growing
13 position at the plant base. Decreasing stem diameter, height and ear length were also used as
14 indicators of tiller order if growing position was not clear. There was at least 0.5 m distance
15 between adjacent sample areas and sampling was avoided within 0.5 m from the ends and
16 edges of plots/treatments to avoid edge effects. Tram lines and drill overlaps were also
17 avoided with the aim of selecting sample areas that were representative of the plot. After
18 sampling ears were stored in sealed plastic bags at 4-6°C for a maximum of 24h prior to
19 sampling of individual grains.

20 The central spikelet on each ear was identified by counting the number of spikelets
21 (fertile and infertile) upwards from the ear collar (alternating from one side of the ear to the
22 other), halving the total number, and then rounding up to the next whole number. Grains were
23 then sampled individually by their location relative to the central spikelet, with grains at the
24 central spikelet designated as occupying zone 0, consecutive grain locations above (distal to

1 the central spikelet) designated +1, +2 etc. and grains below (basal to the central spikelet)
2 designated -1, -2 etc. Cultivar Quench is a two-row barley variety where only the median
3 spikelets at a given node on the rachis are fertile (Kirby and Appleyard, 1984). A spikelet was
4 defined as possessing a 'grain' once it had swollen to twice the width of the two lateral
5 infertile spikelets or if it had developed an awn and was not sampled unless it satisfied these
6 criteria. The grains were sampled by removing bulk florets (including lemma, palea and awn)
7 from each zone location and combining grains from a given zone from all 10 ears. The
8 number of grains per zone was also counted so data would provide an accurate estimation of
9 grain number ear⁻¹. Material was then dried at 70°C for 48h (or to a constant mass) before the
10 dry weight was recorded to 0.1 mg.

11 At harvest ripeness, a quadrat (0.72 m²) of above ground crop material was sampled at
12 random from the previously designated final sampling areas and was air-dried prior to
13 processing. A 40% sub-sample (by shoot number) was obtained for above ground dry matter
14 determination and a further 20% was separated into ears and straw. Ears were counted and
15 tissue fractions dried at 70°C for 48h for dry weight determination. Ears were then hand-
16 threshed between two pieces of foam board and sieved over a mechanically operated 1.0 mm
17 slotted sieve (Glasbläserei, Institute for Fermentation and Biotechnology, Berlin, Germany)
18 to separate into chaff and grain portions. Material was re-dried before the dry weight of each
19 portion was recorded. MGW was calculated for each plot using an automated grain counter
20 (Pfeuffer GmbH, Kitzingen, Germany) by counting the number of grains in an approximate
21 25 g grain sample. After counting, grain weight was determined to the nearest 0.1 mg. Hand
22 threshed grain yield (t ha⁻¹) was then expressed at 100% dry matter, grain number m⁻²
23 calculated as yield divided by MGW (expressed at 100% dry matter), and grain number per
24 ear calculated as grain number m⁻² divided by ear number m⁻².

1 Distribution of individual grain weight was measured for shaded and unshaded
2 treatments in 2011 from the hand threshed grain samples at final harvest. Samples were
3 poured onto a tray, mixed well (samples were not shaken prior to pouring to avoid
4 stratification of grain size) and spread across the tray. Working from one end of the tray to
5 the other one hundred grains per replicate were then removed and weighed individually to 0.1
6 mg.

7 To test whether hand threshed grain samples contained some non-viable grain, imbibed
8 caryopses of defined size class were stained with 2,3,5 triphenyltetrazolium chloride (TTC)
9 (Sigma-Aldrich Co. UK). Samples were taken at harvest ripeness from an accompanying
10 field experiment conducted on cv Quench at a neighbouring site in 2011 in which plots were
11 sown at seed rates ranging from 40 to 1280 seeds m⁻². This provided grain samples with a
12 wide range of MGW. Other than the contrasting seed rates, the plots were grown under the
13 same husbandry regime as that described above. Air dried ears from a sample of 6 x 1 m row
14 lengths were counted and subsampled (20%) before hand-threshing and sieving over a 1.0
15 mm slotted sieve as described above to separate chaff from grain. Grains were then sieved
16 over a 1.75 mm slotted sieve to separate suspected non-viable grains from the grain sample.
17 All suspect non-viable grains (i.e. those not meeting the criteria used to define a grain when
18 sampling by location on the ear; above) passed through the 1.75 mm sieve. The resultant 1.0-
19 1.75 mm size class consisted of approximately 30-60 suspected non-grains and grains. These
20 were imbibed in distilled water for 18h at 20°C before removing from the water and cutting
21 longitudinally to expose each half of the caryopsis. Tissue was then stained with 1.0% v/v
22 TTC for 3h at 30°C in the dark. Viable embryo tissue was stained red and was distinct from
23 the non-stained endosperm. Grains (with viable embryo) and non-viable grains were
24 separated, counted and dried at 70°C for 48h and weighed. The % of non-viable grains in the
25 sample, per unit ground area and per ear were calculated.

1 2.4 Statistical analysis

2 Harvest yield, yield component and biomass data obtained from the harvest ripe quadrat
3 samples were analysed statistically for effects of shading using one way anova in GenStat
4 (14th Edition, VSN International Ltd., Hemel Hempstead, UK). Grain number per ear data
5 from the detailed grain weight assessments were similarly analysed. In 2012 an additional
6 early shading treatment was included in the experimental design. All treatments were
7 included in the statistical analysis to increase its power, but only the treatments of relevance
8 to this paper (i.e. shading from GS55/61 +14 days and controls) are presented here. Results
9 from the early shading treatment will be included in a following publication. Following
10 ANOVA, relevant means for treatments of interest were compared using the standard error of
11 the difference (SED) between means, on the residual degrees of freedom (df) from the
12 ANOVA, thus invoking the least significant difference (LSD) at the $P=0.05$ level of
13 significance. Estimations of grain heterogeneity at harvest were conducted by box-plot
14 analysis of individual grain weight for each treatment in 2011 after first pooling data from
15 each replicate plot and removing those in size classes <5.0 mg. Mean grain weights and
16 coefficients of variation were then calculated for each replicate plot and differences between
17 shading treatments tested by one-way analysis of variance.

18 Repeated measures ANOVA was carried out on data from detailed grain weight
19 assessments at harvest in each year. Grain location within an ear was used as the repeated
20 measure to account for possible correlations between grain weight at different spikelet
21 positions. Main stem data for 2011 were first analysed to determine shading, zone (grain
22 location), and shading x zone interaction effects on grain weight at harvest. Due to missing
23 values at ear extremities (MS ears varied in their number of spikelets and grains per ear) data
24 were restricted to zones +14 to -11. Split-line regression analysis (Genstat 14th Edition, VSN
25 International Ltd., Hemel Hempstead, UK) was utilised to evaluate the relationship between

1 grain weight and time after GS55/61 and to estimate the rate and duration of grain filling for
2 grains at selected locations on the ear. A bilinear model with a plateau was fitted following
3 Miralles et al., (1996) as:

$$4 \quad Y = a + bx, \text{ if } x < c; \quad Y = a + bc, \text{ if } x \geq c;$$

5 Where Y is grain dry weight; a is the Y -intercept; b is the slope (the rate of grain filling); x is
6 the time after GS55/61 (taken to be time after anthesis) and c is the effective duration of grain
7 filling.

8 Repeated measures ANOVA was used to analyse effects of shading in 2012 on
9 individual grain weight at harvest by position on the ear and by tiller hierarchy. All data were
10 checked for normal distribution of residuals and equality of variance to ensure it conformed
11 to the assumptions of parametric analysis.

12 **3. Results**

13 3.1 Climatic conditions and crop development

14 Plots established well in each year; the percentage plant establishment from the seed rate of
15 330 seeds m^{-2} was 89 % and 96 % in 2011 and 2012, respectively. The 2011 season was
16 characterised by having a warmer and drier than average spring (March and April), but a
17 cooler and drier August, whilst solar radiation was close to the long term average for the
18 whole growing season (Fig. 1). By contrast, spring was cooler in 2012, and the summer
19 considerably wetter. The levels of solar radiation in 2012 were lower than the long term
20 average for April, June and July.

21 The mini-meteorological stations installed in shaded and unshaded plots in 2011 showed
22 that the temperature just above the canopy was on average 0.4°C cooler in the shaded plots
23 than the unshaded plots and the relative humidity was on average 0.3 % higher (hourly data

1 averaged across the whole shading treatment period). Gravimetric soil moisture content in the
2 0–30 cm profile at the end of the shading period in 2011 was 21% for the shading treatment
3 and was significantly greater than the unshaded control value of 15 % ($P < 0.001$); there was
4 no difference in 2012. Shading had no significant effect on crop height in either season (data
5 not shown). The reduction in PAR incident at the top of the canopy due to shading was
6 consistent on each measurement occasion, with average reductions of 59% in 2011 and 2012.

7 Shading tended to delay canopy senescence with the % green area declining to <10%
8 around four or five days later than controls in both 2011 and 2012 (Fig. 2). In 2011 shading
9 increased the % of stems that brackled from 2% to 12% ($P=0.002$) and those that leaned
10 slightly from 1% to 4% ($P=0.038$) (data not shown). Although leaning ($5-45^\circ$ from the
11 vertical) and brackling occurred in 2012 (7% and 6% respectively on average across
12 treatments) there was no significant difference between shaded and control plots ($P>0.05$).
13 Leaning and brackling occurred after grain maturity and thus will have had negligible effect
14 on yield.

15 3.2 Yield and yield components

16 Shading reduced yield by 20% in 2011 and 19% in 2012 ($P<0.05$; Table 1). In each case this
17 was associated mostly with a reduction in MGW (16 and 12% for 2011 and 2012
18 respectively; $P<0.05$). By comparison the effects on grain number m^{-2} were small (5-8%) and
19 not statistically significant ($P>0.05$). Neither of the two components of grain number (ear
20 number m^{-2} and grain number ear^{-1}) were significantly affected by shading although there
21 was a reduction in ear number m^{-2} , of comparable size in each year (5-11%), that accounted
22 for the lower grain numbers m^{-2} (Table 1). Total above ground biomass and harvest index
23 were also reduced ($P<0.05$) by shading in each year. Again the magnitude of the effect was
24 comparable in 2011 and 2012.

1 Detailed grain weight assessments made at harvest on MS ears from 2011 and MS plus
2 T1 and T2 ears in 2012 (Table 2) also showed that shading had no effect on grain number ear⁻¹
3 in either season. There was a significant effect of shoot hierarchy on grain number ear⁻¹
4 ($P < 0.001$) where tillers had fewer grains per ear than the MS, but this was not accompanied
5 by a shading x shoot interaction effect, implying that grain number of shoots of contrasting
6 hierarchy did not differ in sensitivity to shading.

7 3.3 Grain growth at different locations on the ear.

8 There were more grains formed above the central spikelet of main stem ears than below it, as
9 a greater number of the basal spikelets immediately above the collar were infertile. At harvest
10 ripeness, grains in central spikelet locations (between +6 and -6) were heavier than those in
11 more distal and basal positions in both the unshaded control and shaded plots in each year
12 (Fig. 3). The decline in grain weight beyond these positions was steeper towards the base of
13 the ear than it was towards the apex, with variability in grain weight at a given location
14 greater at the distal and basal positions than the central positions. In 2011 there was an
15 overall effect of shading ($P < 0.001$) and grain location ($P < 0.001$) on final MS grain weight,
16 but no shading x grain location interaction ($P = 0.23$).

17 When a repeated measures analysis was conducted for 2012 data, including shoot
18 hierarchy as a factor, shading was found to reduce grain weight at harvest ($P < 0.001$)
19 compared to unshaded controls, (32.7 mg vs 37.9 mg), but there were no significant
20 interactions between shading and shoot hierarchy or grain position ($P > 0.05$). This indicates
21 that shading had the same effect on grain weight regardless of the type of shoot the ear was
22 on or the position of the grain on the ear. There was a significant shoot hierarchy effect on
23 grain weight ($P < 0.001$) where the main stem (MS) MGW of 38.9 mg (averaged across
24 shading treatments and grain position) was greater than that of tiller 1 (T1) and tiller 2 (T2)

1 MGW's of 34.0 and 33.0 mg, respectively. There was also a significant shoot hierarchy x
2 grain position interaction ($P < 0.001$; Fig. 4). Thus grain weights in central locations on the ear
3 were comparable in MS, T1 and T2 ears, but differences were observed in distal and, to a
4 greater extent, the basal positions. At these locations, grains on the MS were heavier than
5 those on T1 and T2 ears (Fig. 4).

6 The weight of grains at all locations on the MS ear increased steadily throughout the
7 grain filling period under shaded and unshaded conditions in 2011, until reaching a plateau at
8 the end of grain filling (representative grain positions shown in Fig. 5). A split-line regression
9 fitted the data well with an $R^2 > 0.82$ at all grain positions (with the exception of the most
10 basal location analysed) with and without shading (Table S1). At spikelet location -11 the R^2
11 was lower (0.46-0.49; Table S1). The effects of shading on final grain weight were associated
12 with a 23-27% reduction in the rate of grain filling at all positions except the most basal
13 where the reduction was smaller (10%). By contrast the effects of shading on the duration of
14 grain filling were generally small and not statistically significant (comparison of 95% CI with
15 the difference between means). The duration of grain filling was around 43-46 days
16 regardless of shading and over a wide range of grain positions. The smaller final weight of
17 grains located at the extremities of the ear was associated with a decline in the rate of grain
18 growth away from the central region.

19 3.4 Effects of shading on heterogeneity of grain weight

20 The frequency distributions of individual grain weight in quadrat samples at harvest in 2011
21 revealed a tail of very light material in the weight classes 0-2.5 and 2.5-5.0 mg (data not
22 shown). Shading did not affect ($P > 0.90$) the proportion of harvested grains below 5.0 or 2.5
23 mg in 2011 (Fig. 6). Vital staining using TTC showed that non-viable grains in hand threshed
24 samples had an average weight of 2.9 mg; grains heavier than this were viable. Values for the

1 weight class <5.0 mg were, therefore, removed from the data set as non-viable grains and the
2 variability in weight of remaining (viable) grains analysed. Grain harvested from shaded
3 crops in 2011 had a lower median (36.8 mg) and a wider variation of weights (50% of grains
4 within a 13.1 mg range from 29.8-42.9 mg) than grain from unshaded crops (median weight
5 43.6 mg; 50% of grains within a 10.3 mg range from 38.2-48.5 mg; Fig. 7). In these samples
6 with the light weight fraction removed, mean grain weight was 16% lower ($P<0.001$) with
7 shading than in unshaded controls and the mean coefficient of variation was increased from
8 20.5% to 27.5% ($P<0.001$).

9 **4. Discussion**

10 There was no evidence from the analysis of yield components or detailed grain growth within
11 ears of a significant reduction in grain number ear⁻¹, ear number m⁻² or grain number m⁻², in
12 response to shading, suggesting that following fertilisation and early development, grains are
13 unlikely to abort even if subject to large reductions in assimilate availability. Also, the
14 absence of a significant shading x shoot interaction effect on grain number ear⁻¹ in 2012
15 indicates that a grain abortion mechanism was not more likely to occur on later formed tillers.
16 These conclusions are supported by the observation that the number of grains in the light
17 weight fractions (<5mg), which included non-viable and empty grains, were not increased by
18 shading. The grain numbers achieved for the unshaded treatments in 2011 and 2012 of 22,347
19 and 20,335 grains m⁻², respectively, reflect crops with a very large sink capacity, even
20 compared to average high-yielding crops from similar environments (Kennedy et al., 2017).
21 As such, assimilate availability per grain in the absence of shading was already likely to be at
22 the lower limit of what is normally experienced in the field, yet despite a 59% reduction in
23 photosynthetically active radiation for an extensive post-anthesis period there was still no
24 significant reduction in grain number per ear compared to unshaded controls.

1 These findings are in contrast to results of some of the previous studies in barley and
2 other crops where significant reductions in grain number were observed following a decrease
3 in post-anthesis assimilation capacity (Habgood and Uddin, 1983; Nicolas et al., 1985;
4 Westgate and Boyer, 1986; Grashoff and d'Antuono, 1997; Zinselmeier et al., 1999; Boyer
5 and Westgate, 2004; Boyer and McLaughlin, 2007; Estrada-Campuzano et al., 2008). Post-
6 fertilisation abortion of ovaries in maize following water deficits has been shown to be
7 triggered by the depletion of ovary sugar pools (Zinselmeier et al., 1999; Boyer and Westgate
8 2004; Boyer and McLaughlin, 2007). Furthermore, shading of bread wheat crops post-
9 anthesis resulted in significant reductions in grain number for crops in Mexico (Sanchez-
10 Bragado et al., 2016). However these water deficit and shading treatments were applied
11 closer to pollination than the shading treatment in this study. Florets are particularly sensitive
12 to environmental stress during meiosis (nuclear and cell division in preparation for anthesis;
13 Kirby and Appleyard, 1984) and thus environmental stress during early reproduction can
14 result in floret sterility or decreased grain set in addition to post-fertilization grain abortion
15 (Nicolas et al., 1985; Saini and Westgate, 2000; Fabian et al., 2011). Given that anthesis does
16 not occur simultaneously across all plants, ears, and spikelets of field grown barley it was
17 reasoned that commencing the shading treatment 14 days after anthesis (when the date of
18 anthesis is assessed as an average across all shoots) would allow all potential grains to be
19 fertilised prior to shading. In such a scenario any down regulation of grain number m^{-2} in
20 response to post-anthesis shading could be attributed to a post-anthesis abortion rather than
21 non-fertilisation. When water deficits were applied to maize for a period similar to the
22 shading period here, grain weight was reduced, but there was little effect on grain number
23 (McPherson and Boyer, 1977; Jurgens et al., 1978). This is in line with the present study and
24 suggests that grain abortion in response to a restricted assimilate availability is more likely to
25 occur closer to anthesis. However, it must be noted that shading from as late as 8 days after

1 anthesis reduced grain number ear⁻¹ in wheat (Sanchez-Bragado et al., 2016). These results
2 suggest that the sensitivity of grain number to variation in early post-anthesis PAR may differ
3 between species.

4 Post-anthesis shading was reported to increase heterogeneity of grain weight in barley
5 and reduce grain malting quality (Grashoff and d'Antuono, 1997), but the cause of the greater
6 heterogeneity was not investigated. Our results show that shading reduced grain weight at all
7 grain locations on the ear. With the exception of one basal spikelet location examined where
8 the variability in grain weight over time was high, the lower grain weight with shading was
9 the result of a slower rate and not a shorter duration of grain growth. The rate of grain filling
10 was reduced by shading to a comparable extent at all spikelet positions and there was no
11 evidence of an early arrest of grain growth at any particular location. These results and the
12 lack of a significant interaction between grain location and shading on final grain weight
13 indicates that when supplies were limited, assimilates were not partitioned preferentially to
14 the central grains at the expense of the more distal and basal grains in either in main shoot
15 ears or tillers. This would be expected to limit any increase in heterogeneity of grain weight
16 when conditions restrict post-anthesis assimilate availability. It is clear, however, that these
17 effects are not absolute as there was an increase in the coefficient of variation for grain
18 weight of bulk samples after shading. In wheat, growth of grains was also reduced by shading
19 more or less equally in different spikelet positions, but growth of florets at different positions
20 within spikelets was altered unequally (Bremner and Rawson, 1978). The results were
21 interpreted in terms of variation in the vascular connections between spikelets and between
22 florets within spikelets and its consequences for the resistance of phloem translocation
23 pathways. Increased heterogeneity of grain size and weight is undesirable in spring barley,
24 because it is associated with variable protein concentrations and inconsistent germination in
25 the malting process (Yin et al., 2002; Passarella et al., 2003).

1 It is important to recognize that, in addition to reducing incident radiation, shading can
2 alter other aspects of the microclimate around crops. However, differences in temperature and
3 relative humidity between shaded and unshaded environments were small and, therefore, the
4 effects of shading on grain filling and yield were unlikely to be a consequence of changes in
5 meteorological conditions other than incident radiation (Fisher, 1985). Although the cooler
6 temperature under the shading might be expected to extend the duration of grain filling
7 (longer calendar period for the same thermal time) the difference was only 11.9°Cd
8 (assuming a base temperature of 0°C) over the period from the start of shading to the end of
9 grain filling. This represents less than a day in the current study and is within the margin of
10 error associated with determination of the grain fill duration (Fig. 5, Table S1). Shading did
11 not reduce the top soil moisture content relative to unshaded controls in either season
12 indicating that shading structures did not obstruct rainfall to the crop.

13 Proposals for increasing yield by increasing the number of grains formed rest on the
14 premise that 1) yield of spring barley is sink-limited and that additional assimilate can be
15 made available to meet the demand of a larger grain number and 2) grain numbers can be
16 increased without reducing their potential storage capacity (potential grain weight). The
17 magnitude of the source-sink imbalance in typical production environments will dictate the
18 extent to which grain numbers can be increased before effects on grain filling occur. Indeed,
19 there is evidence that in some production environments barley yield may be source rather
20 than sink-limited (Alvarez Prado et al., 2013). As our results indicate that grain numbers are
21 not reduced in response to reductions in post-anthesis radiation, crops whose source and sink
22 capacities are in relatively close balance at anthesis could be at greater risk of poor grain
23 filling and a greater heterogeneity of grain weight in those environments where restrictions to
24 post-anthesis assimilation are commonplace (e.g. in response to drought or dull wet weather).
25 There is evidence, however, that photosynthesis and RUE during grain filling of wheat can be

1 upregulated in response to an increase in sink demand (Reynolds et al., 2005). In the present
2 study reducing radiation interception per unit grain number through shading also appeared to
3 result in some compensatory adjustment in assimilate supply to the grain. The reduction in
4 radiation interception (59%) greatly exceeded the reduction in yield (19-20%) and MGW (12-
5 16%) and similar observations have been made by other authors from shading experiments
6 (Willey and Holliday, 1971; Grashoff and d'Antuono, 1997; Arisnabarreta and Miralles,
7 2008a; Serrago et al., 2013). The delayed onset or slower initial rate of canopy senescence in
8 shaded plots may have contributed to this, although the duration of grain filling was not
9 increased appreciably and rapid grain filling ceased before complete canopy senescence.
10 There may have also been increases in RUE and utilization of storage reserves for grain
11 filling under shading, but these were not measured in the current study. It remains to be seen
12 whether similar adjustments are also possible in barley if radiation interception per grain is
13 reduced through an increase in grain number rather than decreased incident radiation.

14

15 **5. Conclusions**

16 Our results show that large scale reductions in post-anthesis incident radiation did not
17 promote grain abortion in spring barley. Thus post-anthesis grain abortion is not an important
18 mechanism for promoting stability of grain weight in barley when assimilate availability is
19 restricted. Mean grain weight was reduced and the heterogeneity of grain weight increased.
20 Final grain weight was reduced to a similar extent at the majority of spikelet locations on the
21 ear through effects on the rate rather than duration of grain filling. This response limits, but
22 does not prevent, an increase in heterogeneity of grain weight in barley when incident
23 radiation is reduced. Further, the reduction in grain weight was considerably less than the
24 reduction in incident radiation, reflecting possible compensatory adjustments in RUE and
25 assimilate partitioning to the grain. Efforts to increase yield through increases in grain

1 numbers could have negative effects on grain size and malting quality depending on the
2 extent of the source-sink imbalance of the crop and its capacity for increasing the supply of
3 assimilates for grain filling when the sink capacity is raised.

4

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1 Table 1. Mean values of yield, yield components and other harvest variables for crops of spring barley treated with post-anthesis shading or unshaded in 2011
 2 and 2012.

3

	2011				2012				4
	Unshaded	Shaded	SEM	P-value	Unshaded	Shaded	SEM	P-value	5
Yield (t ha ⁻¹ ; 100% DM)	9.35 ^a	7.48 ^b	0.41	0.025	6.8 ^a	5.5 ^b	0.25	0.026	
Grain number m ⁻²	22347	21266	1192.6	0.550	20335	18685	802.5	0.253	6
MGW (mg; 100% DM)	41.9 ^a	35.4 ^b	0.71	0.001	33.3 ^a	29.3 ^b	1.02	0.033	7
Ear number m ⁻²	1208	1156	55.3	0.531	997	892	34.0	0.168	
Grain number ear ⁻¹	18.4	18.4	0.29	0.947	20.4	21.0	0.93	0.623	8
Harvest Index %	59.9 ^a	56.1 ^b	0.55	0.005	50.9 ^a	48.4 ^b	0.60	0.004	
Ear number plant ⁻¹	4.1	3.9	0.27	0.531	3.1	2.8	0.11	0.168	9
Total biomass (t ha ⁻¹ DM)	15.7 ^a	13.7 ^b	0.50	0.039	13.4 ^a	11.2 ^b	0.48	0.049	10

11 Means with different letter superscripts are significantly different (P<0.05); for 2012 based on Fishers least significant difference test. DM = dry matter;
 12 MGW = mean grain weight.

13

14

15

- 1 Table 2. Effects of post anthesis shading on spring barley grain number per ear at harvest for main
- 2 shoots (MS) in 2011 and main shoots, tiller 1 (T1) and tiller (2) in 2012. Within a column means
- 3 followed by a different letter are significantly different at ($P < 0.05$) based on Fisher's LSD

	Grain number ear ⁻¹		
	2011	2012	
<i>Post-anthesis shading</i>			
Unshaded	24.4	22.0	6
Shaded	24.2	21.7	7
<i>Shoot hierarchy</i>			
MS	-	24.3 ^a	8
T1	-	21.4 ^b	9
T2	-	19.9 ^c	10
S.E.M.	0.38	0.42	11
<i>Significance (P value)</i>			
Shading	0.698	0.244	12
Shoot hierarchy	-	<0.001	13
Shading x shoot hierarchy	-	0.912	13

1 Supplementary Table 1. Estimations of effective duration of grain filling (bilinear model parameter *c*) and rate of filling (bilinear model parameter *b*) (\pm 95%
 2 confidence interval) for grains at different positions on the ear; analysis of data by split-line regression for crops of spring barley either grown unshaded or
 3 shaded post-anthesis in 2011.

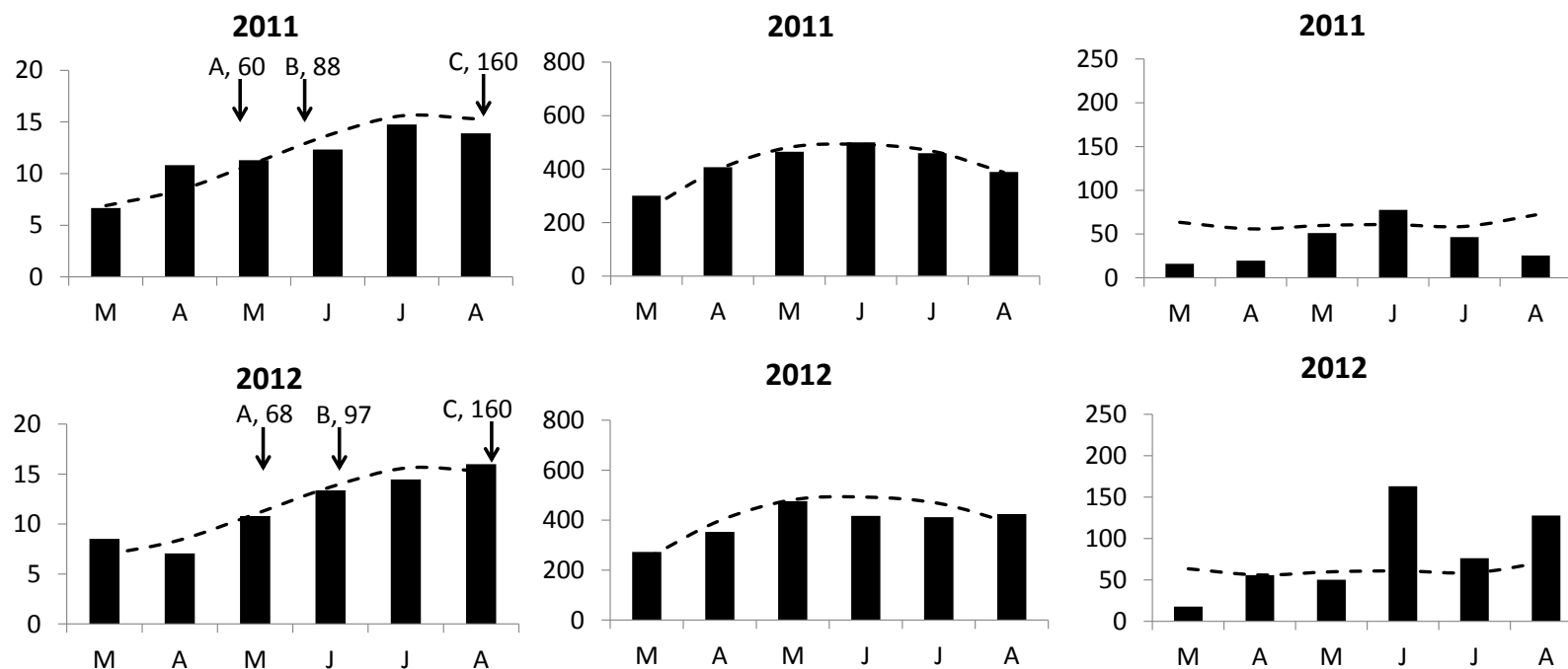
4

Grain Position	Shading	Significance of fit		Estimated grain filling duration (days)		Estimated grain filling rate (mg/day)	
		R ²	P-value	Duration (\pm CI)	% change when shaded	Rate (\pm CI)	% change when shaded
+1	Unshaded	0.95	<0.001	43.6 (\pm 2.32)	+5%	1.40 (\pm 0.135)	-25%
	Shaded	0.94	<0.001	45.9 (\pm 2.91)		1.05 (\pm 0.117)	
-1	Unshaded	0.96	<0.001	44.5 (\pm 2.28)	+4%	1.46 (\pm 0.135)	-23%
	Shaded	0.96	<0.001	46.3 (\pm 2.32)		1.13 (\pm 0.101)	
+6	Unshaded	0.95	<0.001	43.6 (\pm 2.32)	+2%	1.33 (\pm 0.127)	-26%
	Shaded	0.94	<0.001	44.6 (\pm 2.61)		0.99 (\pm 0.107)	
-6	Unshaded	0.95	<0.001	45.9 (\pm 2.51)	0%	1.39 (\pm 0.135)	-24%
	Shaded	0.95	<0.001	46.0 (\pm 2.67)		1.05 (\pm 0.109)	
+9	Unshaded	0.95	<0.001	43.1 (\pm 2.34)	+2%	1.24 (\pm 0.121)	-26%
	Shaded	0.94	<0.001	43.9 (\pm 2.51)		0.92 (\pm 0.095)	
-9	Unshaded	0.89	<0.001	43.7 (\pm 3.56)	0%	1.07 (\pm 0.158)	-25%
	Shaded	0.82	<0.001	43.7 (\pm 4.71)		0.80 (\pm 0.156)	
+12	Unshaded	0.92	<0.001	42.8 (\pm 2.77)	1%	1.06 (\pm 0.123)	-27%
	Shaded	0.84	<0.001	43.3 (\pm 4.35)		0.77 (\pm 0.137)	
-11	Unshaded	0.49	<0.001	42.0 (\pm 0.73)	-17%	0.67 (\pm 0.214)	-10%
	Shaded	0.46	<0.001	34.7 (\pm 6.08)		0.60 (\pm 0.279)	

Mean daily temperature (°C)

Monthly solar rad. (MJ m⁻²)

Monthly rainfall (mm)

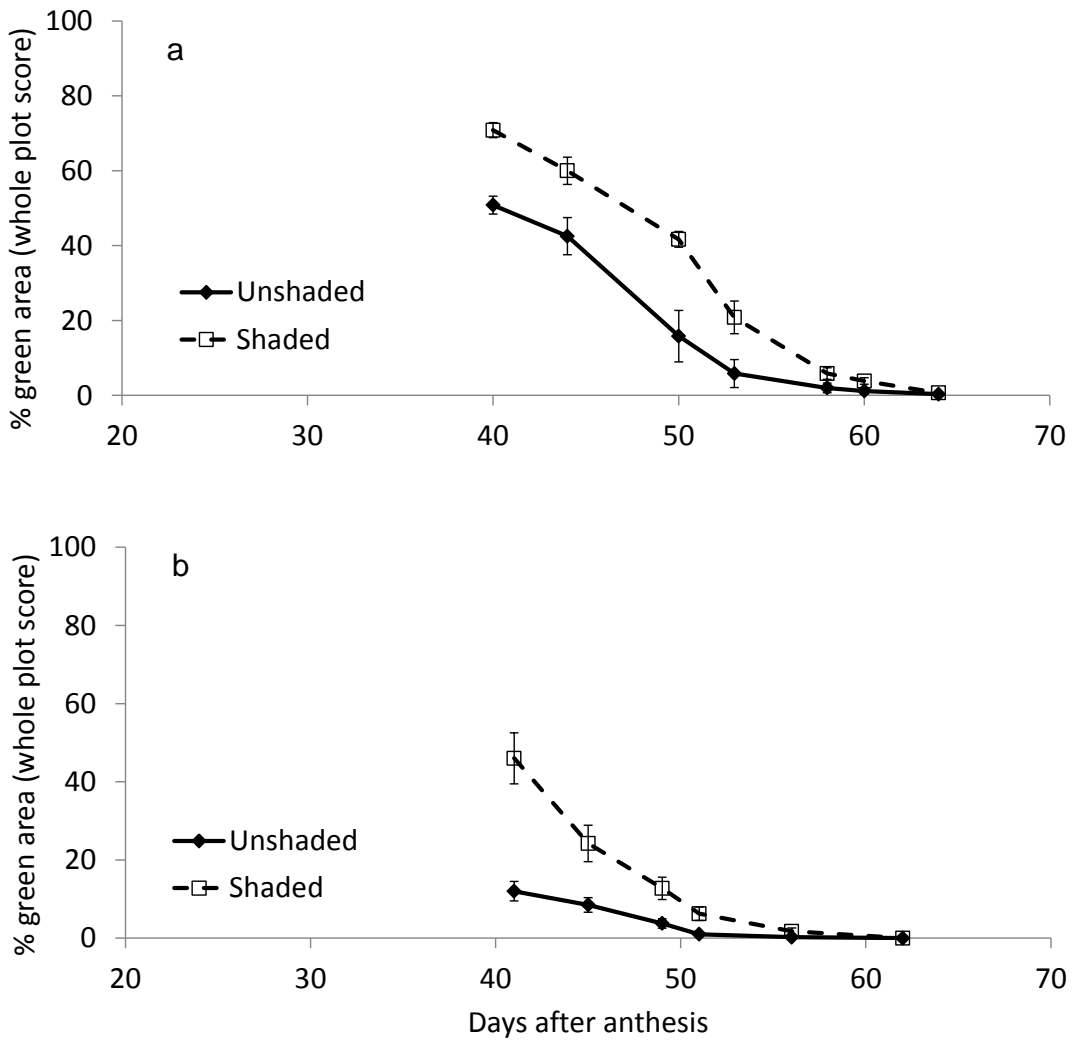


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3 Figure 1. Mean monthly weather data for March to April at field sites in 2011 and 2012 (bars). Broken line gives the long-term average (2005-2012) for the
 4 site. In the mean daily temperature panels letters A-C refer to Zadoks growth stages 31, 55 and 92 respectively; numbers following the letter are the
 5 number of days after sowing the growth stage was reached.

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3 Figure 2. Canopy senescence for spring barley grown unshaded or shaded from GS 55/61 + 14 days
4 until crop maturity in 2011 (a) and 2012 (b). Values are means \pm SEM of % green area scored on a
5 whole plot basis over the latter stages of senescence.

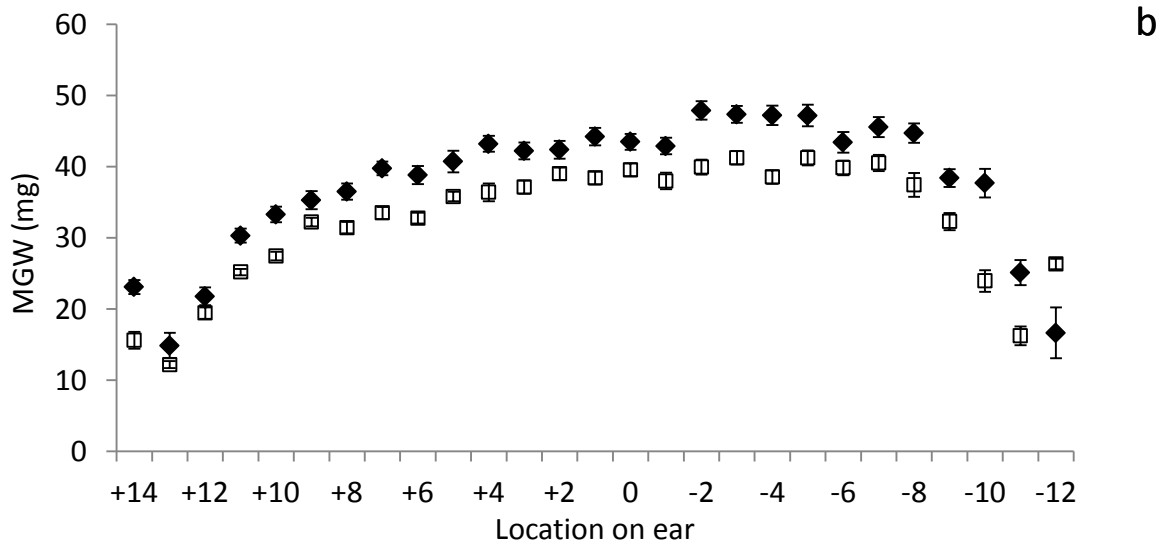
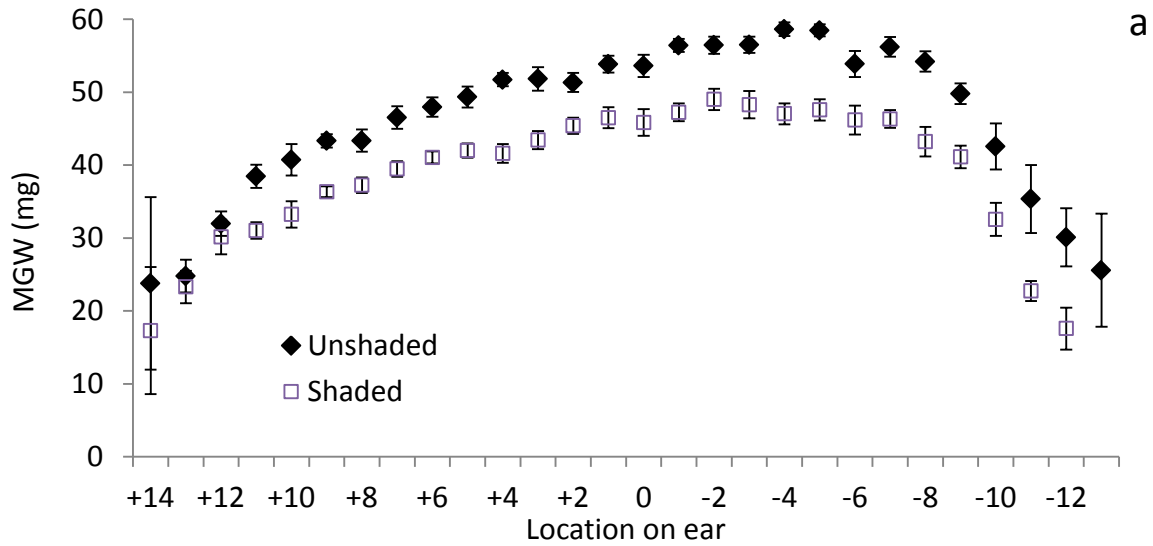
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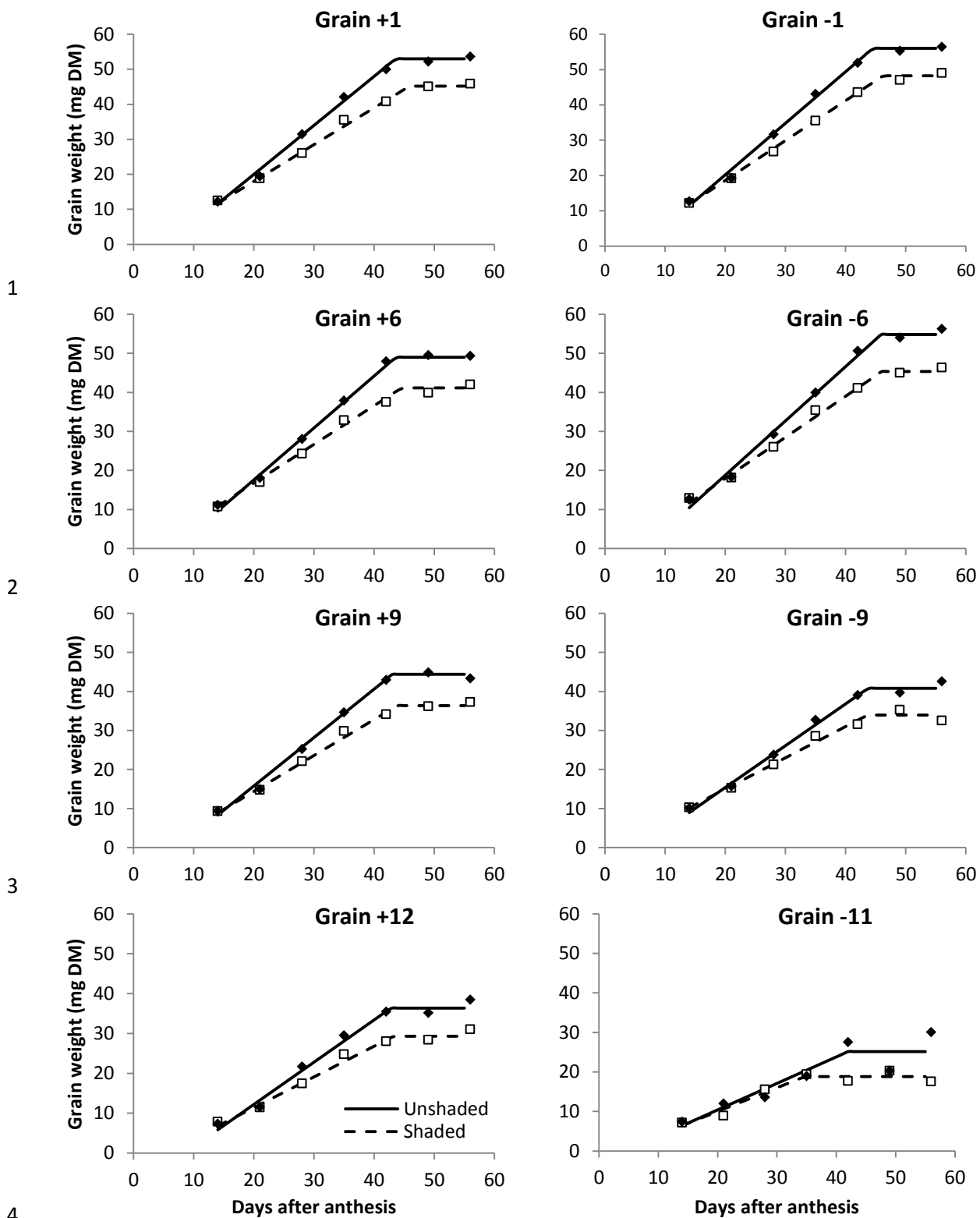
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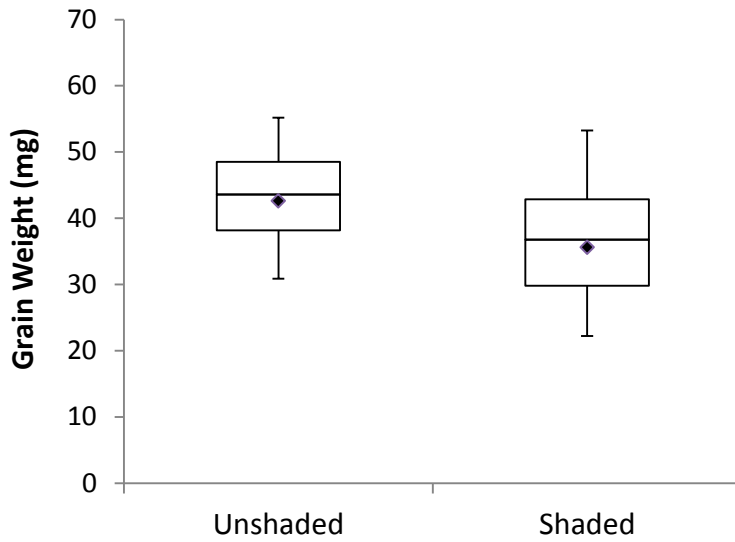
4 Figure 3. Plots of grain weight at individual spikelet locations on main stem ears at harvest for spring
5 barley grown unshaded or shaded from GS 55/61 + 14 days until crop maturity in (a) 2011 and (b)
6 2012. Values are means \pm SEM of ten sampled ears from each of 4-6 replicate plots, with locations
7 referring to grains above (+) or below (-) the central spikelet on each ear. MGW includes the weight
8 of the lemma and palea.

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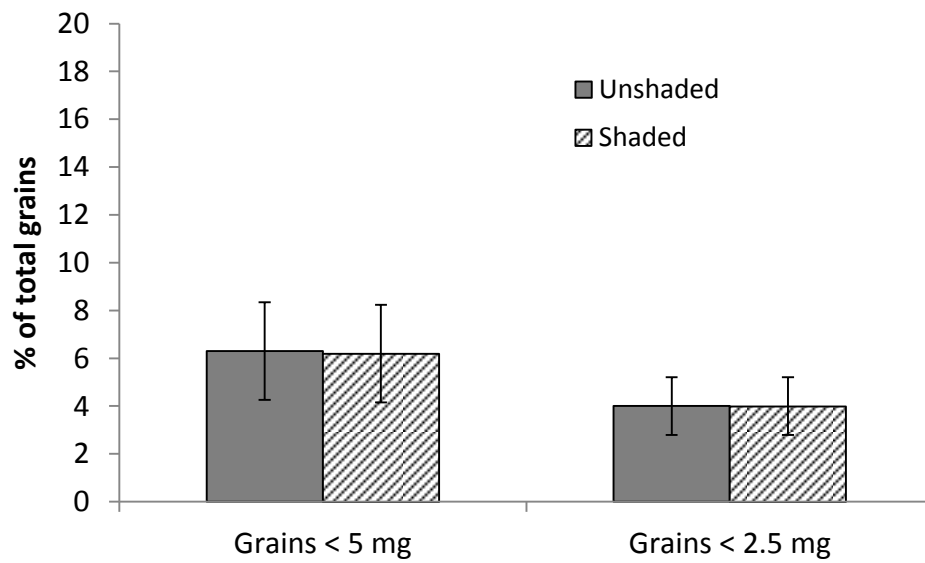
5 Figure 5. Split-line regression plots of the relationship between time after anthesis and the individual
6 grain weight at specific spikelet positions on the main stem ear for spring barley crops grown in 2011
7 either unshaded or shaded from GS 55/61 + 14 days until crop maturity. At all time points grain
8 weight includes the weight of the lemma and palea. Values are means of 10 ears from each of 6
9 replicate plots. Parameter values of the bilinear models are given in Table S1.

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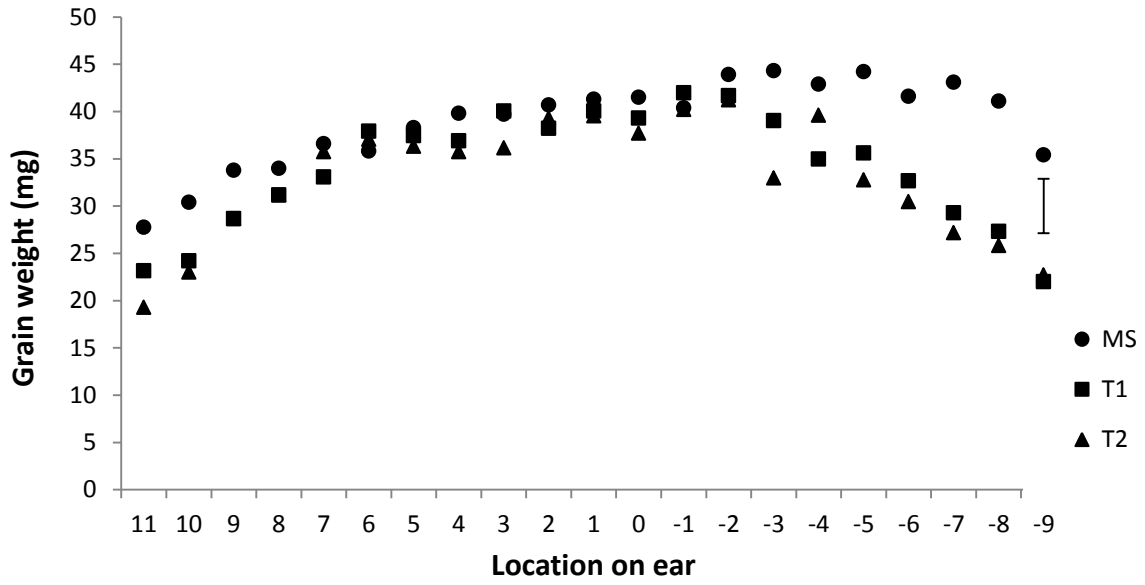
Figure 7. Box plot of individual grain weight in samples of 600 grains from spring barley crops harvested in 2011 grown either unshaded or shaded from GS55/61 + 14 days until crop maturity. Grains <5.0 mg were excluded from the analysis as non-viable. Boxes represent the central quartiles of data distribution whilst the ends of the whisker bars reflect the 90th (upper) and 10th (lower) percentiles respectively; horizontal line is the median value and the solid symbol the mean.



1

2 Figure 6. Proportion of grains weighing below 5 and 2.5 mg for spring barley crops harvested in 2011
 3 either shaded from GS55/61 + 14 days until crop maturity, or grown unshaded. Error bars represent
 4 the standard error of the mean of 100-grain samples from six replicate plots.

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2 Figure 4. Grain weight on main shoot (MS), tiller 1 (T1) and tiller 2 (T2) ears at individual spikelet
 3 locations relative to the central spikelet (location 0). Data are means across the two shading
 4 treatments (unshaded, shaded) at harvest in 2012. Error bar is LSD 5% for the grain zone x tiller
 5 interaction effect following repeated measures ANOVA.

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