Use of spike moisture content to define physiological maturity and quantify progress though grain development in wheat and barley

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A single measurement is useful for determining how far a crop has progressed through grain development, and whether it has reached physiological maturity. Grain development is commonly assessed using subjective, qualitative methods that describe the look and feel of the kernel or the color of the straw. Physiological maturity in cereal crops can be determined more accurately by the grain moisture content, but the moisture content of whole spikes is potentially quicker and easier to assess compared to individual kernels, and with a greater degree to accuracy. The dry matter and water content of whole spikes of 5 wheat and 5 barley cultivars sown at six times of sowing was determined at weekly intervals throughout the period of grain development from anthesis to harvest ripeness. Using regression analysis, it was determined that the spike moisture content at physiological maturity was 43% (95% CI 41-45%) for wheat and 50% (95% CI 49-51%) for barley, irrespective of differences in cultivar morphology, phenology and growing conditions. We demonstrate that progression through kernel development in wheat and barley can be assessed objectively and quantitatively using spike moisture content and provide guidelines to accurately determine the grain development stage using spike moisture.

Keywords

Grain development, grain filling, physiological maturity, moisture dynamics.

Introduction

Accurate knowledge of the timing of physiological maturity in wheat and barley is critical for both researchers and grain growers. Physiological maturity corresponds to the cessation of dry matter accumulation in the grain; at this point, grain fill is complete and maximum dry matter, and thus yield, is reached (Bewley and Black 1994; Calderini *et al.* 2000). This is distinct from harvest maturity or harvest ripeness, which occurs at a moisture content considerably lower than at physiological maturity (Bewley and Black 1994). Knowing exactly when physiological maturity occurs would increase the accuracy with which this key stage of development can be measured in phenological studies and would improve confidence around pre-harvest management decisions. However, published methods for estimating physiological maturity tend to be highly subjective and not repeatable because they rely on the interpretation of qualitative morphological traits. For example, Zadoks' decimal code (Zadoks *et al.* 1974; Tottman and Broad 1987) aligns physiological maturity with the hard dough stage (Z87) when the contents of the kernel are dry and the exterior can be dented by a fingernail. Others align this stage with the absence of green color in the peduncle, chaff or kernel (Bell and Fischer 1994; McMaster 1997).

The moisture content (or water percentage) of grain kernels, expressed as a percentage of total grain fresh weight, is one method by which physiological maturity in wheat and barley can be quantitatively estimated with greater accuracy and precision. It has been found that there is a universal relationship between grain moisture content and grain dry weight that applies to many crops, including the temperate cereals (Slafer *et al.* 2014). That is, crops of a given species growing under non-stressful conditions will reach physiological maturity at a similar grain moisture content (Slafer *et al.* 2014; Menendez *et al.* 2019). Grain moisture has the benefit of being quantitative, objective, and easy to measure in the laboratory (using the gravimetric method) or field (using a commercial grain moisture meter).

Grain moisture at physiological maturity has been found to occur at approximately 38% kernel moisture content in wheat (*Tritium aestivum*) (Calderini *et al.* 2000; Alvarez Prado *et al.* 2013) and 48% kernel moisture content in two-row barley (*Hordeum vulgare*) (Alvarez Prado *et al.* 2013). However, published estimates of kernel moisture content at the key development stages vary by as much as ±10% in wheat and barley: moisture at the end of anthesis could range from 70-80%, and at physiological maturity could range from 33-45% in wheat and 35-52% in barley (Schnyder and Baum 1992; Calderini *et al.* 2000; Pepler *et al.* 2006; Bingham *et al.* 2007; Alvarez Prado *et al.* 2013; González *et al.* 2014).

Most authors have studied this relationship using individual grains dissected from the center of the spike (Schnyder and Baum 1992; Saini and Westgate 1999; Calderini *et al.* 2000; Bingham *et al.* 2007; Alvarez Prado *et al.* 2013; González *et al.* 2014), but only Bingham et al. (2007) has compared individual grains to whole intact spikes inclusive of rachis, husks and grains. Bingham et al. (2007) noted that the moisture dynamics of whole spikes followed a similar to pattern to individual grains, but occurred over a narrower range of moisture contents. This is due to two reasons. First, the intact spike contains non-grain architectural components that will affect the relative proportions of dry matter and water. Second, grains in different positions on the spike do not flower, fill and mature at the same time and rate (Baillot *et al.* 2018). The fact that whole spikes develop over a smaller range of moisture contents suggests that this metric might serve as a more robust measure of grain development stage compared to the moisture content of individual kernels. Additionally, measuring moisture content of whole spikes would be quicker and easier than doing so with individual grains as it does not require a sample to be threshed (Menendez *et al.* 2019).

This field experiment aimed to characterize the moisture dynamics of whole intact wheat and barley spikes during the grain development phase and identify the spike moisture content that corresponded to physiological maturity for both species. A phenologically diverse selection of five wheat and five barley cultivars were sown on six different dates over an 11-week period to generate a broad range of environments in which to observe grain development from anthesis to harvest ripeness. The identification of critical moisture content values of whole spikes complements the work of others who studied the same process in individual kernels and enables the publication of objective, quantitative guides to progression through grain development and timing of physiological maturity that are based on measurement of spike moisture.

Method

Site, treatments and agronomy

This study was opportunistically undertaken on a sub-set of treatments of a larger field experiment designed to measure crop development in a diverse range cultivars over a broad range of sowing dates.The field experiment was conducted in 2019 at the Melbourne Polytechnic Farm at Yan Yean in central Victoria (37°32'37.6"S, 145°05'40.0"E) where long term average annual rainfall is 660 mm (April-October growing season rainfall 460 mm; Bureau of Meteorology station no. 086131). Plot size was three rows wide by 1.80 m long with a target plant density of 50 seeds per metre and row spacing of 20 cm. Plots were sown in a north-south direction with 100 kg/ha flutriafol-treated monoammonium phosphate. In the complete field experiment there were 96 genotypes, 64 wheat (*Triticum aestivum*) and 32 two-row barley (*Hordeum vulgare*), sown at 8 times of sowing: 5 March, 1 April, 15 April, 1 May, 15 May, 31 May, 14 June and 16 July 2019. The experiment layout consisted of 8 blocks randomly allocated to each time of sowing. Partially replicated design, *p*-design, (Cullis *et al.* 2006) was used, where for each time of sowing 78 randomly selected genotypes (52 wheat and 26 barley) were replicated 1¼ times, 12 randomly selected genotypes (8 wheat and 4 barley) were replicated 1⅛ times and 6 control genotypes (4 wheat and 2 barley) were replicated 1½ times. Of the 96 genotypes sown in the complete field experiment, a subset of 10 commercially available cultivars (5 wheat and 5 barley) were selected for weekly sampling of spikes from anthesis to physiological maturity (Table 1). These subset cultivars were selected to represent the diverse range of phenology types and development speeds in the complete experiment, thus they included both winter and spring types (*i.e.* do or do not require a period of vernalization to trigger reproductive development) and varied from very quick (~115 days to heading) to mid (~130 days to heading) to slow (~150 days to heading) in their development. Four of the five wheat cultivars were hard white

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milling quality with awns, and one (SQP Revenue) was red feed quality and awnless. All barley cultivars were two-row and had awns.

<Table 1>

Measurements

Rainfall and temperature at the field site was monitored from the first time of sowing (5 March 2019) to the date of final spike collection (19 December 2019). Daily rainfall was measured using a manual rain gauge and air temperature was monitored at 0.5 h intervals with two Tinytag Plus 2 data loggers (Gemini Data Loggers, Chichester, UK) housed in radiation shields at 1.2 m above the soil surface.

Collection of spikes in each plot began when anthesis was observed and continued until harvest ripeness, which was judged to be when the grain was hard and dry and unable to be dented by fingernail and the peduncles were completely devoid of chlorophyll (Z92; Zadoks *et al.* 1974). In wheat, anthesis was defined as when anther extrusion was observed in one floret in the middle of the spike (Kirby and Appleyard 1987). For barley cultivars, spike collection began at awn peep as a proxy for anthesis, because many barley cultivars flower inside the leaf sheath well before heading (Alqudah and Schnurbusch 2017). Five representative spikes were collected from each plot every Monday in the early afternoon (1200 to 1400 h) and placed in a hermetic plastic bag for transport to the laboratory.

The fresh weight (FW, g) and qualitative grain development stage of each spike was recorded immediately upon returning to the laboratory. The qualitative grain development stage (Z69 flowered, Z71 watery ripe, Z73 early milk, Z75 medium milk, Z77 late milk, Z83 early dough, Z85 soft dough, Z87 hard dough and Z92 fully ripe) was evaluated on a central kernel of each spike as per Zadoks *et al*. (1974) and Tottman and Broad (1987). Spikes were then oven dried at 70°C for 72 hours and the dry weights (DW, g) were recorded.

Any spikes that were compromised during the period of grain development, such that normal grain expansion, filling and ripening processes did not occur (e.g. frost- or heat-affected plants or cultivars flowering outside the optimal period that aborted grain fill), were excluded from the analysis because stressors that shorten grain-filling duration are known to disrupt the normal patterns of grain development (Rondanini *et al.* 2007). In addition, no spikes were harvested from the first or final times of sowing.

Data analysis

Temperature data were used to calculate thermal time accumulation after anthesis, measured as degree days (°Cd, *i.e.* the mean daily temperature) using a base temperature (*T*min) of 9.2°C and maximum temperature (*T*max) of 35.4°C (Porter and Gawith 1999). Because of the close association between heading and anthesis dates at Yan Yean in 2019 (±5 days), heading date was used as a proxy for anthesis date, and was calculated as the date on which 50% of the population had reached full head emergence in wheat (Z59) or first awns visible in barley (Z49) (Zadoks *et al.* 1974).

Spike water content (WC, g) of each sample was calculated as $WC = FW - DW$ where FW is spike fresh weight (g) and DW is spike dry weight (g). Spike water concentration, hereafter referred to as moisture content (M, %) of each sample was calculated as $M = (WC \div FW) \times 100$. Non-linear regression analysis was carried out to describe the dynamics of DW, FW, WC and M over thermal time after anthesis. Curve fitting was done for wheat and barley separately in a sequential manner with increasing complexity using cultivar as grouping factor. The sequent models were compared by assessing the statistical significance of the change in any two sequent models and by comparing the percentage variance accounted for by each of the models. Fresh weight was modelled using a quadratic-by-quadratic function presented by the equation $y_i = a + \frac{b + cx_i}{1 + dx_i + gx_i^2} + \varepsilon_i$ where y_i is the measured trait FW and x_i is the thermal time after anthesis. Dry weight and moisture were both modelled using a Gompertz curve of equation $y_i = a + ce^{-e^{-b(x_i - m)}} + \varepsilon_i$ where y_i is the measured trait DW or M and x_i is the thermal time after anthesis. Water content was modelled using a Gaussian curve presented by the equation $y_i = a + \frac{b}{\sqrt{2\pi}} e^{-\frac{(x_i - m)^2}{2s^2}} + \varepsilon_i$ where y_i is the measured trait WC and x_i is the thermal time after anthesis. For all models ε_i is the error term associated with the non-linear regression model.

Maximum spike dry weight for each cultivar at physiological maturity (*MaxDW*, g) was estimated by fitting a split-line (broken stick) regression of the form $y_1 = a_1 + b_a x,\ y_2 = a_2$ to the relationship between spike moisture (*M*) and dry weight (*DW*) and identifying the breakpoint at which dry weight reached a maximum. This breakpoint defines the maximum dry weight reached by a given cultivar; below this threshold value the grain is still accumulating dry matter. The value for *MaxDW* estimated using this method was approximately equal (± 0.20 g) to the mean dry weight of spikes for each cultivar harvested at the fully ripe grain development stage (Z92).

By standardizing differences in final spike dry weight and expressing it as a function of moisture, the moisture content at which physiological maturity was reached in wheat and barley could be estimated. Relative spike dry weight (*RSDW*, %) was calculated as $RSDW = (DW \div MaxDW) \times 100$. Moisture content at physiological maturity was estimated by fitting a split-line (broken stick) regression of the form $y_1 = a_1 + b_a x,\ y_2 = a_2$ to the relationship between spike moisture (*M*) and relative spike dry weight (*RSDW*) and identifying the breakpoint at which 100% relative spike dry weight was reached. This breakpoint defines moisture content at physiological maturity; at moisture contents below this threshold value the spike has not reached physiological maturity. This was calculated separately for each cultivar and then collectively for each species.

All regression analyses and curve fitting were carried out in Genstat 20 (VSN International 2019). Figures were created in Microsoft Excel and R (R Core Team 2013) using the packages *ggplot2* (Wickham 2018), *gridExtra* (Auguie 2017) and *RColorBrewer* (Neuwirth 2014).

Results

Seasonal conditions and anthesis dates sampled

Average daily air temperature experienced by plants during development was affected by sowing date and cultivar development speed, effectively creating a variable range of environments for observing cereal phenology and grain development in the ten cultivars studied (Fig. 1). Rainfall conditions in 2019 were non-limiting for development with 418 mm of total annual rainfall and 386 mm of this falling within the growing season from the start of April to the end of November. An additional 18 mm of irrigation was applied on 6 March, 18 March, 4 April and 17 April to assist seedling establishment in the earlier times of sowing.

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<Figure 1>

The subset of cultivars chosen for spike collection had viable spikes harvested from plants sown at the second through to the seventh time of sowing, corresponding to flowering dates ranging from 19 July (Emu Rock sown 1 April) to 3 November (SQP Revenue sown 14 June) in wheat, and from 8 August to (Rosalind sown 1 April) to 15 October (Oxford sown 14 June) in barley (Fig. 2). Each cultivar displayed distinctive patterns of development depending on its relative sensitivity to day length and requirement for vernalization. The ten cultivars reached anthesis and physiological maturity on different dates depending on their individual phenology and sowing date. The number of days from sowing to anthesis ranged from 109-185 days in wheat and 108-175 days in barley, and from sowing to physiological maturity ranged from 161-208 days in wheat and 147-189 days in barley.

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<Figure 2>

Dynamics of spike development after anthesis

All wheat and barley cultivars showed common patterns of grain development despite differences in phenology, flowering date, grain filling duration and spike size (Fig. 3). For all cultivars, the initial grain expansion period was accompanied by a rapid increase in spike fresh weight, which was overlapped and followed by the grain filling period where dry weight increased. The cessation of dry weight gain was followed by a period of maturation drying where water content rapidly declined.

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On average, fresh weight, dry weight and water content of wheat spikes reached a maximum at a higher value compared to barley spikes (mean weight 3.5, 2.2 and 2.0 g compared to 2.5, 1.4 and 1.3 g, respectively). Fresh weight and water content peaked around 200-250°Cd after anthesis and were followed by the peak in dry weight at physiological maturity, 500-600°Cd after anthesis. The dynamics of spike moisture were best described by fitting quadratic-by-quadratic functions to fresh weight over time, Gompertz functions to dry weight over time and Gaussian functions to water content over time (Fig. 3, Table 2).

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The quadratic-by-quadratic curve reflects the pattern observed for fresh weight, as a cubic curve with an asymptotic maximum falling to an asymptote. For wheat the model with separate linear parameters *a*, *b* and *c* for each cultivar and common parameters *d* and *g* was the best fit, whereas the five barley cultivars were more diverse and required a model with separate parameters for each cultivar. These patterns are reflected in the percentage variance accounted for by the model of each crop, 77.5% for barley and 67.7% for wheat. The spikes of SQP Revenue peaked at a higher fresh weight and achieved this peak sooner after anthesis when compared to the other wheat cultivars. The barley cultivar Oxford also peaked earlier than the other cultivars, but at a comparatively lower maximum fresh weight.

Dry weight over thermal time was best described by a Gompertz curve with a positive parameter *b* to specify rate of growth. The best model for barley had common non-linear parameters *b* and *m* but a separate shift parameter *a* and scale parameter *c* for each cultivar, whereas the best model for wheat had all separate parameters due to the greater variability between cultivars. The dynamics of dry weight over time were similar for all barley cultivars. In contrast, the wheat cultivars Bolac, Whistler and Emu Rock grouped together, followed by Derrimut, but SQP Revenue had different parameters and peaked earlier at a higher dry weight. The model for barley accounted for 79.6% of the variation and similarly 78.4% of the variation in dry weight was explained for wheat.

The most appropriate curve to model water content was the bell-shaped Gaussian curve. This trait varied between the cultivars of each species and was modeled with separate parameters *a*, *b*, *s* and *m* for each cultivar of wheat and barley. SQP Revenue was notably different to the other wheat cultivars in all parameters, peaking at a higher water content sooner after anthesis. The barley cultivars were more consistent and reached maximum water content at similar thermal time after anthesis.

The model for water content in the wheat varieties explained 74.9% of the variation and for barley varieties, respectively 75.1%.

<Figure 3>

<Table 2>

Maximum spike dry weight at physiological maturity as estimated by split-line regression was highest in the winter wheat SQP Revenue (2.41 ± 0.04 g), followed by Emu Rock (2.32 ± 0.08 g), Derrimut (2.25 ± 0.07 g), Bolac (2.01 ± 0.05 g) and then Whistler (1.95 ± 0.05 g). In barley, winter cultivar

Cassiopèe (1.48 ± 0.03 g) had the highest maximum spike dry weight followed by Fleet (1.48 ± 0.03 g), Westminster (1.45 ± 0.02 g), Rosalind (1.28 ± 0.02 g) and then Oxford (1.27 ± 0.02 g).

For both wheat and barley, the increase in spike dry weight over time was accompanied by a sigmoidal decrease in spike moisture content (Fig. 4). Spike moisture over thermal time was best described by a Gompertz curve with a negative parameter *b* to specify the rate of decay; in this case, the rate of moisture loss or maturation drying (Table 2). For both wheat and barley, the best model had separate parameters for *a*, *b*, *c* and *m* for each cultivar. The curves fit explained extremely well the moisture dynamics in time and accounted for 96.2% of the variation for wheat and 95.5% for barley. Barley cultivars had *ca.* 4% higher moisture content than wheat cultivars at all stages of grain development. At anthesis, spike moisture content was *ca.* 63% in wheat and *ca.* 68% in barley. Moisture decreased continuously during the grain expansion, filling and ripening phases and reached a minimum of *ca.* 5% in both species once maturation drying was complete. Maximum grain dry weight was approached when spike moisture had declined to <50% in wheat and <55% in barley. These patterns of spike moisture after anthesis were well described using Gompertz growth functions (Fig. 4, Table 2) and demonstrate the consistency in spike moisture content within species compared to the other parameters.

<Figure 4>

Spike moisture content at qualitative grain development stages

Spike moisture content continuously declined as wheat and barley cultivars progressed through the grain development stages from anthesis to fully ripe (Fig. 5). Moisture content at each grain development stage, from milk through to dough and then ripening, was found to vary by 10-25%. For example, spikes judged to be in the soft dough stage in wheat corresponded to moisture contents anywhere in the range of 20 to 45%; moisture contents that spanned medium milk to fully ripe stage. Although the trend of declining moisture content was clear it was not reasonable to define the moisture content that was characteristic of a given stage due to the variability observed and the fact that any given moisture content could relate to several grain development stages concurrently.

<Figure 5>

Modelling spike moisture content at physiological maturity

Spike moisture content at physiological maturity was found to be 42.9 ± 0.7% (R^2 = 64%, n = 775) for wheat and 49.8 ± 0.3% (R^2 = 77%, n = 1065) for barley (Fig. 6, Table 3). Considering 95% confidence intervals of the breakpoint of the fitted functions, spike moisture content at physiological maturity ranged from 41-45% for wheat and 49-51% for barley.

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Amongst the wheat cultivars, the breakpoint which defined moisture content at physiological maturity was similar for Derrimut, Bolac, Emu Rock and SQP Revenue, which reached maximum dry weight at 40-43% moisture. In comparison, the fast winter wheat Whistler reached maturity at a considerably higher moisture content of 51%. There was less difference between barley cultivars, with all five reaching physiological maturity at 46-52% spike moisture content (Table 3).

<Figure 6>

<Table 3>

Discussion

The results of this study demonstrate that progression through kernel development and timing of physiological maturity in wheat and barley can be determined in a repeatable, objective and quantitative manner using spike moisture content. Despite large differences in phenology, morphology and growing conditions, all cultivars of wheat and barley followed the same predictable pattern of grain development and exhibited the same relationship between relative spike dry weight and moisture content as has been observed for individual kernels (Bewley and Black 1994; Slafer *et al.* 2014). The pattern of grain development of all cultivars, visualized in terms of changes in spike fresh weight, dry weight and water content, followed the characteristic three-phase model of grain expansion, filling and ripening described by Bewley and Black (1994). Dynamics of fresh weight, dry weight and water content over thermal time were modelled by quadratic-by-quadratic functions, Gompertz functions and Gaussian functions, respectively. In the initial expansion phase immediately following anthesis, kernels rapidly accumulated water and some solids to reach maximum kernel size and fresh weight (Bewley and Black 1994). This was followed by a grain filling phase, characterized by the deposition of solids until maximum grain dry weight is reached at physiological maturity. During the maturation drying phase the kernels ripened and fresh weight and water content declined (Bewley and Black 1994). When differences in final spike dry weight were standardized, all cultivars displayed the same bi-linear pattern whereby relative spike dry weight reached a maximum at a given moisture content (Slafer *et al.* 2014). Our results showed that physiological maturity in wheat and barley is reached at 43% and 50% spike moisture content, respectively.

Whole spikes of wheat and barley were observed to follow a similar pattern of development to individual kernels, but over a narrower range of moisture contents. This is in agreement with a previous study by Bingham et al. (2007) where whole intact barley spikes mimicked the pattern of individual kernels, but at comparatively lower moisture content. We observed moisture content in wheat spikes of 61-65% at anthesis declining to 43% at physiological maturity, and in barley spikes of 67-69% at anthesis declining to 50% at physiological maturity. These ranges of moisture are indeed narrower than published values for individuals wheat kernels (70-80% moisture at anthesis declining to 30% at physiological maturity; Calderini *et al.* 2000; Alvarez Prado *et al.* 2013) and barley kernels (70-80% moisture at anthesis declining to 48% at physiological maturity; Bingham *et al.* 2007; Alvarez Prado *et al.* 2013). These differences in whole spike and individual kernel moisture dynamics occur because the whole spike comprises non-kernel components (e.g. rachis, glumes, awns) that dilute the effect of the kernels on overall spike moisture. This is also why barley cultivars in the present study were consistently observed to have smaller spikes but greater moisture content than wheat cultivars: the spike of two-row barley has simpler architecture compared to the spike of wheat, resulting in the barley kernels themselves contributing proportionally more to spike weight and moisture content.

All five barley cultivars reached physiological maturity at approximately the same threshold moisture content of ~50%, regardless of whether they were winter or spring habit. However, there was some variation amongst the wheat cultivars, with Whistler reaching physiological maturity at a threshold moisture content of 51%, much higher than the other four cultivars at ~43%. There was no indication why this was the case for Whistler as it was not the only winter wheat cultivar, nor did it vary from the expected patterns of grain development observed in all other cultivars. These findings suggest that there may be within-species variation in moisture dynamics that were not captured in the present study. However, this variation may not be due to obvious morphological differences between cultivars, as the red-grained awnless cultivar SQP Revenue reached physiological maturity at similar moisture content to the white-grained awned spring wheat cultivars in this study, despite clear differences in spike moisture dynamics. It would be valuable for future studies to investigate a more diverse range of wheat and barley cultivars and look for factors explaining differences in moisture dynamics during grain development. Nevertheless, moisture contents in the range of 41-45% for wheat and 49-51% for barley were found to be a robust indicator of physiological maturity in these species.

It may also be valuable for future studies to compare the effects of standardizing spike size on predictions of moisture content at physiological maturity. Variation in relative spike dry weights, ranging from 50-150% of maximum spike dry weight for a given cultivar, reflects the wide range of spike sizes sampled in the field. Differences in spike size and weight can occur both between and within cultivars: between cultivars, due to differences in breeding and pedigree; and within cultivars due to differences in growing conditions, flowering date, grain filling rate or duration and yield components (Slafer 2003; Baillot *et al.* 2018). Spike weight could be normalized by adjusting for the number of spikelets or grains per spike to control for this inherent source of variation.

It is possible to estimate the grain development stage of wheat and barley qualitatively using the scales of development defined by Zadoks et al. (1974), Feekes (Large 1954) and others. However, the subjective and qualitative nature by which the stages of grain development are described and evaluated results in uncertainty around identification of each stage and therefore variability in the moisture content that corresponds to a given stage. It was clear from this study that it is not possible to conclusively identify a value of moisture content that defines each qualitative development stage due to the large degree of variability observed. For this reason, it is necessary to define objective and quantitative methods of assessing grain development that are reliable and repeatable.

Given that kernel (Calderini *et al.* 2000; Alvarez Prado *et al.* 2013) and spike moisture contents at the beginning and end of grain development have now been estimated, it is possible to publish guidelines to accurately determine the progress of a wheat or barley crop through the grain development phase with a single measurement of kernel or spike moisture (Table 4). In this way, subjective observations of grain development that are defined in the Zadoks' decimal code (Zadoks *et al.* 1974; Tottman and Broad 1987) and elsewhere (e.g. Large 1954; Calpouzos *et al.* 1976; Lancashire *et al.* 1991; Bell and Fischer 1994; McMaster 1997) can be replaced with objective assessments of moisture content that clearly define the start and end of this phase. The moisture content of a sample of threshed grain or whole spikes can be measured in the laboratory using the gravimetric method (as per this study) or in the field using a grain moisture meter. Note that the guide to grain development presented in Table 4 is not applicable to plants that do not follow normal patterns of grain development, such as those affected by frost and heat stress.

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<Table 4>

Conclusion

We have demonstrated that progression through kernel development in wheat and barley can be assessed objectively and quantitatively using spike moisture content. This method should improve accuracy and repeatability of assessments in agronomic studies of crop development. It could also be used as a method to more accurately time pre-harvest management decisions like the application of desiccants. Whole spikes are easier to sample and assess compared to individual kernels and have a smaller range of moisture contents between anthesis and physiological maturity. Grain development begins immediately after anthesis when the whole spike is at 61-65% (wheat) or 67-69% (barley) moisture. As the kernels expand, fill and ripen, the moisture content of the spike declines. Spike moisture contents in the range of 41-45% for wheat and 49-51% for barley can be used to indicate that the crop has reached physiological maturity.

Conflict of Interest

The authors declare no conflicts of interest.

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Table 1. Wheat and barley cultivars used in the experiment. Phenology type, qualitative development speed and days to heading (mean of all sowing dates) at Yan Yean shown in parentheses.

Wheat	Barley
Emu Rock (spring, very fast, 116)	Rosalind (spring, fast, 115)
Derrimut (spring, mid-fast, 122)	Fleet (spring, mid-fast, 119)
Bolac (spring, mid, 132)	Westminster (spring, mid, 125)
Whistler (winter, fast, 145)	Oxford (spring, mid-slow, 133)
SQP Revenue (winter, slow, 164)	Cassiopèe (winter, slow, 143)

Table 2. Results of curve fitting to describe spike moisture dynamics in wheat and barley cultivars over thermal time after anthesis.

Fresh weight				**Dry weight**			
Quadratic-by-quadratic, $y_i = a + \frac{b+cx_i}{1+dx_i+gx_i^2} + \varepsilon_i$				Gompertz curve, $y_i = a + ce^{-e^{-b(x_i-m)}} + \varepsilon_i$			
Crop	Parameter	Estimate	SE	Crop	Parameter	Estimate	SE
Barley	*d* cultivar Cassiopèe	-0.00759	0.000742	Barley	*b*	0.012233	0.000545
	g cultivar Cassiopèe	2.08E-05	2.95E-06		*m*	161.24	3.12
	b cultivar Cassiopèe	-0.213	0.119		*c* cultivar Cassiopèe	1.307	
	c cultivar Cassiopèe	0.00334	0.000765		*a* cultivar Cassiopèe	0.2783	
	a cultivar Cassiopèe	1.039	0.169		*c* cultivar Fleet	1.303	
	d cultivar Fleet	-0.00763	0.00051		*a* cultivar Fleet	0.2751	
	g cultivar Fleet	1.90E-05	1.76E-06		*c* cultivar Oxford	1.079	
	b cultivar Fleet	-0.2229	0.0782		*a* cultivar Oxford	0.2813	
	c cultivar Fleet	0.002786	0.000528		*c* cultivar Rosalind	1.114	
	a cultivar Fleet	1.105	0.132		*a* cultivar Rosalind	0.2418	
	d cultivar Oxford	-0.00981	0.0011		*c* cultivar Westminster	1.322	
	g cultivar Oxford	3.33E-05	5.42E-06		*a* cultivar Westminster	0.2356	
	b cultivar Oxford	-0.3523	0.0828	Wheat	*b* cultivar Bolac	0.00993	0.00249
	c cultivar Oxford	0.003656	0.00068		*m* cultivar Bolac	162.6	22.5
	a cultivar Oxford	1.149	0.115		*c* cultivar Bolac	1.718	0.248
	d cultivar Rosalind	-0.00803	0.000843		*a* cultivar Bolac	0.415	0.183
	g cultivar Rosalind	2.26E-05	3.08E-06		*b* cultivar Derrimut	0.00767	0.00194
	b cultivar Rosalind	-0.2525	0.0919		*m* cultivar Derrimut	184.9	20.1
	c cultivar Rosalind	0.003088	0.000801		*c* cultivar Derrimut	2.323	0.338
	a cultivar Rosalind	0.922	0.154		*a* cultivar Derrimut	0.293	0.187
	d cultivar Westminster	-0.0071	0.000564		*b* cultivar Emu Rock	0.01247	0.00229
	g cultivar Westminster	1.71E-05	2.03E-06		*m* cultivar Emu Rock	200.62	9.93
	b cultivar Westminster	-0.164	0.105		*c* cultivar Emu Rock	2.056	0.154
	c cultivar Westminster	0.002559	0.000518		*a* cultivar Emu Rock	0.4007	0.0751
	a cultivar Westminster	0.955	0.156		*b* cultivar Revenue	0.01867	0.00383
Wheat	*d*	-0.00795	0.00029		*m* cultivar Revenue	161.1	10.2
	g	2.18E-05	1.22E-06		*c* cultivar Revenue	1.464	0.118

	b cultivar Bolac	-0.2921			*a* cultivar Revenue	0.974	0.101
	c cultivar Bolac	0.004043			*b* cultivar Whistler	0.00796	0.00229
	a cultivar Bolac	1.462			*m* cultivar Whistler	122.5	43.7
	b cultivar Derrimut	-0.5882			*c* cultivar Whistler	2.097	0.506
	c cultivar Derrimut	0.004947			*a* cultivar Whistler	0.112	0.404
	a cultivar Derrimut	1.757					
	b cultivar Emu Rock	-0.8162					
	c cultivar Emu Rock	0.005931					
	a cultivar Emu Rock	1.817					
	b cultivar Revenue	0.2377					
	c cultivar Revenue	0.002159					
	a cultivar Revenue	2.024					
	b cultivar Whistler	-0.1517					
	c cultivar Whistler	0.003868					
	a cultivar Whistler	1.341					

Water Content

Gaussian curve, $y_i = a + ce^{-e^{-b(x_i-m)}} + \varepsilon_i$

Moisture

Gompertz curve, $y_i = a + ce^{-e^{-b(x_i-m)}} + \varepsilon_i$

Crop	Parameter	Estimate	SE	Crop	Parameter	Estimate	SE
Barley	*s* cultivar Cassiopèe	104.46	6.32	Barley	*b* cultivar Cassiopèe	-0.01464	0.000908
	m cultivar Cassiopèe	211.53	3.15		*m* cultivar Cassiopèe	348.29	3.65
	b cultivar Cassiopèe	365.2	32.7		*c* cultivar Cassiopèe	59.4	1.35
	a cultivar Cassiopèe	0.0888	0.0599		*a* cultivar Cassiopèe	7.858	0.925
	s cultivar Fleet	107.67	5.59		*b* cultivar Fleet	-0.01182	0.000761
	m cultivar Fleet	226.04	2.87		*m* cultivar Fleet	372.04	4.08
	b cultivar Fleet	383.3	28		*c* cultivar Fleet	60.28	1.52
	a cultivar Fleet	0.1139	0.0506		*a* cultivar Fleet	7.521	0.973
	s cultivar Oxford	111.26	7.35		*b* cultivar Oxford	-0.01329	0.00075
	m cultivar Oxford	192.14	3.77		*m* cultivar Oxford	333.78	3.49
	b cultivar Oxford	317.1	33.4		*c* cultivar Oxford	61.11	1.4
	a cultivar Oxford	0.0743	0.0601		*a* cultivar Oxford	7.404	0.961
	s cultivar Rosalind	102.37	6.32		*b* cultivar Rosalind	-0.01121	0.000749
	m cultivar Rosalind	208.81	3.85		*m* cultivar Rosalind	340.48	4.08
	b cultivar Rosalind	289.2	24.9		*c* cultivar Rosalind	62.34	1.7

	a cultivar Rosalind	0.096	0.0459		*a* cultivar Rosalind	7.641	0.886
	s cultivar Westminster	108.72	6.8		*b* cultivar Westminster	-0.01328	0.000779
	m cultivar Westminster	219.02	3.22		*m* cultivar Westminster	355.65	3.72
	b cultivar Westminster	354	35		*c* cultivar Westminster	60	1.42
	a cultivar Westminster	0.1032	0.0643		*a* cultivar Westminster	7.79	1.02
Wheat	*s* cultivar Bolac	102.83	7.01	Wheat	*b* cultivar Bolac	-0.01836	0.00128
	m cultivar Bolac	210.79	4.36		*m* cultivar Bolac	353.38	3.2
	b cultivar Bolac	471.8	46.6		*c* cultivar Bolac	53.91	1.05
	a cultivar Bolac	0.1162	0.0835		*a* cultivar Bolac	8.301	0.735
	s cultivar Derrimut	106.69	9.34		*b* cultivar Derrimut	-0.01381	0.000902
	m cultivar Derrimut	218.68	5.18		*m* cultivar Derrimut	365.47	4.42
	b cultivar Derrimut	465.4	63.6		*c* cultivar Derrimut	56.48	1.46
	a cultivar Derrimut	0.178	0.112		*a* cultivar Derrimut	7.78	1.09
	s cultivar Emu Rock	77.48	7.43		*b* cultivar Emu Rock	-0.01559	0.00117
	m cultivar Emu Rock	243.32	5.03		*m* cultivar Emu Rock	378.19	5.22
	b cultivar Emu Rock	328.8	42.9		*c* cultivar Emu Rock	59.7	2.04
	a cultivar Emu Rock	0.4601	0.0908		*a* cultivar Emu Rock	5.03	1.77
	s cultivar Revenue	126.52	9.15		*b* cultivar Revenue	-0.01265	0.000968
	m cultivar Revenue	161.3	6.08		*m* cultivar Revenue	325.55	3.81
	b cultivar Revenue	803.1	83.2		*c* cultivar Revenue	63.35	2.14
	a cultivar Revenue	-0.041	0.118		*a* cultivar Revenue	5.37	1.01
	s cultivar Whistler	106.6	6.54		*b* cultivar Whistler	-0.01524	0.000885
	m cultivar Whistler	200.65	3.66		*m* cultivar Whistler	350.35	3.27
	b cultivar Whistler	547.6	51.6		*c* cultivar Whistler	59.33	1.21
	a cultivar Whistler	0.0682	0.0929		*a* cultivar Whistler	6.742	0.873

Table 3. Results of split-line regression analysis to estimate moisture content (M) at physiological maturity (100% RSDW).

Cultivar	Breakpoint_X (M)	SE	Breakpoint_Y (RSDW)	SE	Slope	SE	95% CI	R^2
All wheat	42.87	0.68	100.31	1.18	-3.033	0.123	40.61, 45.08	64.2
Bolac	41.68	0.61	100.00	2.38	-2.865	0.193	37.21, 46.86	61.6
Derrimut	40.25	2.67	100.00	2.89	-3.032	0.364	34.33, 44.28	69.6
Emu Rock	42.18	2.22	100.00	3.34	-3.257	0.327	36.57, 46.17	73.5
SQP Revenue	43.14	0.42	100.00	1.71	-2.232	0.137	39.30, 50.24	58.9
Whistler	50.86	0.48	100.00	2.52	-4.436	0.302	47.45, 55.31	58.7
All barley	49.79	0.27	99.60	0.73	-4.1319	0.0928	48.40, 50.55	77.1
Cassiopèe	50.24	0.99	100.00	1.88	-4.245	0.283	48.27, 52.51	76.7
Fleet	52.19	1.02	100.00	1.69	-5.050	0.423	49.99, 54.08	74.4
Oxford	48.16	0.24	100.00	1.50	-3.639	0.133	46.35, 50.15	78.7
Rosalind	46.14	1.31	99.99	1.77	-3.362	0.242	44.18, 49.33	76.4
Westminster	48.96	0.98	100.00	1.71	-4.056	0.244	46.33, 50.73	79.9

Table 4. Guide to grain development stage of wheat and barley based on moisture content of individual threshed kernels or whole spikes. Values for spike moisture content are from this study. Values for kernel moisture content are from Alvarez Prado *et al.* (2013) and Calderini *et al.* (2000).

Stage of development	Moisture content (water concentration, %)			
	Wheat		Barley	
	Kernel	Spike	Kernel	Spike
Anthesis complete; beginning of grain expansion	~70 - 80%	61 - 65%	~70 - 80%	67 - 69%
Grain filling 50% complete	56%	52%	62%	58%
Grain filling complete; physiological maturity	38%	43%	48%	50%
Grain ripening complete; harvest ripe	~12.5 - 20%	~12.5 - 20%	~12.5 - 20%	~12.5 - 20%

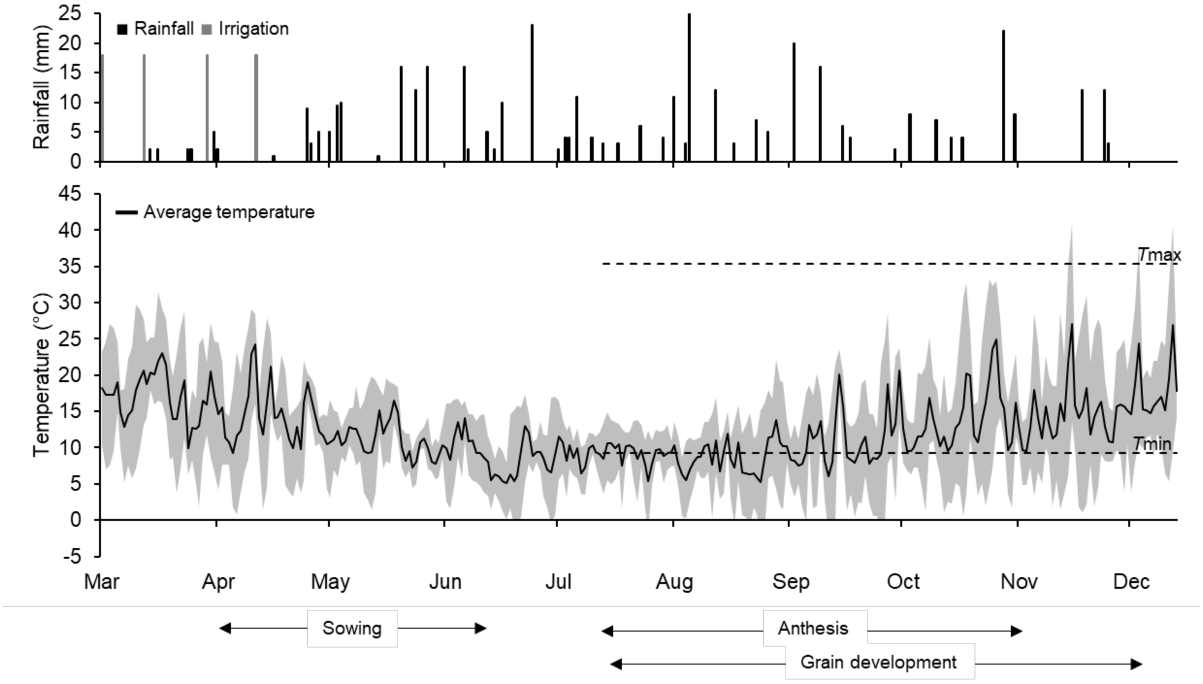

Fig. 1. Daily temperatures and rainfall at the Yan Yean field site in 2019 in relation to the periods of sowing, anthesis and grain development. Shaded area represents the range of daily minimum and maximum temperatures. Dashed lines represent the base (*T*min, 9.2°C) and maximum (*T*max, 35.4°C) temperatures for grain filling as per Porter and Gawith (1999).

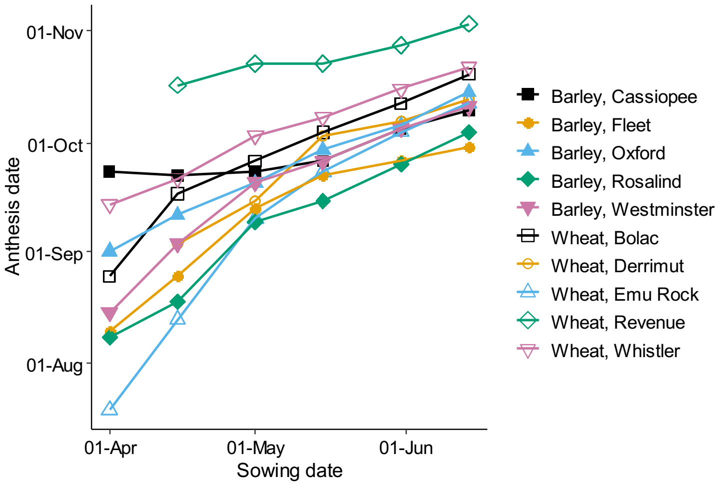

Fig. 2. Range of sowing and anthesis dates for the ten cultivars grown at Yan Yean in 2019 from which spikes were harvested.

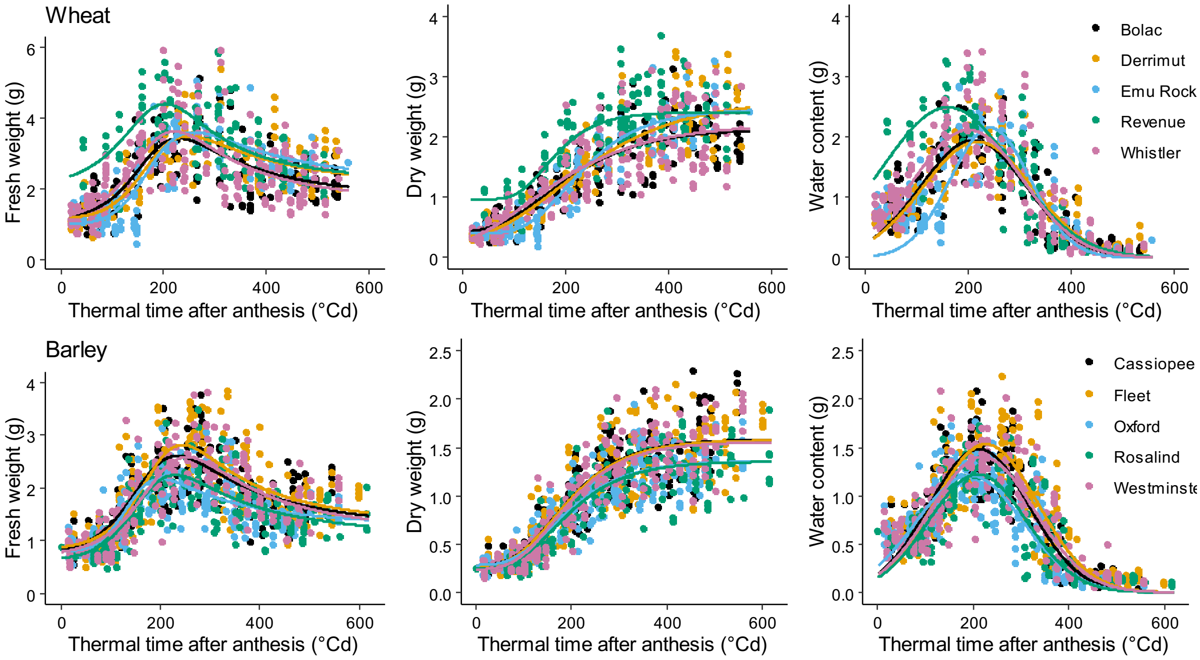

Fig. 3. Pattern of grain development showing the changes in whole spike fresh weight (FW), dry weight (DW) and water content (WC) during the grain development phase from anthesis to harvest ripeness. Fitted quadratic-by-quadratic functions to FW over thermal time (R^2 wheat = 60%, R^2 barley = 60%), Gompertz functions to DW over thermal time (R^2 wheat = 78%, R^2 barley = 80%) and Gaussian functions to WC over thermal time (R^2 wheat = 75%, R^2 barley = 75%) are shown.

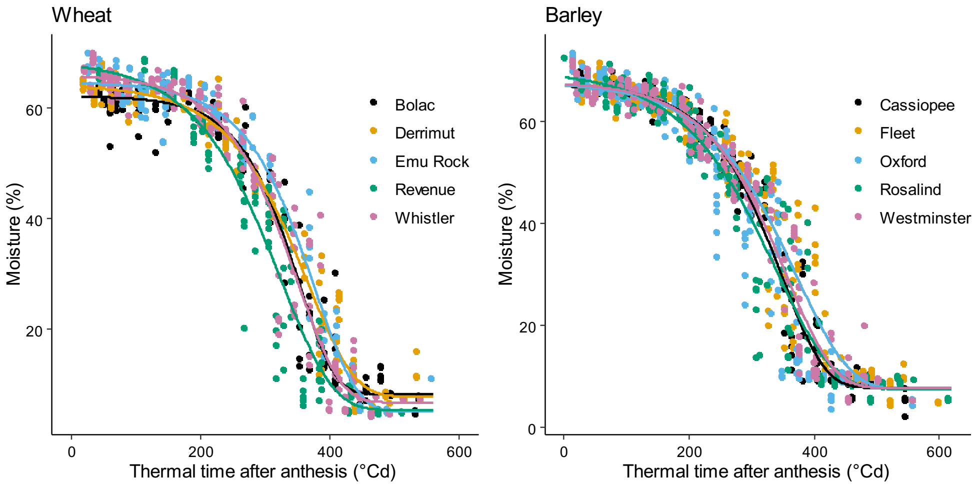

Fig. 4. Dynamics of dry weight (DW) and moisture content (M) of wheat and barley spikes as a function of thermal time during the grain development phase from anthesis to harvest ripeness. Fitted Gompertz functions are shown for wheat (R^2 = 96%) and barley (R^2 = 96%).

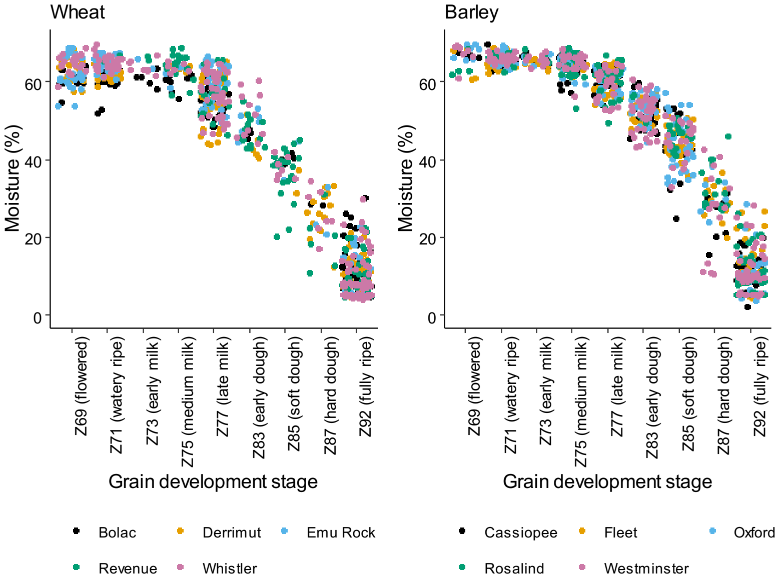

Fig. 5. Moisture content (M) of wheat and barley spikes at key qualitative grain development stages defined by Zadoks *et al.* (1974).

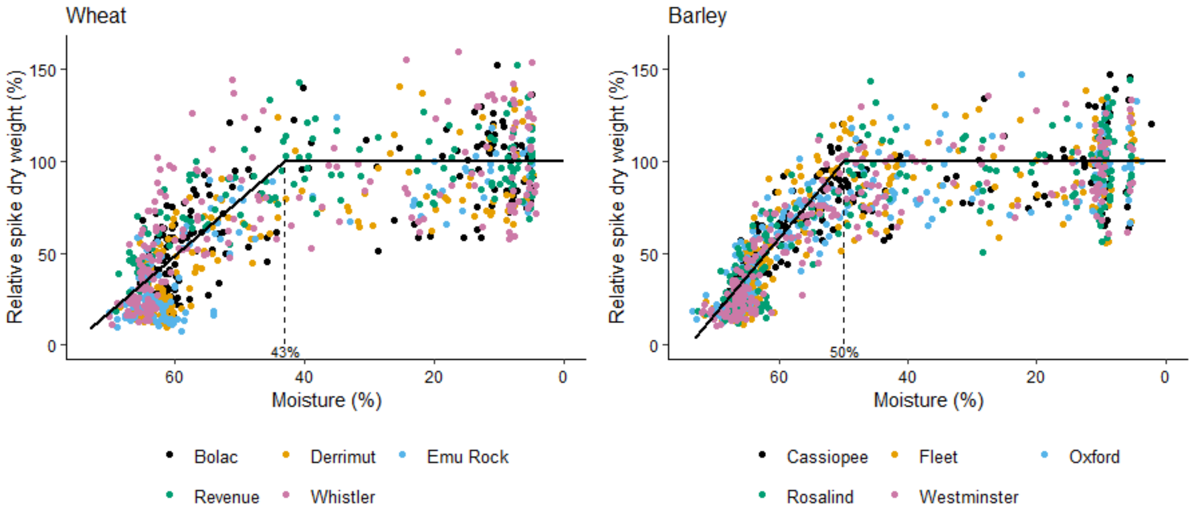

Fig. 6. Relationship between moisture content (M) and relative spike dry weight (RSDW) of wheat and barley spikes during grain development. The fitted functions show the split-line regression analysis of this relationship for wheat ($R^2 = 64\%$, $n = 775$) and barley ($R^2 = 77\%$, $n = 1065$). The dashed line indicates the spike moisture content corresponding to physiological maturity of the pooled data (wheat 42.9 ± 0.7%; barley 49.8 ± 0.3%).