Quantifying progress through grain development in wheat and barley using spike moisture content

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Abstract
It would be useful to have a single, quantitative metric to determine how far a wheat or barley crop has progressed through grain development, and whether it has reached physiological maturity. Current development scales such as Zadoks' decimal code are highly subjective and qualitative and do not define the grain expansion, filling and ripening phases well. Alternatively, progress through grain development and the timing of physiological maturity can be determined using grain moisture content, but to date this has only been defined for individual grains and not for whole spikes. Using data from five wheat and five barley cultivars sampled over a broad range of flowering dates, we determined that the spike moisture content that indicates physiological maturity was 43% in wheat and 50% in barley. Grain or spike moisture content can be used to accurately determine the progress of a wheat or barley crop through grain development, from anthesis to harvest ripeness.

Keywords
Grain filling; physiological maturity; moisture dynamics.

Introduction
Accurate knowledge of the progression of grain development and the timing of physiological maturity in wheat and barley is critical for both researchers and grain growers. Physiological maturity refers to the cessation of dry matter accumulation in the grain; at this point, the grain has expanded to maximum size and completed grain filling, and maximum dry matter (*i.e.* yield) is achieved (Bewley and Black 1994; Calderini et al. 2000). Many of the published methods for estimating the timing of this stage are problematic because they rely on the subjective interpretation of qualitative morphological traits like 'hard dough' or 'yellow peduncle' (Zadoks et al. 1974; Bell and Fischer 1994).

The moisture content of grains is one method by which grain development in wheat and barley can be objectively and quantitatively estimated with much greater accuracy and precision. There is a universal relationship between grain moisture content and grain dry weight that applies to both wheat and barley, whereby all crops of a given species will reach physiological maturity at a similar grain moisture content (Slafer *et al.* 2014). Physiological maturity occurs at approximately 38% grain moisture content in wheat (Calderini et al. 2000; Alvarez Prado et al. 2013) and 48% grain moisture content in two-row barley (Alvarez Prado et al. 2013).

The moisture dynamics of whole intact spikes are known to follow a similar pattern to individual grains, but occur over a narrower range of moisture contents (Bingham et al. 2007). Measuring the moisture content of whole spikes is quicker and easier than measuring individual grains as it does not require a sample to be threshed (Menendez et al. 2019). For this reason, spike moisture content might serve as a more robust measure of grain development compared to the moisture content of individual grains. 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. This study is reported in full in Celestina et al. (2021).

Methods
This study was opportunistically undertaken on a subset of cultivars and sowing dates from one of the GRDC National Phenology Initiative field validation experiments. The field experiment was conducted in 2019 at the Melbourne Polytechnic Farm at Yan Yean (VIC) and was designed to monitor crop development in a diverse range of cultivars over a broad range of sowing dates. In the complete field experiment, there were 64 wheat (*Triticum aestivum*) and 32 barley (*Hordeum vulgare*) genotypes 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 in a partially replicated design (Cullis et al.

2006). Of the 96 genotypes sown in the complete field experiment, a subset of five wheat and five barley cultivars were selected for weekly sampling of spikes from anthesis to physiological maturity. These subset cultivars were selected to represent the diverse range of phenology types and development speeds in the complete experiment. The wheat cultivars were Emu Rock (spring, very quick-quick), Derrimut (spring, mid), Bolac (spring, slow), Whistler (winter, quick) and SQP Revenue (winter, slow). The barley cultivars were Rosalind (spring, very quick), Fleet (spring, quick), Westminster (spring, quick-mid), Oxford (spring, quick-mid) and Cassiopèe (winter, quick).

Rainfall and temperature at the field site was monitored from the first time of sowing to the date of final spike collection. Temperature data were used to calculate thermal time accumulation after anthesis using a base temperature of 9.2°C and maximum temperature of 35.4°C (Porter and Gawith 1999). Five representative spikes were collected from each plot every week from anthesis to harvest ripeness. The fresh weight (FW, g) of each spike was recorded and then spikes were oven dried at 70°C for 72 hours to determine dry weight (DW, g). Spike water content (WC, g) 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, %), 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. Fresh weight was modelled using a quadratic-by-quadratic function, dry weight and moisture were both modelled using a Gompertz function and water content was modelled using a Gaussian function. Maximum spike dry weight for each cultivar at physiological maturity ($MaxDW$, g) was estimated by fitting a split-line (broken stick) regression to the relationship between spike moisture (M) and dry weight (DW) and identifying the breakpoint at which dry weight reached a maximum. 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 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. All regression analyses and curve fitting were conducted in Genstat 20 (VSN International) and all figures were created in R (R Foundation for Statistical Computing).

Results and Discussion

Despite large differences in phenology, morphology and growing conditions, all wheat and barley cultivars 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 grains (Bewley and Black 1994; Slafer et al. 2014).

The pattern of grain development, visualized in terms of changes in spike fresh weight, dry weight and water content (Figure 1), was the same for wheat and barley. This pattern followed the characteristic three-phase model of grain expansion, filling and ripening described by Bewley and Black (1994): (1) an initial grain expansion period characterised by a rapid increase in spike water content and fresh weight; (2) a grain filling period that overlapped and followed grain expansion, characterised by an increase in dry weight and then cessation of dry weight gain; and (3) a period of maturation drying where water content rapidly declined. Spike fresh weight and water content peaked around 200-250°Cd after anthesis. Spike dry weight peaked at physiological maturity, 500-600°Cd after anthesis.

For both wheat and barley, the increase in spike dry weight over time corresponded to a sigmoidal decrease in spike moisture content. At anthesis, spike moisture content was 61-65% in wheat and 67-69% in barley. Spike moisture decreased during the grain expansion, filling and ripening phases to reach a minimum of around 5% once maturation drying was complete. Whole spikes of wheat and barley were observed to follow a similar pattern of development to individual grains, but over a narrower range of moisture contents.

By standardizing differences in final spike dry weight and expressing the relative spike dry weight as a function of moisture, the moisture content at which physiological maturity was reached could be estimated (Figure 2). Spike moisture content at physiological maturity was found to be 43% for wheat and 50% for barley. 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. This breakpoint defines the moisture content at physiological maturity; at moisture contents below this threshold the spike is still accumulating dry matter and has not yet reached physiological maturity.

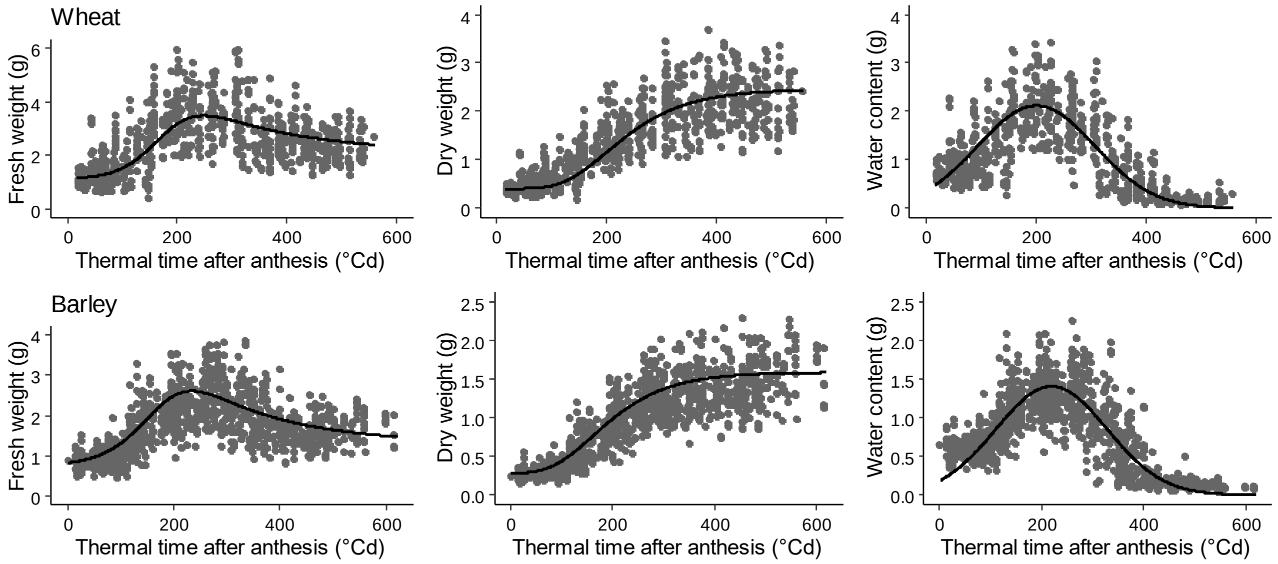

Figure 1. Pattern of grain development showing the changes in whole spike fresh weight (FW), dry weight (DW) and water content (WC) during the grain development phase from anthesis to harvest ripeness. Fitted quadratic-by-quadratic functions to FW over thermal time (R^2 wheat = 59.6%, R^2 barley = 60.0%), Gompertz functions to DW over thermal time (R^2 wheat = 78.4%, R^2 barley = 79.6%) and Gaussian functions to WC over thermal time (R^2 wheat = 74.9%, R^2 barley = 75.1%) are shown. Adapted from Celestina et al. (2021).

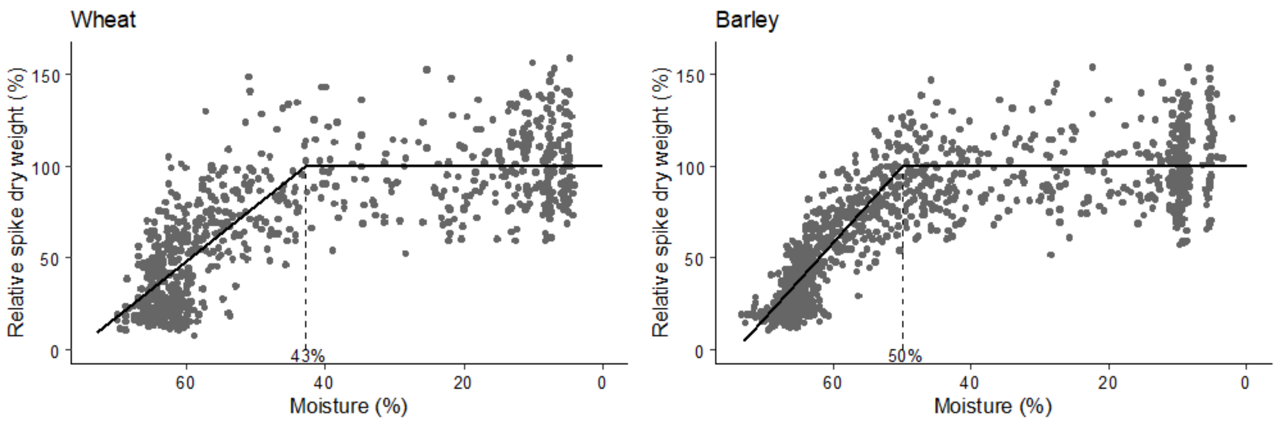

Figure 2. Relationship between moisture content (M) and relative spike dry weight (RSDW) of wheat and barley spikes during grain development. The fitted functions show the split-line regression analysis of this relationship for wheat (R^2 = 64.2%, n = 775) and barley (R^2 = 77.1%, n = 1065). The dashed line indicates the spike moisture content corresponding to physiological maturity of the pooled data (wheat 42.9 ± 0.7%; barley 49.8 ± 0.3%). Adapted from Celestina et al. (2021).

All five barley cultivars reached physiological maturity at approximately the same threshold moisture content of ~50% but there was some variation amongst the wheat cultivars, with Whistler reaching physiological maturity at a moisture content of 51%, much higher than the other four cultivars at ~43%. There was no indication why this was the case as Whistler was not the only winter wheat cultivar, nor did it appear to be due to obvious morphological differences (e.g. awned *vs* awnless, red *vs* white grain). These findings suggest that there may be within-species variation in moisture dynamics that were not captured in the present study.

Given that grain 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 grain or spike moisture (Table 1). The moisture content of a sample of threshed grain or whole spikes can be measured in the laboratory using the gravimetric method or in the field using a grain moisture meter. Note that the guide to grain development presented here is not applicable to plants that do not follow normal patterns of grain development, such as those affected by frost and heat stress.

Table 1. Guide to grain development stage of wheat and barley based on moisture content of individual threshed grains or whole spikes. Values for spike moisture content are from this study. Values for grain moisture content are from Alvarez Prado et al. (2013) and Calderini et al. (2000).

Stage of development	Moisture content (water concentration, %)			
	Wheat		Barley	
	Grain	Spike	Grain	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%

Conclusion

Progression through grain development and the timing of physiological maturity in wheat and barley can be determined in a repeatable, objective and quantitative manner using spike moisture content. Compared to individual grains, whole spikes are easier to sample and assess and have a smaller range of moisture contents between anthesis and physiological maturity. Grain development begins immediately after anthesis when the moisture content in whole spike is at 61-65% (wheat) or 67-69% (barley). Spike moisture content declines to 41-45% (wheat) or 49-51% (barley) at physiological maturity and <12.5% at harvest ripeness.

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