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Understanding the Components of Specific Weight in Barley Grains:
Opportunities for improving grain quality and processing efficiency

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Thesis submitted for the degree of Doctor of Philosophy
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Declaration of Authorship

1. I declare that the thesis has been composed by myself and that the work has not be submitted for any other degree or professional qualification. I confirm that the work submitted is my own, except where work which has formed part of jointly-authored publications has been included. My contribution and those of the other authors to this work have been explicitly indicated below. I confirm that appropriate credit has been given within this thesis where reference has been made to the work of others.

   The work presented in Chapter 2 was previously published in the *Journal for the Science of Food and Agriculture* as "Specific Weight of Barley Grains is Determined by Traits Affecting Packing Efficiency and by Grain Density" by Aaron Hoyle, Maree Brennan, Gail Jackson and Steve Hoad.

   The work presented in Chapter 3 was previously published in the *Journal of Cereal Science* as "Increased Grain Density of Spring Barley (*Hordeum vulgare* L.) is Associated with an Increase in Grain Nitrogen" by Aaron Hoyle, Maree Brennan, Gail Jackson and Steve Hoad.

   The work presented in Chapter 4 was previously submitted and is currently under-review with The Crop Journal titled "Specific Weight of Spring Barley is Maintained Under Post-anthesis Water Stress" by Aaron Hoyle, Maree Brennan, Logan Rees, Gail Jackson and Steve Hoad.

   The work presented in Chapter 5 was previously submitted and is currently with the editor with the *Journal of Cereal Science* titled "Relationship Between

Specific Weight of Spring Barley and Malt Quality" by Aaron Hoyle, Maree Brennan, Nicholas Pitts, Gail Jackson and Steve Hoad.

All of these studies were conceived by myself under supervision and with the feedback from my PhD supervisors. I defined the necessary hypotheses in each study, designed the experiments, carried out all experimental work, performed the data analyses and the interpretation of the results. Supervisors gave feedback on written work during supervisory team meetings.

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Signed: A. Hoyle

Date: 27/03/20

Abstract

Spring barley is the primary cereal crop grown in Scotland, 35% of the crop is used for malting and 55% for animal feed. There is a clear distinction between barley destined for malting or feed, this is a result of the higher quality grain demanded for malting and consequently a premium is paid for this. For example, in the UK during September 2018 malting barley reached prices of £46/t more than that of feed barley. Quality requirements for malting barley include: germination rate, per cent admixture, nitrogen levels, cultivar, moisture content, uniformity, skinning level, disease/weathering damage and specific weight (SW). Therefore different agronomic approaches are taken when a grower is striving for either malting or feed barley. The majority of these malting barley quality requirements are well understood, SW is well established however its impact on malting outputs or efficiency are not well understood. Specific weight is one of the longest standing measures of grain quality for cereals and oilseeds, it is a measure of the weight of grain per unit volume and is reported in kilograms per hectolitre (kg hl^{-1}). An increased SW is thought to be beneficial for malt output. The aim of this thesis is to enhance the understanding of SW as a measure of grain quality, and to establish what aspects of barley grain determine this measure. Following establishing these grain traits, the aim is then to relate these to the malting process and outputs, to understand how SW influences malting. Firstly, SW has been demonstrated to have two components: grain density and packing efficiency. This is a key part of the thesis, because both components can change independently. Different grain parameters influence each of the components, therefore both need to be considered together when investigating SW differences or similarities between samples. The packing efficiency and grain density of nine spring barley cultivars was investigated, this demonstrated that grain density contributed 48.5% to the variation in SW and packing efficiency 36.5% to the variation in SW. It

was hypothesised that the packing efficiency of grains was primarily influenced by grain morphometrics, and grain density influenced by composition. Investigating how composition changes with grain density was investigated by first stratifying grains by density, resulting in groups of grains with different densities. Compositional analyses were then carried out on these groups which showed that grain nitrogen level and the proportional volume of starch B-type granules contributed 47% to the observed variation in grain density. Specific weight is also known to be affected by growing conditions, with year to year variation observed. Such year-on-year variations might be a result of changing climatic conditions between years, therefore the effect of a moderate, but prolonged water stress was investigated under glasshouse conditions. Plant development was altered by the stress, but SW was maintained through compensatory mechanisms. To investigate how changes in SW affect malt quality parameters, SW was manipulated through selection for different grain size and weights. Specific weight was shown to be strongly correlated with the predicted spirit yield and hot water extract of the malt. These are two fundamental measures of malt quality. Grain density also correlated with these two measures, but packing efficiency of the grains did not. This indicates that it is grain density rather than the packing efficiency of the grain that is the beneficial component of SW for malting. Therefore if breeding of elite malting cultivars is continued to enhance malt quality through increasing SW, this should be done so through increasing the grain density component rather than packing efficiency.

Lay Summary

Barley is a common cereal crop, the grain produced by barley is a rich source of carbohydrates. Barley ranks fourth in terms of global cereal production behind wheat, maize and rice. It is the main crop grown in Scotland, and is of particular importance in Scotland because of its use as a raw material in the brewing and distilling supply chains. Prior to use in these industries, barley has to first undergo malting. Malting is the process by which cereal grains are germinated and dried to produce malt. Barley is most frequently malted cereal crop in the UK. This is integral to the brewing and distilling industries because during malting the physical structure of the grain is modified and an array of enzymes activated. These both allow the utilisation of the carbohydrates, specifically the conversion of starch into alcohol during brewing and distilling. In particular it is spring-sown barley which is used for malting, this is commonly referred to as spring barley. The reasons why spring barley is favoured for this process are due to its physical and biochemical characteristics. These characteristics benefit the malting process and lead to the production of a high quality malt product. The characteristics of the barley grain that are beneficial for malting are called 'grain quality traits'. One of these grain quality traits is the focus of this thesis, specific weight. Specific weight is the weight of grain in a given volume and is recorded in kilograms per hectolitre (kg hl^{-1}). Specific weight is the centre of this thesis because there is a lack in understanding of how variation in specific weight impacts upon the malting process as a whole and also the quality of the malt product. Maltsters (people who malt grain to produce malt) pay growers a premium for grain which attains pre-arranged target values for grain quality traits, specific weight being one of these. Therefore growers strive to grow barley with a high specific weight, and maltsters may pay more for this, without necessarily knowing the impact this will have upon malting. The overall aim of this thesis is to enhance the understanding of specific

weight as a grain quality measure. This will be addressed in terms of: what grain characters contribute to this measure and also how this measure influences the malting process. These have been addressed in four separate experimental chapters with the following titles:

i. Specific weight of barley grains is determined by traits affecting packing efficiency and by grain density

ii. Increased grain density of spring barley (*Hordeum vulgare* L.) is associated with an increase in grain nitrogen

iii. Specific weight of spring barley is maintained under post-anthesis water stress

iv. Relationship between specific weight of spring barley and malt quality

The first experimental chapter demonstrated that specific weight could be broken down into two different components: the packing efficiency of the grain and grain density. When the values of these two traits for a given sample are multiplied together, the resultant value is equal to the specific weight of the sample. The second experimental chapter further investigated one of these components, grain density. Through separating grains by ascending density, it was possible to observe how the composition of these grains changes with density. As density increased so did protein content and the volume of small starch B-type granules. In the third experimental chapter specific weight was maintained when barley plants were exposed to a water stress treatment. Despite the apparent static specific weight under this environmental stress many other developmental, plant and grain characteristics were altered. These included a reduced: ear length, ear number, yield, plant biomass and grain carbon to nitrogen ratio. The final experimental chapter used samples of varying specific weights and put them through a scaled down malting process. Specific weight correlated well with two measures of malt output: hot water extract and predicted spirit yield. Interestingly, it was the grain density component of specific weight that

correlated with these and not the packing efficiency component. This indicates that it is the grain density component of specific weight which is important in changing the malting process and product, rather than packing efficiency of the grain.

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Abbreviations

AHDB	Agriculture and Horticulture Development Board
ANOVA	Analysis of variance
C	Carbon
C:N	Carbon to Nitrogen ratio
DMS	Dimethyl Sulphide
DP	Diastatic power
EBC	European Brewery Convention
GD	Grain Density
ha	Hectare
HSD	Honestly Significant Difference
HWE	Hot water extract
kg hl^{-1}	Kilograms per hectolitre
LA	Litres of alcohol
LMM	Linear mixed-effects model
N	Nitrogen
NIR	Near-infrared spectroscopy
PC	Principal component
PCA	Principal component analysis
PE	Packing Efficiency
PSY	Predicted spirit yield
QTL	Quantitative trait locus
REML	Restricted Maximum likelihood
RL	Recommended List
RVA	Rapid Visco Analyser
SEM	Scanning electron microscope
SGD	Single Grain Density

SW   Specific Weight

v/v   Volume per volume

Wc   Number of whole corns

1 Chapter One

Introduction

Chapter 1. *Introduction*

1.1.1 Overview

Barley is the main cereal crop used in the malting industry. The quality of barley grain used for malting is of utmost importance, ensuring both an efficient malting process and a high quality product. Therefore prior to malting, barley grain is assessed for certain quality attributes which are indicative of future malt quality. One of these quality attributes is specific weight (SW); the weight of grain in a given volume. The underlying grain characteristics associated with this trait are not well understood. Detailed links between SW and the malting process have yet to be shown, however it is currently used as a quality attribute.

In this thesis there are six chapters in total, the work presented in which enhances the current understanding of SW in barley, and identifies links between SW and malting. This introductory chapter (Chapter 1) provides background and context for the entire thesis. The following four experimental chapters (Chapter 2 to 5) are from either: published, submitted or intended to submit research papers. The stage of publication for each chapter will be explicitly stated at the beginning of each chapter. The thesis will then be concluded with a final discussion chapter (Chapter 6), highlighting important results from the thesis and discussing these in context of the wider industry.

1.1.2 Background

Barley (*Hordeum vulgare* L.) has been an important cereal crop since its domestication 10,000 years ago (Badr et al., 2000). Barley ranks fourth globally in terms of both production quantity and land area, behind wheat, maize and rice (FAOSTAT, 2020). This equates to a global production of 142 million tonnes over 48 million hectares (FAOSTAT, 2020). The success of barley as a cereal crop is a result

of both its ability to be grown across a diverse range of environments, from 70ºN to 65ºS, and also its wide variety of uses (Schildbach, 1986). Its primary uses are for livestock feed and for malting, these account for roughly two thirds and one third of its usage, respectively. In addition to this, 2% of the global barley crop is grown for direct human consumption, with the majority of this consumption occurring in areas of Asia and North Africa (Baik and Ullrich, 2008). Barley can be subdivided into two types depending on its growing season. Winter barley is planted in autumn and harvested the subsequent summer, requiring a vernalization period. Spring barley is planted in spring and harvested the same summer. Winter barley is characteristically high-yielding, with a longer growing season in comparison to spring barley. In the UK, spring barley typically yields 20% less than winter barley (AHDB, 2015). In addition to winter and spring barley cultivar types, cultivars can also be either two-row or six-row. These differences between two-row and six-row cultivars arise as a result of spikelet fertility. In two-row barley only the central spikelet is fertile, whereas in six-row barley all three spikelets are fertile (AHDB, 2015).

1.1.3 Scottish Context

Barley and its downstream uses are of particular importance to Scotland. In 2019, spring barley accounted for 48% (241,000 ha) of the total cereal crop by area in Scotland, and winter barley for 12% (49,000 ha) (The Scottish Government, 2019). Since 2010, the production area, yield and consequently production quantity of spring barley in Scotland have been relatively stable (**Figure 1-1**). The prime arable land in Scotland is mainly situated on the eastern coast (**Figure 1-2**), with both reduced rainfall and increased hours of sunshine in comparison to the west. This makes eastern Scotland some of the highest value land for growing barley in the UK, in particular malting barley. Despite the yield differences between spring and winter

barley, spring barley is preferred for malting. This is because it is typically only spring barley cultivars that meet the stringent requirements for malting. The grains process well and lead to a more efficient malting process. Therefore, of the tonnage of barley bought in 2018 by the Scottish members of the Maltsters Association of Great Britain (MAGB), 96.4% of this was spring barley (www.ukmalt.com). However, there is one example of a winter barley which was particularly prominent in the malting industry during the 1970s, Maris Otter. Due to the higher yielding nature of winter barley, there is a potential to increase the malt output per hectare of barley grown. As a result of the need to strive for increased sustainability, there has been a recent resurgence of interest in breeding a winter barley of malting quality. Researchers have started to try to transfer malting quality attributes from spring to winter barley, in an attempt to reduce or even eliminate this gap in quality between the two types (Thomas and Impromalt Consortium, 2018).

Malt and associated products are used in the food industry, brewing sector and distilling sector. However it is the distilling sector that is of particular importance to Scotland. At the time of writing this thesis, there are currently 133 Scotch Whisky distilleries throughout the country, resulting in the highest concentration of whisky producers in the world (O'Connor, 2018; Scotch Whisky Association, 2019). Scotch whisky exports in 2019 were worth £4.9bn to the economy, and account for 70% of the total Scottish food and drinks exports (Scotch Whisky Association, 2019). It is not only these direct measures which are of value to the economy, the whisky industry also benefits tourism and supports jobs in related supply chains.
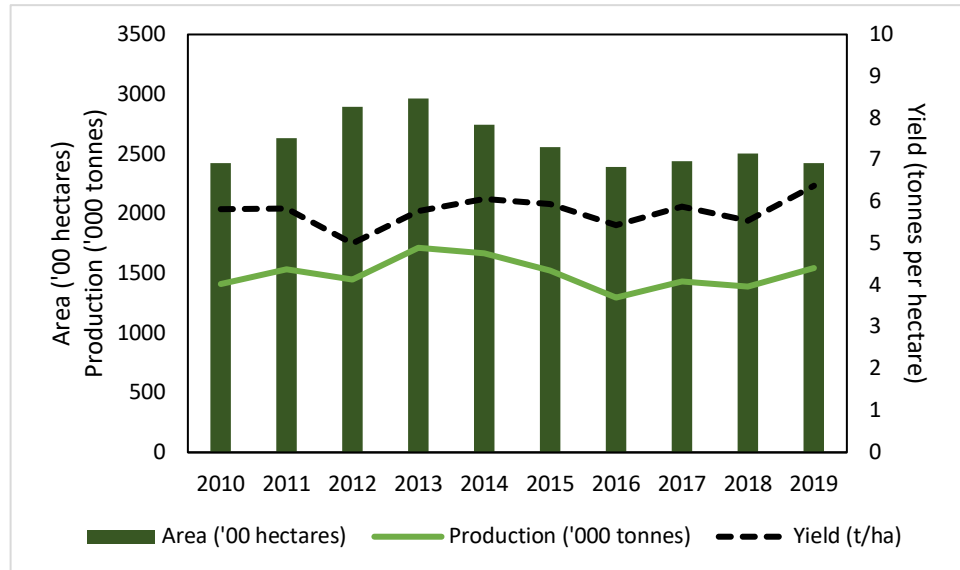
Figure 1-1 Spring barley growth area, production quantity and yield in Scotland from 2010 to 2019 (Scottish Government, 2019).

Land Capability for Agriculture in Scotland

- Arable
- Mixed agriculture
- Grassland
- Rough Grazing
- Built up areas
- Inland water

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Map produced by Steven Thomson
SRUC (2016)

Figure 1-2 Land use capability map developed by the Macaulay Institute which uses soil, climatic and other landscape factors to classify land into different categories depending on its potential productively and suitability for different crops (Birnie et al., 2010).

1.2 The Barley Grain

1.2.1 Barley Grain Anatomy

Throughout this section and the entire thesis, major grain tissues within the barley grain will be referred to, as different tissues have important roles in malting, therefore a schematic of a barley grain has been included for reference (**Figure 1-3**) (Gupta and Varshney, 2013).

Barley is harvested for its fruit, more commonly referred to as the caryopsis or grain. In this thesis it will be solely described as the grain. A typical barley grain is composed of an embryo, endosperm, nucellus, testa, pericarp, lemma and palea (Evers and Millar, 2002). The embryo and the endosperm are filial tissues making up the next generation. The remaining tissues are the maternal tissues which envelope the filial tissues. Early in the developmental stages of the grain the maternal tissues form the majority of the grain bulk. Then throughout development these are superseded by the filial tissues which comprise most of the grain weight in comparison to the negligible maternal tissues. The embryo consists of two parts, the embryonic axis and the scutellum. The embryonic axis is a filial tissue containing the shoot, mesocotyl and radicle. The scutellum is positioned between the embryonic axis and endosperm, and is involved in the secretion and absorption of both water and solutes during germination (Evers et al., 1999). This regulates the supply of nutrition to the embryonic axis (Evers et al., 1999). The largest tissue within barley grains is the endosperm, most of which is the starchy endosperm, the main storage tissue of the grain. The main constituents of the starchy endosperm are starch and protein. The second part of the endosperm is the aleurone, a layer which surrounds the starchy endosperm consisting of between two and four thickly walled cells; it is typically three cells thick.

This layer is high in proteins and lipids, and is responsible for the production of endosperm cells. It also plays an important role in germination through the secretion of hydrolytic enzymes which solubilise carbohydrate reserves within the starchy endosperm (Evers et al., 1999). Adjacent to the aleurone layer is the nucellus, a maternal tissue within which the endosperm and embryo developed (Evers and Millar, 2002). The testa is the true seed coat and is composed of a single layer of cells, which is enclosed by an outer cuticle. Grain tissues which are outside of the testa are therefore no longer part of the seed, but part of the fruit. The pericarp originates from ovary walls which have ripened. Finally, in typical barley (with a husk) two further layers are adhered to the grain, the lemma and palea, these act to protect the grain.

1.2.2 Barley Grain Composition

The endosperm is the grain tissue responsible for starch storage, and is composed of a cell wall-protein matrix with semi-crystalline starch granules embedded within the cells (Chandra et al., 1999). Starch is the most abundant constituent of barley grains, accounting for roughly 60% of grain weight (Fox, 2010). Starch is composed of two polysaccharides amylose and amylopectin (James et al., 2003). Amylose is composed of a chain of α-glucose units, which are primarily unbranched, amylopectin is also composed of α-glucose units, but these are highly branched. Barley starch is stored within the endosperm in granules which come in two distinct forms; A-type and B-type starch granules (**Figure 1-4**). Although some studies suggested that there are three forms of granules, small, medium and large it is largely agreed that in barley there are only two distinct types (Takeda et al., 1999). Despite this disagreement, it is well established that larger granules (A-type) are initiated earlier in grain development and have a higher proportion of amylose than later developing, smaller (B-type) granules (Takeda et al., 1999). The matrix within which these granules are embedded,

can either be densely or loosely packed, leading to grains with either a 'steely' or 'mealy' texture respectively (Chandra et al., 1999). Differences in endosperm texture as a result of this influences the downstream processing of barley. Barley endosperm cell walls are primarily composed of β-glucans (75%) and arabinoxylans (20%) (Fox, 2010). The protein content of barley is typically between 8 and 13%, with different requirements within this range for different end-uses (Fox, 2010). In brewing a protein content of between 10% and 10.9% crude protein is typically demanded, whereas in distilling a lower range of 9.4% to 10.3% crude protein is demanded (MAGB, 2020). Protein content is important for these end users because it is the valuable starch granules that are embedded within the matrix, and there is typically an inverse relationship between starch content and protein content. The main proteins in barley grains are hordeins, but albumins, globulins and glutelins also contribute to the overall protein content (Fox, 2010). Additional minor constituents of barley grains include lipids, which are between 2% and 4% of the total grain weight. These are present in the forms of nonpolar lipids, glycolipids and phospholipids (Shewry and Ullrich, 2014).
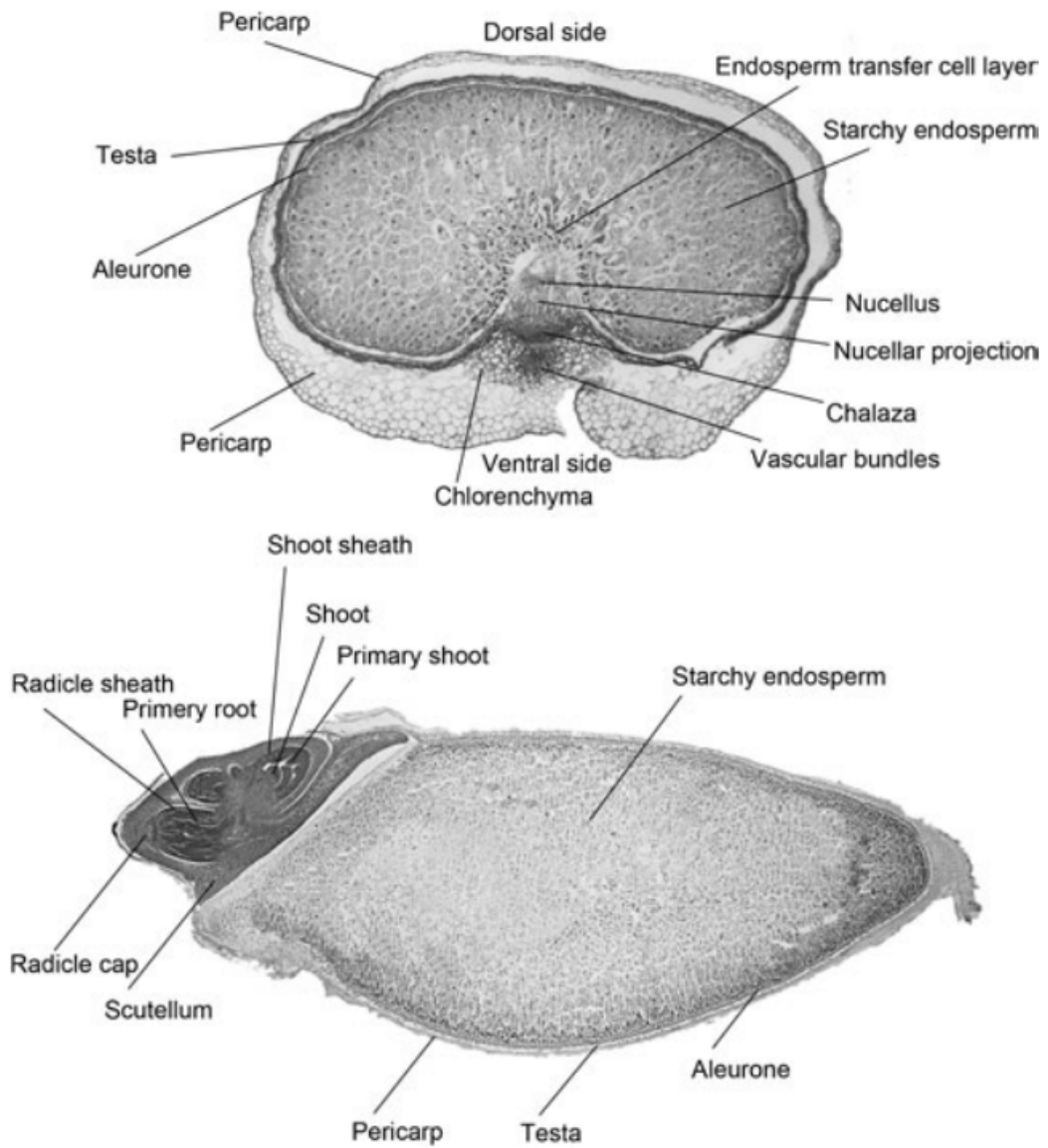
Figure 1-3 Diagrams of transverse (top) and longitudinal (bottom) sections of a typical barley grain, with the major grain tissues labelled (Gupta and Varshney, 2013).
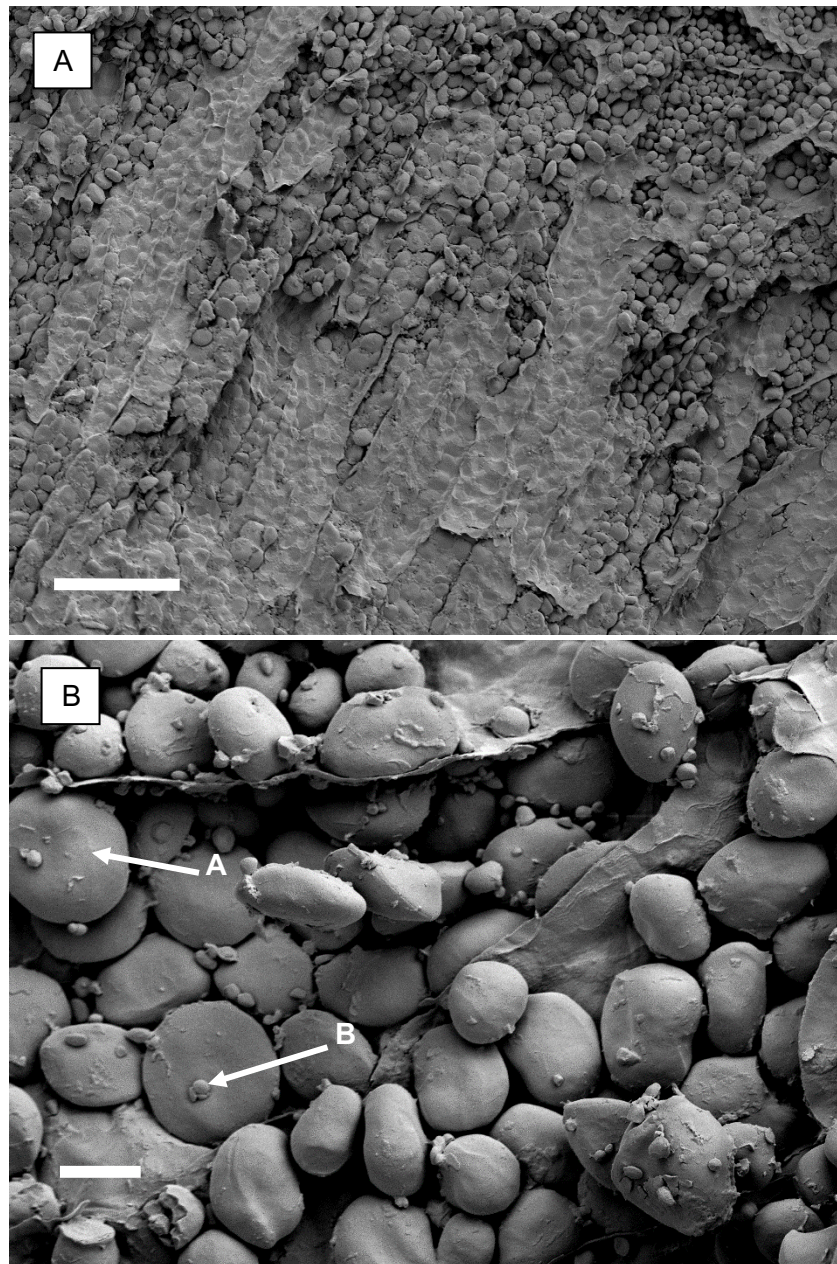
Figure 1-4 Scanning electron micrographs of the barley endosperm taken as part of work in Chapter 3 from the cultivar Laureate at two different scales, demonstrating the presence of starch granules within endosperm cells. In **B** larger A-type granules and smaller B-type granules are labelled. Scale bar for **A** is 100µm and for **B** is 10µm.

1.3 Grain and Malt Quality

In the UK, barley is the main crop used for malting, with both its biochemical composition and physical characteristics contributing to a desirable malt. The AHDB's (Agriculture and Horticulture Developmental Board's) RL (Recommended List) for cereals and oilseeds provides crop and cultivar specific information for market options (feed, brewing and distilling), yield, agronomy, grain quality and disease resistance. The above information in the yearly updated RLs aid the decision making procedure undertaken by growers and maltsters in the selection of cultivars to sow. Appropriate cultivar selection helps to ensure the grain harvest will be acceptable for the intended end market. In the malting industry, barley cultivars are selected on additional 'grain quality' traits which influence the malting process. The grain quality traits that are listed in AHDB's RLs are: screenings (a measure of broken or poorly filled grains), specific weight (SW) and nitrogen content. The RL does not contain an exhaustive list of grain quality characteristics, there are many more grain quality characteristics that contribute to malt quality and malting efficiency (**Table 1-1**).

In both the literature and industry the term 'malting quality' groups factors that influence the efficiency of the malting process, output and the quality of the end product. Therefore it is often confused what factors or traits lead to either a high quality product, an efficient malting process or high malt output. Here malting quality has been split into i) Grain traits that influence quality and efficiency in **Table 1-1** and ii) Malt quality parameters that can be used to define the quality of the malt product in **Table 1-2**. The target values for the parameters listed in **Table 1-2** will vary depending on the product being made, however these are the target values for malt used in the brewing industry. Knowledge of how grain traits relate to malting efficiency and output

is essential, so maltsters can make informed decisions about the cultivars they

demand from growers.

Table 1-1 Factors affecting malt quality and their desirable levels, information collected from a range of sources.

Grain Traits	Desirable Level	References
Grain Condition	No frost or heat damage	(Brewing and Malting Barley Research Institute (BMBRI), 2010)
	Bright grains, free from disease	(Gupta et al., 2010; Martin, 2015)
	No admixture of weeds/insects/chemicals	(Martin, 2015)
	No tolerance of mycotoxins e.g deoxynivalenol (DON)	(Brewing and Malting Barley Research Institute (BMBRI), 2010; Martin, 2015; Nielsen et al., 2014)
	Pure batch of one cultivar	(Brewing and Malting Barley Research Institute (BMBRI), 2010)
	<5% of skinned/broken grains	(Brewing and Malting Barley Research Institute (BMBRI), 2010)
Grain Size	90% retention through 2.5mm Screening	MAGB
	Favour high SW	AHDB RLs
	Favour uniform grains (homogeneity)	(Wade and Froment, 2003)
Germination	No pre-harvest germination	(Martin, 2015)
	Fully mature grains	(Brewing and Malting Barley Research Institute (BMBRI), 2010; Martin, 2015)
	>98% germinative energy	MAGB
Moisture Content	<13.5%	(Brewing and Malting Barley Research Institute (BMBRI), 2010)
Composition	Protein Content 11-12.5% (dry basis)	(Brewing and Malting Barley Research Institute (BMBRI), 2010)

Table 1-2 Malt quality parameters and target values for good brewing malt, where not stated values are on a dry weight basis (adapted from Verstegen et al. 2014; Brennan et al. 1997).

Malt quality parameter	Target values
Protein content	<10.8%
Kolbach Index, or soluble protein	38% to 42%
Hot water extract	305-315 L°/kg
Extract difference	1.2% to 1.8%
Viscosity	<1.55 mPa s
β-Glucan	<300 mg/l
Wort colour	<3.4 EBC (European Brewery Convention)
Boiled wort colour	<5.0 EBC
Soluble Nitrogen (dry matter)	>0.65g/100 g MTrS
Friability	>87%
Viscosity 65 °C	<1.65 mPa s
β-Glucan 76 °C	<400 mg/l
DMS-P (Dimethyl sulphide)	<6 ppm

1.4 Malting

Malting is the controlled germination of cereal grains. It has been suggested that malting is the oldest biotechnology in the world. Since the cultivation of barley, accidental germination probably lead to noticeable flavour changes in products made from the grain. This would have led to the deliberate germination of cereal grains, then ancient methods of producing bread and beer (Briggs, 1998). Malting is batch process, meaning the product is not produced continuously. Malting broadly occurs in three stages: i) steeping, ii) germination and iii) kilning. Steeping involves the soaking of grains in water to increase their moisture content from <12% to >40% (Gupta et al., 2010). In a maltings the temperature of the steep water is often controlled because this will have knock on effects on germination time. The steep water becomes dirty and is changed at least once during a steep, therefore this initial stage in malting can be a very water intensive part of the malting process. In striving to reduce the environmental impacts of the high water use of this step, some maltings filter and re-use steep water. The whole steeping process takes between 48 and 72 hours. Water uptake by the grain is affected by endosperm structure. Grains with a less dense matrix or 'mealy' texture are likely to have more uniform uptake of water during the steeping process and later movement of enzymes (Gupta et al., 2010).

Once barley grains have imbibed enough water to increase the moisture content to >44%, steeping is complete. The water is drained and the grain enters the next stage of the process, germination. This can either be in: a different vessel, the same vessel or traditionally the grain was spread across a maltings floor. In either of these methods germination is triggered and there is a cascade of biochemical changes within the grain. Hydrolytic enzymes such as α-amylase, which are produced in the scutellum and aleurone layer begin to degrade components of the endosperm (Briggs, 1998).

This results in the hydrolysis of cell walls by β-glucanases and pentosanases, weakening of the endosperm structure. Consequently previously bound starch granules are released from the matrix (Gupta et al., 2010). The combination of these changes to the grain are termed 'modification'. Both the accumulation of enzymes and modification of the endosperm are crucial to produce a good quality malt. However, during the natural process of uncontrolled germination, the plant would further degrade the starch and use the resultant sugars to begin growth. Therefore the degree of modification and accumulation of enzymes needs to be balanced with embryo growth. Otherwise the sugars that are required to make the downstream products (e.g. beer, whisky, malt extract) from malt will be metabolised by the embryo, equating to malting losses. When fermentable sugars are lost as a result of this, the endosperm has undergone 'overmodification'. Consequently germination is arrested before full degradation and excessive embryo growth occurs, by the final stage of the process, kilning. The time taken for the required level of germination in a maltings can vary between 84 and 144 hours.

After germination is judged to be complete by the maltster the final stage of malting is started, kilning. Kilning dries the grains and stabilises the changes in biochemistry so all of the necessary starch degrading enzymes are still present in the resulting malt, the product of the malting process (Gupta et al., 2010). Kilning regimes can vary depending on the type of malt being produced. Kilning can produce large differences in characteristics of malt, from pale lager malts through to darker ale malts. Colour and flavour can be further enhanced by roasting malts, this is typical for caramel or chocolate malts. However, it is integral for many types of malt that the initial kilning temperature is not too high, as this causes enzyme denaturation while the moisture content of the malt is still relatively high. These enzymes are then utilised in the aforementioned downstream uses. Upon rehydration and elevated temperatures the

enzymatic degradation of starch through amylase activities continues, producing fermentable carbohydrates (Gupta et al., 2010). This highlights the need for only this partial degradation of starch and the maintenance of starch degrading enzyme integrity during the malting process. Kilning is the most energy intensive part of malting. Maltings have reduced energy consumption through heat recovery and the introduction of continuous kilning. However there is a lot more scope for decreasing the environmental impact of the malting industry.

1.5 Specific Weight

1.5.1 Definition and Applications

Specific weight is defined as the mass of grain per unit volume and is measured in kilograms/hectolitre (kg hl^{-1}). Specific weight describes the bulk density of grain and is thought to be primarily determined by: grain weight, grain density (GD) and packing efficiency (PE) of a bulk (Clarke et al., 2004). However, on a finer scale these are in turn thought to be influenced by the following grain traits: size, morphology, compaction, composition, surface friction and moisture content. These traits are influenced by both genotype and environment (Pushman and Bingham, 1975). Specific weight is a measurement used on all cereal grains; however with the different end uses of grain it has more relevance for certain processes. For example, SW has come under criticism in terms of its use for valuing animal feed (McCracken et al., 2002). This study demonstrated that there was no relationship between the SW of wheat grain and the feed value for poultry. However, SW has applications in the transportation and storage of grain around the world, describing the mass of grain that can be transported in a given container (Grain Trade Australia, 2013).

Chapter 1. *Introduction*

The terminology surrounding SW is inconsistent in both the literature and industry, where it can be referred to as 'grain density', 'test weight', 'bushel weight', 'hectolitre mass' or 'hectolitre weight' (Manley et al., 2009). Throughout this thesis and all subsequent work this grain quality measurement shall be referred to as SW. In addition to this, units for measuring SW and techniques often vary, further complicating this measurement (Wychowaniec et al., 2013). The typical piece of equipment used to measure SW is a chondrometer. "Chondro-" originates from the Greek word 'khondros' meaning grain, and "-ometer" is an instrument used in measuring something. Hence, a chondrometer is an instrument used for measuring grain. In the UK, this consists of two stacked cylinders separated by a sliding gate. The upper cylinder is filled with grain, the separating gate is withdrawn and re-inserted once the grain has fallen into the bottom portion. The grain in the lower cylinder, of known volume (500 ml), is weighed and from this SW in kg hl^{-1} is calculated. However the exact apparatuses of this equipment vary from country to country. Some use funnels of differing diameters to pour the grain; others use a collection cylinder of varying shapes and sizes. It has been demonstrated that the use of these different techniques and equipment across different countries leads to different SW values (Manley et al., 2009). Furthermore, when different personnel use the equipment, different results can be obtained (Manley et al., 2009). This highlights the need for an increased awareness of this variation. In this work, only one scaled-down version of a chondrometer was used, with only one operator, which allowed work on smaller grain samples, and ensured consistency between measurements. The absolute values obtained are therefore not directly comparable with the industry standard. However this method will allow comparisons to be made between samples when this method is used to estimate SW.

1.5.2   Specific Weight and Malting

At present SW is considered a desirable characteristic of barley cultivars approved for malting. Hypothetically, SW could play a role in the amount of extract produced per batch and consequently efficiency. If grains of a high SW are purchased; an increased weight of grains could be included in the malting vessels, increasing the output per processed batch.

The link between SW and malt quality parameters in **Table 1-2** has yet to be made. In particular, any quantitative relationship between SW and hot water extract (HWE) or predicted spirit yield (PSY), the main predictors of malt output used in industry, remains to be shown. Specific weight has been included as a grain quality characteristic in AHDB's RLs without the necessary evidence to support that this is indicative of malt output. Therefore SW has been included in **Table 1-1** without the knowledge of what SW is beneficial for in terms of either malting efficiency or quality. Work in this thesis addressed this link between SW and malt quality parameters. Its inclusion may be a result of it being one of the longest standing measures of grain quality and the simplicity and speed in measuring it.

The literature is vague when describing links between SW and potential malting benefits. Often grains with a high SW are thought to be plumper and therefore have a larger proportion of endosperm, particularly the starchy endosperm (Dimmock and Gooding, 2002), resulting in more starch available for hydrolysis to maltose. However, Yu et al. (2017) has recently shown that a high SW does not result in increased starch content in barley grains. Therefore it is important to know if an increased SW is due to a change in the PE of grains, grain composition or a combination of both.

If SW is altered by changing the proportion of the protein matrix in the starchy endosperm, is SW a good measure of malt quality? If a higher proportion/density of endosperm protein increases SW, a lower SW may result in more efficient malting and higher quality malt. The ranges of acceptable levels of protein have been previously mentioned. High protein contents can lead to a slow rate of endosperm modification during malting and also a reduced extract yield from the malt produced (Agu, 2003). Not only does this reduce the output of this batch of malt, but because of the slower rate of germination, the next batch of malt will be delayed. Therefore the throughput of batches of malt would be reduced in the maltings. However, if a lower protein content increases SW the opposite may occur with enhanced levels of modification, increased extract yield and higher throughput. This lack of knowledge of which grain traits contribute to SW needs to be addressed, to be confident that SW is a relevant quality indicator for malting, and for which aspects of malting.

As mentioned previously, grain with a high SW is thought to be associated with well-filled plump grain (Gooding et al., 2003). However, the precise ways in which grain morphology and composition contribute to SW are not fully understood. Furthermore variation in SW is observed among barley cultivars and between growing locations, demonstrating SW is influenced by both the genotype and the environment (AHDB, RL harvest data 2016). This highlights another avenue of interest, how the environment influences grain traits and consequently SW. The environmental conditions during the growth of barley are known to influence both composition and morphology. Starch biosynthesis under different environmental stressors has been widely studied. The effect of an environmental stress on starch biosynthesis and accumulation is dependent upon the severity of the stress, timing and duration of the stress, and also the sensitivity of the genotype to the stress (Thitisaksakul et al., 2012). Globally, water stress is the most common stress, responsible for most of the

observed reductions in yield, with starch content correlating well with yield (Worch et al., 2011). Alongside yield impacts, water stress is also known to affect the malt quality of barley grain changing composition (β-glucan) and enzyme activity (β-amylase) (Wu et al., 2017).

In addition, the use of SW as an indicator of potential malt quality needs to be tested to determine the effect of different SWs on the malting process. One of the difficulties in testing this is that SW is thought to be influenced by numerous grain characteristics simultaneously. A mixture of experimental and statistical work will aim to quantify the key contributors to SW, and examine which are likely to impact on malt quality. This thesis will enhance the understanding of whether or not SW is an important grain trait to measure when evaluating the malting potential of spring barley cultivars. This aims to provide the malting industry with a clear description of the contributing factors to SW and the consequences which these may have for both malt quality and efficiency.

1.6 Thesis Outline

The overarching goals of this thesis, which will be further dissected into more detailed aims and hypotheses in the following experimental chapters, are as follows:

1. Describe how grain packing efficiency and grain density contribute to SW (Chapter 2)
2. Investigate associations between grain composition and the grain density component of SW (Chapter 3)
3. Study the effect of a water stress treatment on SW through examining the components of SW and plant development (Chapter 4)

4. Explore the effects of a changing SW as a result of manipulated grain size and weight on malting quality

In Chapter 2 "grain dimensions, weight, volume and two-dimensional area of 100 individual grains of nine cultivars [were measured] to develop a detailed grain-level understanding of cultivars with a range of SWs". This described the contribution of grain packing efficiency and grain density to SW. Through detailed grain and bulk analysis, it was shown that SW is the product of grain density and packing efficiency. The findings of this chapter provide the basis for all following chapters. It highlights that in all future work on SW (in all cereal species), both components grain density and packing efficiency should be taken into consideration.

Chapter 3 builds on Chapter 2 by further investigating the components of SW. In this chapter the grain density component of SW which was not dissected in the previous chapter is examined. It was hypothesised that unlike packing efficiency, which is influenced by grain morphology, grain density will be related to the composition of the barley grain. This was investigated by addressing the three following aims:

1. "examine the correlations between quantitative changes in grain composition and single grain density"
2. "build an equation to predict single grain density from grain composition to understand the contributions of compositional aspects to single grain density"
3. "test the accuracy and efficacy of the equation using an independent grain sample"

Analysis of the compositional changes across a range in grain densities related single grain density within a cultivar to the composition of these grains. Grain density and composition in barley have not been linked before. This chapter therefore demonstrated real novel progression in enhancing the understanding of SW in barley.

Alongside variation in SW as a result of different genotypes, environmental growing conditions are known to influence SW. Therefore Chapter 4 aims to further develop on this aspect of SW, whereas Chapters 2 and 3 did not. Chapter 4 specifically focuses on the effect of a prolonged, but moderate water stress on three spring barley cultivars with varying SWs. This was examined using the following three objectives:

1. "establish how water stress modified plant development, yield components and grain composition that impact on SW"

2. "evaluate changes in SW according to its components and traits which affect them"

3. "investigate associations between grain parameters and the components of SW"

In this glasshouse study SW was not influenced by the water stress treatment. However plant development was significantly affected as well as other grain attributes which could impact upon the malting process.

Chapter 5 utilises information gained across the previous three experimental chapters, particularly on the components of SW and what influences these. This chapter investigates links between SW and the malting process, in terms of either efficiency or output. To study how SW and its components; grain density and packing efficiency effect malting three aims were addressed:

1. "alter SW and its components through the manipulation of grain size and grain weight"

2. "determine the malting quality of grain samples with different SWs and/or components"

3. "examine correlations between grain parameters and malt quality parameters to establish links between SW and malt quality"

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2 Chapter Two

Specific Weight of Barley Grains is Determined by Traits Affecting Packing Efficiency and by Grain Density

Specific Weight of Barley Grains is Determined by Traits Affecting Packing Efficiency and by Grain Density

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Chapter 2. *Specific Weight of Barley Grains is Determined by Traits Affecting Packing Efficiency and by Grain Density*

Abstract

Background

Specific weight influences the market value of barley grain, and in malting barley a high specific weight is thought to result in an increased malt output. However, links between specific weight and malt output have not yet been established. I hypothesised that packing efficiency and grain density will each contribute to specific weight. These traits would have implications for the malting process, highlighting the need for understanding what grain traits contribute to specific weight, before I can predict its effect on malting performance and efficiency.

Results

We report that specific weight is a product of grain density and packing efficiency, in our study proportionally contributing 48.5% and 36.5% to variation in specific weight, respectively. We report that packing efficiency is determined by grain dimensions, and is negatively correlated with the sum of grain length and depth. Therefore shorter, thinner grains can result in an increased specific weight, which is likely to be detrimental for malting performance. We also demonstrate that among cultivars which have grains with contrasting size traits, the same specific weight can be achieved through differing grain densities.

Conclusions

Our results demonstrate that both grain dimensions and grain density must be considered jointly to optimise specific weight, and that the relationship between specific weight and malting performance and efficiency needs to be carefully considered with respect to how a high specific weight is achieved.

Keywords: *Hordeum vulgare*, Grain quality, Malting barley, Grain dimensions

Chapter 2. *Specific Weight of Barley Grains is Determined by Traits Affecting Packing Efficiency and by Grain Density*

2.1 Introduction

Specific weight (SW) is a measure of the weight of grain per unit volume and is used as a grain quality criterion for major cereals and oilseeds. Confusion can arise from the use of inconsistent terminology surrounding this criterion in the literature. 'Test weight', 'grain density', 'bushel weight', 'hectolitre mass, 'hectolitre weight' and 'bulk density' have all been used to describe this criterion. The traditional industry standard for measuring SW is using a chondrometer, which consists of two stacked cylinders separated by a sliding gate. The upper cylinder is filled with grain, the gate withdrawn and re-inserted once the grain has fallen. The grain in the lower cylinder of known volume is weighed and used to calculate SW in kilograms per hectolitre (kg hl^{-1}). Additional industry standards used to measure SW include a Dickey-John analyser or prediction using near-infrared spectroscopy (UK National Trials, 2018).

In barley (*Hordeum vulgare* L.) SW influences the price of grain for both the feed and malting industries. Malting is the process of controlled grain germination in order to make the starch stored within the endosperm available for later enzymatic hydrolysis to maltose and glucose (Brennan et al., 1997). In the UK, spring barley is the main crop used for malting, as the grains have a high proportion of starchy endosperm and are therefore ideal for securing a good malt yield. The malt industry demands grain of a high SW, as it is assumed that a bulk of grain with high SW will contain a high proportion of endosperm biomass (Bayles et al., 1978). Grain 'plumpness' is one trait that is believed to positively contribute to SW and also benefit the malting process resulting in good extract levels due to higher levels of starch in the endosperm (Dimmock and Gooding, 2002; Edney et al., 2005). However a recent study showed that there is no significant correlation between starch content and SW in barley grains (Yu et al., 2017). Grain bulks with a low SW incur penalties from industry and in

extreme cases can even lead to rejections at a maltings. However correlations between barley SW and hot water extract, the main predictor of malt yield used in industry, have yet to be shown.

The very definition of SW indicates that it will be influenced by grain weight, and how well the grains pack into a volume. Indeed, dividing a sample's specific weight by grain density has previously been used to estimate the packing efficiency (PE) in cereal grains (Doehlert and McMullen, 2008; Pushman and Bingham, 1975). This relationship between SW, grain density and PE has not been applied to barley grains to the same extent as it has to oats and wheat. Determining that this relationship holds true among cultivars of spring barley would allow the examination of how each of the components, PE and density, contribute to SW differences among genotypes. This would be valuable information for barley breeders as SW is an important breeding target for malting barley. The ability to define SW by these two components will allow each one to be investigated individually not only to enhance our understanding of the formation of SW, but to assess their impact on malting performance.

It is clear there is a knowledge gap in identifying what attributes of spring barley grains influence SW. This needs to be addressed prior to investigating the effect of grain attributes on the malting process and product. In this study, we measured grain dimensions, weight, volume and two-dimensional area of 100 individual grains of nine cultivars to develop a detailed grain-level understanding of cultivars with a range of SWs. Grain size was manipulated through sieving altering SW, grain density and PE were calculated to determine how these contribute to the SW of barley grains. Correlations among all measured grain traits were also examined to understand links among traits and between them and SW.

2.2 Methods

2.2.1 Grain Samples

Nine spring barley malting cultivars from the Agriculture and Horticulture Development Board's (AHDB's) Recommended List (RL) 2016/17 were used in this study: KWS Irina, Octavia, Odyssey, Laureate, Origin, Concerto, Olympus, Propino and Sienna (https://cereals.ahdb.org.uk). These cultivars were chosen due to their phenotypic range in SW and varying levels of screenings, according to AHDB's RL 2016/17. The purpose of including multiple cultivars with a range of SWs was to extend the phenotypic variation in SW and its components, in order to better characterise relationships among SW and grain characteristics. All grain samples were grown in Docking, Norfolk under natural rainfall conditions during the 2016 season for the AHDB's RL crop trials. Prior to analysis grain was stored in cloth bags at ambient temperature and humidity, samples were then cleaned by shaking over a 2.50 mm slotted sieve, with 19.05 mm long slots for 20 seconds. Grain retained by the sieve was used for analysis.

2.2.2 Specific Weight

To achieve a detailed grain-level analysis of how differently shaped grains pack within a volume, and influence SW, it is necessary to have a scaled-down procedure for measuring SW which corresponds to the industry standard measurements, similar to that described by Gooding et al. (2003). Therefore, an accurate scaled-down method for measuring SW was developed in this study. Grain was poured from a height of 2 cm into a 25 ml measuring cylinder until it overflowed and superficial grains were removed by striking across the top of the cylinder with a straight edge. The total volume of the cylinder (39.16 ml) was obtained by weighing the amount of water

required to fill the cylinder (Kern analytical balance PLJ 750-3N, accuracy ± 0.01 g). The weight of grain in the cylinder was divided by cylinder volume and multiplied by 100 to give an estimate of SW in kg hl^{-1}. The results from this scaled-down method were highly correlated with an industry standard measurement of SW in a trial ($r^2 =$ 0.84, P < 0.001). This technique of estimating SW is similar to that described by Gooding et al. (2003) and Walker and Panozzo (Walker and Panozzo, 2011).

2.2.3 Representative Sampling

Grain samples (350 g) were sieved sequentially into the following size fractions using a stack of slotted 3.25, 3.00, 2.75 mm sieves, with 19.05 mm long slots: large (>3.25 mm), medium (3.25 to 3.00 mm), small (3.00 to 2.75 mm) and very small (<2.75 mm). The weight of grain in each fraction was recorded (Kern analytical balance PLJ 3500-2NM, accuracy ± 0.01 g) and where the fraction size was greater than 25 g SW was measured in triplicate using the scaled-down SW measurement described above. A 100 grain sample was taken from each fraction, and the mean grain weight from each fraction was used to estimate the total grain number in each size fraction and in the whole sample. A number of grains proportional to the total number of grains from each fraction were chosen at random, to give a 100-grain sample that was representative of the grain size distribution within the larger bulk sample.

2.2.4 Grain Size Parameters and Image Analysis

On the representatively sampled 100 grains from each of the nine cultivars the following measurements were taken. The grain dimensions length (L), width (W) and depth (D) were measured (see Appendix **Figure A-1**) using a hand-held digital caliper (accuracy ± 0.01 mm). These dimensions were used to calculate grain sphericity which was calculated as the cube root of L × W × D divided by L (Coşkuner and

Karababa, 2007). This value was multiplied by 100 to give a percentage, with a value of 100% representing a sphere. The two-dimensional (2-D) area of grains was measured using ImageJ (National Institutes of Health, USA, https://imagej.nih.gov/ij/). All of these measures describe grain "size", which in this study refers solely to physical dimensions of the grain, whereas "weight" refers to mass. Individual grain area density is a measure of the mass per unit area (mg mm^{-2}), a combination of size and weight, and was calculated by dividing grain weight by 2-D area.

2.2.5 Packing Efficiency and Grain Density

Grain volume and density were measured on the same 100-grains as above. Grain volume was measured by water displacement, with the weight of water displaced being equal to the volume of the grain (Archimedes' Principle) (Hughes, 2005). Grains were individually weighed using a Mettler AE 160 electronic balance (Mettler, Toledo, accuracy ± 0.0001 g) then submerged using a 0.5 mm x 25 mm hypodermic needle (BD Microlance) into a beaker of water using the same balance. The weight was recorded after 5 seconds and the grain immediately patted dry with paper towel. Grain density (g cm^{-3}) was calculated by dividing the grain mass by grain volume. Packing efficiency was defined as the proportion of space occupied by the grain in the 25 ml cylinder above, and was calculated by multiplying mean grain volume by the mean grain number in the cylinder, divided by the cylinder volume. Mean grain number was calculated from three cylinder re-fills.

2.2.6 Data Analysis

All data analysis was carried out using R software version 3.4.1 (R Core Team, 2017). An analysis of variance (α = 0.05) was done to determine whether the choice of

different cultivars was successful in achieving significant differences in measured grain traits, thereby extending the phenotypic range within the analysed samples. Cultivar was found to be a significant factor in all grain traits apart from volume. *Post-hoc* Tukey's Honestly Significant Difference ($\alpha = 0.05$) tests were done to determine which cultivars were significantly different from each other to gain insight into whether differences in grain traits among samples corresponded with sample differences in SW. For sequential sieve analysis the effect of fraction size and cultivar among SW samples was analysed using a multiple linear model. Calculation of 95% confidence intervals using the 'emmeans' package was used to compare the SW between grain fractions both within and between cultivars (Lenth, 2018). The effect of the product of PE and grain density on SW among the three replicated samples measured was analysed using a simple linear regression. For this model the y-intercept was removed as it can be assumed that when SW is equal to zero the product of PE and grain density is also zero. A two-way ANOVA was done with SW as the dependent variable and PE and grain density as the two independent variables. To determine the relative contribution of both PE and density to the variance in SW the proportion of the sums of squares (SS) for each variable to total SS was calculated. Principal component analysis (PCA) was carried out using mean individual grain dimensions (L, W and D), plots of scores were created to investigate grain shape among the nine cultivars. The associations among all measured traits describing both individual grains and grain bulks were studied using a correlation matrix of Pearson correlation coefficients, which was produced using the 'corrplot' package (Wei and Simko, 2016).

2.3 Results

2.3.1 Grain Traits

Grain traits were measured on 100 representatively sampled grains from each cultivar; the mean values and standard error of the mean for the 100-grain samples are presented in **Table 2-1** for each cultivar as 'Individual Grain Analyses'. Significant differences in traits among grain samples were achieved in this case through use of cultivar selection within this 2016/17 field trial, providing a wide range of grain phenotypes with which to investigate performance of grain bulks. The 'Bulk Analysis' traits were measured on the larger bulk sample of each cultivar as supplied from AHDB, and the mean and standard deviation of these technical repeat measurements are presented in **Table 2-1** to give a measure of variation within the bulk for these measurements. Cultivar samples are listed in order of descending bulk SW, from Sienna with the highest (69.40 kg hl^{-1}) to KWS Irina with the lowest (64.53 kg hl^{-1}). Among the grains sampled, Concerto had the lowest grain weight (47.49 mg) which was significantly lower than grains of Sienna ($P < 0.05$), Propino ($P < 0.05$) and Laureate ($P < 0.001$). Concerto also had the shortest (7.79 mm) and least wide (3.80 mm) grains, which were significantly shorter than grains from all other cultivars and less wide than Origin ($P < 0.0001$), Olympus ($P < 0.0001$), Laureate ($P < 0.01$) and Propino ($P < 0.05$). Grain volume and 2-D area were lowest in Concerto (37.85 mm^3, 21.71 mm^2), although its volume was not significantly smaller than any other cultivars its 2-D area was significantly smaller than Laureate ($P < 0.0001$), KWS Irina ($P < 0.0001$), Origin ($P < 0.001$) and Odyssey ($P < 0.05$). Sphericity was significantly higher in Concerto (57.62%) than all other cultivars. In terms of bulk analyses Concerto had the highest number of grains in the measuring cylinder (555.5). Laureate had the heaviest grains (52.45 mg) which was significantly heavier than Octavia ($P < 0.05$), Olympus ($P < 0.01$) and Concerto ($P < 0.001$). Laureate also had the highest volume and density (40.37 mm^3, 1.31 g cm^{-3}), although its volume was

not significantly larger than any other cultivars its density was greater than Octavia (P < 0.01), Concerto (P < 0.01), KWS Irina (P < 0.001) and Odyssey (P < 0.0001). In terms of bulk analyses Laureate had the lowest mean grain number in the cylinder (492.2) and packing efficiency (50.7%), compared to all other cultivars. Despite grains within the Laureate and Concerto samples having significantly different dimensions and weight, the SWs of 66.33 kg hl^{-1} and 66.84 kg hl^{-1} of each cultivar sample respectively, are very similar to one another. These results demonstrate that among grain bulks, the same SW can be achieved through different combinations of grain traits.

Table 2-1 Measured[a] grain traits for the nine spring barley cultivars[b] examined.

	Cultivar								
	Sienna	Propino	Olympus	Concerto	Origin	Laureate	Odyssey	Octavia	KWS Irina
Individual Grain Analysis									
Weight (mg)	51.20 ± 0.79 ab	50.97 ± 0.79 ab	48.32 ± 0.75 bc	47.49 ± 0.78 c	49.36 ± 0.72 abc	52.45 ± 0.81 a	50.01 ± 0.73 abc	48.61 ± 0.85 bc	49.67 ± 0.75 abc
Depth (mm)	2.98 ± 0.02 bc	3.06 ± 0.02 a	2.91 ± 0.02 d	3.03 ± 0.02 ab	2.88 ± 0.02 d	3.03 ± 0.02 ab	2.95 ± 0.02 cd	3.01 ± 0.02 abc	2.91 ± 0.01 d
Length (mm)	8.12 ± 0.06 d	8.22 ± 0.06 cd	8.22 ± 0.06 bcd	7.79 ± 0.07 e	8.56 ± 0.06 a	8.53 ± 0.06 a	8.48 ± 0.05 ab	8.33 ± 0.07 abcd	8.45 ± 0.06 abc
Width (mm)	3.82 ± 0.02 cd	3.90 ± 0.02 abc	3.94 ± 0.02 a	3.80 ± 0.02 d	3.95 ± 0.02 a	3.93 ± 0.02 ab	3.85 ± 0.02 bcd	3.80 ± 0.02 d	3.89 ± 0.02 abcd
Volume (mm^3)	39.61 ± 0.65 a	39.61 ± 0.63 a	38.01 ± 0.62 a	37.85 ± 0.70 a	38.71 ± 0.57 a	40.37 ± 0.70 a	40.17 ± 0.57 a	38.39 ± 0.66 a	39.59 ± 0.66 a
Density (g cm^{-3})	1.30 ± 0.01 ab	1.29 ± 0.01 abc	1.27 ± 0.01 abcd	1.26 ± 0.01 cd	1.28 ± 0.01 abcd	1.31 ± 0.01 a	1.25 ± 0.01 d	1.27 ± 0.01 bcd	1.26 ± 0.01 cd
2-D Area (mm^2)	22.26 ± 0.25 cd	22.53 ± 0.26 bcd	22.72 ± 0.27 bcd	21.71 ± 0.28 d	23.37 ± 0.24 ab	24.02 ± 0.25 a	22.94 ± 0.22 abc	22.38 ± 0.26 bcd	23.88 ± 0.26 a
Sphericity (%)	55.77 ± 0.20 bc	56.14 ± 0.21 b	55.44 ± 0.22 bcd	57.62 ± 0.27 a	53.81 ± 0.24 e	54.77 ± 0.20 def	54.07 ± 0.21 ef	54.97 ± 0.28 cde	54.16 ± 0.19 f
Area Density (mg mm^{-2})	2.29 ± 0.02 a	2.25 ± 0.02 ab	2.12 ± 0.02 cd	2.18 ± 0.02 bc	2.11 ± 0.02 cd	2.17 ± 0.02 c	2.17 ± 0.02 c	2.16 ± 0.02 c	2.07 ± 0.02 d
Bulk analysis									
Grain Number	544.67 ± 2.08	523.00 ± 4.36	549.50 ± 3.46	555.50 ± 5.63	527.17 ± 3.33	492.17 ± 4.16	522.50 ± 8.79	522.33 ± 0.58	520.33 ± 4.54
PE (%)	55.09 ± 0.21	52.90 ± 0.44	53.34 ± 0.34	53.69 ± 0.54	52.11 ± 0.33	50.73 ± 0.43	53.60 ± 0.90	51.20 ± 0.06	52.60 ± 0.46
SW (kg hl^{-1})	69.40 ± 0.38	68.05 ± 0.25	66.95 ± 0.28	66.84 ± 0.38	66.53 ± 0.37	66.33 ± 0.69	65.93 ± 0.24	65.53 ± 0.55	64.53 ± 0.67

[a]Individual grain analysis (n=100) values are expressed as mean ± standard error of the mean and bulk analyses expressed as ± standard deviation.

[b]Cultivars which do not share a letter for each of the measured traits are significantly different from one another.

2.3.2 The Effect of Grain Fraction Size on Specific Weight

To examine how grain size correlates with specific weight among bulks, samples from each of the cultivars were sequentially sieved into different grain size fractions, creating a total of 25 samples with different grain sizes. Not all fractions were represented within each cultivar since not enough grain was retained of every size fraction for a SW estimate to be measured. Analysis of the SW of grain size fractions produced indicated significant differences between the largest and smallest fractions present for five out of the nine cultivar bulks (**Figure 2-1**), these were: KWS Irina, Octavia, Laureate, Concerto and Propino. For these five cultivars, the smallest size fraction yielded grain with a higher SW than the largest fraction size. KWS Irina, Origin and Olympus only had the three smallest size fractions, whereas Octavia, Laureate, Concerto and Propino had the three largest size fractions. Both Odyssey and Sienna only had enough grain for estimates to be made on the middle two size fractions. This demonstrates that within these bulk samples, these two cultivars have a more uniform grain size than the other seven when grown in the conditions of this trial. This may vary when cultivars are grown under different environmental conditions during another season or location. Specific weight was not consistent for size fractions among samples from different cultivars. For example, the medium size fraction for Sienna which had a SW of 70.1 kg hl^{-1}, which was significantly greater than the medium size fractions of all other cultivars. These data demonstrate that grain size alone is insufficient to determine SW among bulks, and that density and packing efficiency of the grains must be taken into account.
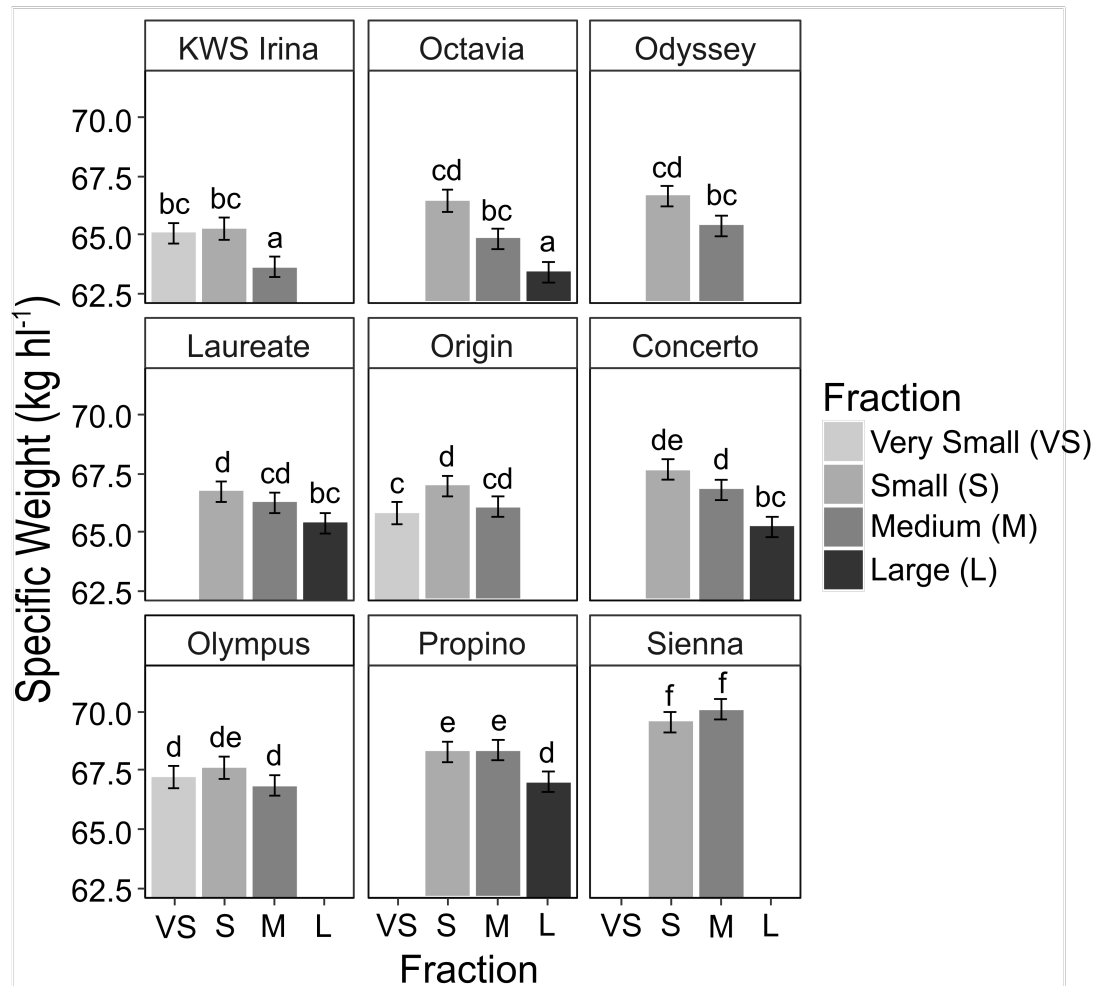
Figure 2-1 Specific weight measured on four size fractions of nine spring barley cultivars (n=3). Size fractions are the following: very small (2.50 to 2.75 mm), small (2.75 to 3.00 mm), medium (3.00 to 3.25 mm) and large (> 3.25 mm). Cultivars are ordered from the lowest mean SW from KWS Irina to the highest mean SW, Sienna. When fractions share a letter the SWs are not significantly different from one another and when a letter is not shared the fractions are significantly different from one another, P < 0.05. Bars are the standard error of the means.

2.3.3 Defining Specific Weight by its Components: Packing Efficiency and Grain Density

Regression analysis showed a strong positive correlation between the product of PE and grain density with SW (r^2 = 0.66, P < 0.01) among the 100-grain samples from each cultivar. The output of the linear regression is shown by the solid black line and the equation SW = 0.988 × (PE × grain density) (**Figure 2-2**). Seven of the nine cultivars appear close to the y=x line, shown by the dashed line, with four of these almost exactly on this line. This demonstrates that for the vast majority of cultivar samples used, the procedure used to estimate SW through PE and grain density was successful. Two cultivar samples however, KWS Irina and Sienna, are beneath the linear regression due to PE × grain density being larger than the SW. Through examining the mean grain weight of the 100-grain sample and mean weight of grains in the cylinder KWS Irina and Sienna had the greatest differences of +1.11 mg and +1.30 mg respectively (see Appendix, **Table A-1**). An ANOVA showed that both PE and grain density had a statistically significant effect on SW at P < 0.01 (**Table 2-2**). Further analysis using the sum of squares to calculate the proportion of variation contributed by each component showed that PE contributed to 36.5% of the variability in SW, and grain density contributed 48.5%. The contribution of the residual error was small at 15.0% (**Table 2-2**).
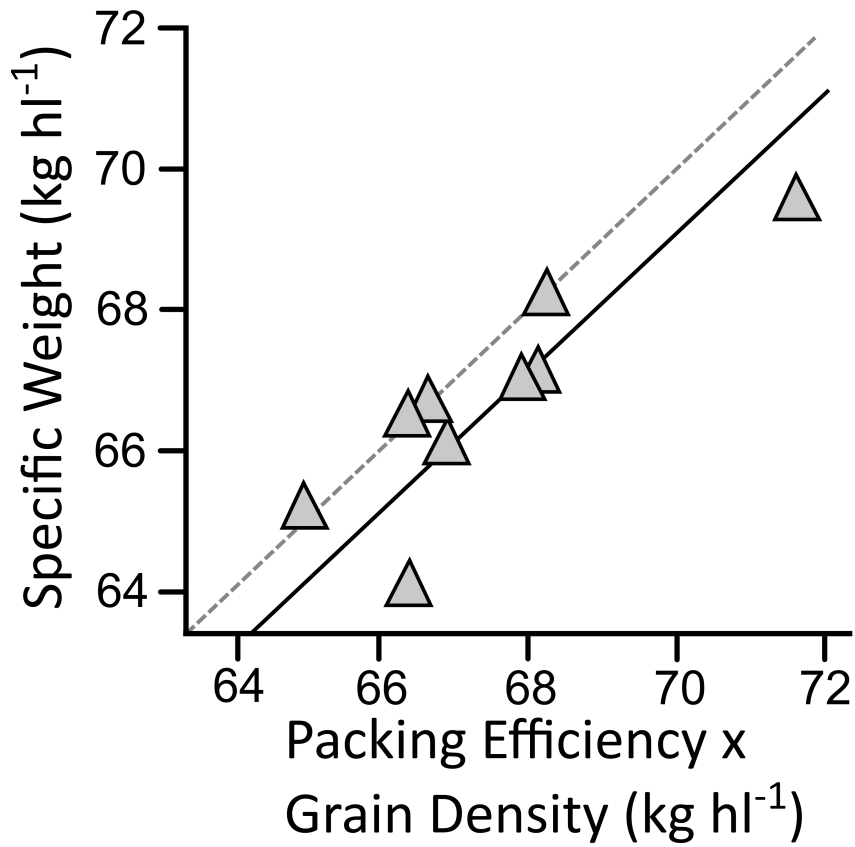
Figure 2-2 The SW of nine barley cultivars plotted against the product of PE and grain density.

The linear regression is shown by the solid black line, whereas the dashed line indicates the

y=x relationship

Chapter 2. *Specific Weight of Barley Grains is Determined by Traits Affecting Packing Efficiency and by Grain Density*

Table 2-2 ANOVA table for specific weight showing the proportional contributiona of packing efficiency and density to SW.

Source of variation	df	Sum of squares	Mean square	*F*-value	*P*-value	Contribution (%)
Packing efficiency	1	5.85	5.85	14.60	0.0088	36.48
Density	1	7.78	7.78	19.42	0.0045	48.52
Residuals	6	2.40	0.40			14.99
Total	8	16.03				

aCalculated as a percentage of the sum of squares for each variable

2.3.4 The Influence of Grain Dimensions on Packing Efficiency

Grain shape was further investigated through principal component analysis (PCA). The loadings and variance explained of the principal components (PCs) are reported in Appendix A **Table A-2**. Principal component 1 (PC1) contributed 91.8% of the total variance, cultivars with a high score in PC1 tended to have shorter grains. Principal component 2 (PC2) contributed 5.3% to the total variance, cultivars with a high PC2 score have deeper grains. The relationship between grain length, width and depth and the PCs are shown in **Figure 2-3**. A principal component biplot of PC1 against PC2 (**Figure 2-3**) shows cultivars with longer grains have a lower PC1 score such as Laureate, Odyssey, KWS Irina and Origin. As cultivars increase in length from Concerto with the shortest grain length to Origin with the longest grain length, they have a higher PC1 score. Further separation occurs by PC2, cultivars with deep grains have a more positive PC2 such as Octavia, Laureate, Propino and Odyssey. Again, this analysis shows the difference in grain size between Laureate and Concerto, which occupy opposite sides of the plot. The plot separates cultivars according to their grain dimensions, which also corresponds to a diagonal gradient of grain number in the cylinder, because a greater number of small grains pack into the cylinder. Therefore Laureate is positioned in the far top left as it has the largest grains and hence fewest in the cylinder (492.2). The next diagonal portion of the plot is occupied by Origin, KWS Irina, Odyssey Octavia and Propino with similar grain numbers of 527.2, 520.3, 522.5, 522.3 and 523.0 respectively. The final diagonal portion in the bottom right of the plot has cultivars with the highest grain numbers Sienna (544.7), Olympus (549.5) and Concerto (555.5). Grain number is one aspect of PE, therefore grain dimensions may help to partly explain PE but not the full extent of this component of SW.
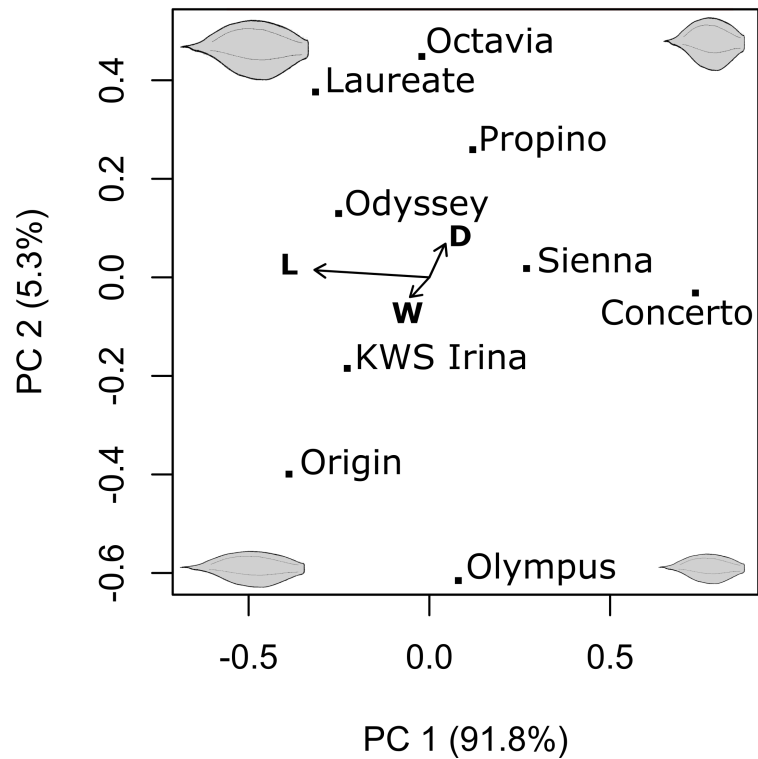
Dimension	PC1	PC2
Length	-0.981	0.185
Depth	0.130	0.185
Width	-0.146	-0.485

Figure 2-3 Biplot of the principal component analysis of grain shape parameters of nine spring malting barley cultivars. Grain dimensions used in this analysis: L, length; W, width and D, depth. Arrows originating at the centre of the biplot represent the loadings of grain dimensions, with the length of these arrows corresponding to the relative importance of each dimension in each axis. Example grain shapes (not to scale) are shown on the plot to indicate which grain shapes have high or low scores in each of the principal components. Loadings for each grain shape parameter are included in a table beneath the biplot.

2.3.5 Combined Correlation Analysis on Grain Parameters

The significance of correlations between measured traits was analysed, and a matrix of Pearson correlation coefficients (r) is given in **Table 2-3**. The significant correlation between sphericity and grain 2-D area (r = -0.77, P < 0.01) highlights that more spherically shaped grains have a reduced 2-D surface area. The negative correlation between grain number and length, (r = -0.77, P < 0.05) confirms the discovery in the previous PCA that fewer longer grains pack into a cylinder. This can also be related to grain volume, since grain number and volume negatively correlate (r = -0.72, P < 0.05). The negative correlation between the grain dimensions, length and depth with grain number was further explored in Appendix A **Figure A-2**. The sum of grain length and depth correlates very strongly with grain number (r = 0.90, P < 0.01) (see Appendix **Figure A-2A**) and with PE (r = 0.75, P < 0.05) (see Appendix **Figure A-2B**). The sum of grain depth and length in this analysis strengthened the correlation between the dimensions and both grain number and PE than just length alone. Another strong positive correlation was observed between area density and SW (r = 0.81, P < 0.05). Area density summarises the weight of grain in a given area and SW is a measure of the weight of grain in a given volume, therefore the strong correlation between these variables was expected.

Table 2-3 Correlation matrix[a] of Pearson correlation coefficients (r) for grain dimensions, shape parameters and components of SW.

	Weight (mg)	Depth (mm)	Length (mm)	Width (mm)	Volume (mm^3)	Density (g cm^{-3})	2-D Area (mm^2)	Sphericity (%)	Grain Number	Area Density (mg mm^{-2})	SW (kg hl^{-1})	PE (%)
Weight (mg)	1	0.26	0.46	0.28	0.89**	-	0.51	-0.20	-0.69	-	0.30	-0.16
Depth (mm)		1	-0.47	-0.47	0.13	0.36	-0.41	-	-0.15	0.68	0.31	-0.11
Length (mm)			1	0.58	0.56	0.02	0.85***	-	-0.77*	-0.44	-0.46	-0.57
Width (mm)				1	0.16	0.31	0.68*	-	-0.35	-0.44	-0.06	-0.36
Volume (mm^3)					1	-	0.58	-0.45	-0.72*	0.27	0.02	-
Density (g cm^{-3})						1	0.16	0.17	-0.28	0.50	0.59	-0.15
2-D Area (mm^2)							1	-0.77**	-0.77*	-	-0.50	-0.57
Sphericity (%)								1	0.59	0.52	0.50	0.40
Grain Number									1	0.13	0.40	-
Area Density (mg mm^{-2})										1	0.81*	0.45
SW (kg hl^{-1})											1	0.59
PE (%)												1

[a]The symbol "-" indicates that one variable was used to calculate the other, therefore no correlation was calculated.

"***", "**", "*" were significant at P < 0.001, P < 0.01 and P < 0.05 respectively.

2.4 Discussion

How grain dimensions, weight, volume and PEs combine to determine the final SW within a grain bulk, or among cultivars, has previously not been established. Since SW is embedded in global grain trade as a measure of grain quality, an enhanced understanding of these traits is essential. Previous assumptions made that SW is a good predictor for the nutritional value of wheat have been upturned (Miller and Wilkinson, 1998). Therefore assumptions made about the value of SW for malting need to be investigated to ensure it is an effective measure of grain quality.

Studies on other cereal species which use SW as a measure of grain quality have used the equation SW = PE × grain density (Doehlert et al., 2009; Pushman and Bingham, 1975). The current work demonstrated that this is also the case for barley grain, where the linear regression nearly mirrored the y=x line. The knowledge that barley SW can be defined by PE and grain density is an integral step towards enhancing our understanding of SW. Analysis of the relative contribution of each of these components to SW highlights that the contribution of one component does not vastly outweigh the other. Therefore both PE and grain density are the two defining contributors to SW and the grain traits that affect both of these components need to be analysed in turn.

In this study, grain traits of individual barley grains and also bulk level grain samples were analysed to investigate SW as a measure of grain quality. We have shown that observing just one grain trait or bulk character is not enough to understand SW. However, combining variables leads to a better understanding of SW and its components. This is highlighted by the non-significant relationships between: grain weight and SW; grain 2-D area and SW; and grain density and SW. However, for the

combined variable 'area density', a strong and significant correlation is observed with SW. Therefore grain shape does not solely determine SW, nor does grain weight or density. Specific weight is influenced by a combination of all of the grain traits examined in this study. A multivariate approach therefore needs to be considered when analysing SW and its components.

The influence of grain dimensions on PE was investigated further through PCA. Here we demonstrated that grain dimensions length and depth strongly influence the number of grains in a vessel. The negative relationship between PE and these two grain dimensions is of borderline significance, which is not improved by including grain width in the analysis. This highlights that grain dimensions as studied here in three planes (L, W and D) cannot fully describe PE. What can be concluded is that cultivars with shorter, less deep grains pack more into a vessel and tend to have an increased PE, but other factors such as grain morphology could influence PE. In oat grains, Doehlert et al., (2006) observed a strong negative correlation between length and SW this could partly be explained by the relationship between grain length and number in this study. Future grain morphological analysis will combine grain size and shape. The analysis of grain shape will involve quantifying shape, describing grains as more rounded or pointed through morphometrics.

Clarke et al. (2004) reported a positive correlation between wheat grain size and SW, although in their study, "grain size" was a principal component vector encompassing grain mass alongside grain dimensions, area and perimeter. In our study, a higher grain size fraction negatively influenced SW in five out of the nine cultivars (Fig. 1), demonstrating that the effect of grain size fraction on SW is not uniform across cultivars. In the remaining four cultivars no significant effects on SW between the

smallest and largest grain size fractions were found. The difference in results between these two studies is likely to be a result of the different methods of grain size manipulation. Clarke et al. (2004) manipulated grain size by irrigation and nitrogen application, but we achieved this through sequential sieving. Sequential sieving influences size and may result in grain fractions of differing densities, but the effect of this is not the same as the environmental effect. Therefore it can be suggested that not only grain size influences SW, but also the environmental conditions or genotype leading to this size change. Other factors such as weathering, awn retention, grain shape and grain density affect SW, further demonstrating the potential environmental and genotypic influences on this trait (Atkinson and Kettlewell, 2008).

When the same technique of sequential sieving was used with oat grains Doehlert et al., (2004) found that smaller grain fractions resulted in increased SW, as found in the current study in five out of the nine cultivars. Doehlert et al. (2004) observed grand means of size fraction SWs of numerous grain samples, so whether this effect is consistent among all cultivars used in their study is unknown. Grain size is a trait that has been suggested to affect malting and the results of this study provide a link between a factor that influences SW and also impacts upon malting (Fox et al., 2006; Wade and Froment, 2003). In particular homogeneity of grain size is thought to be beneficial for malting to ensure uniform rates of water uptake by the grain, and consequential germination and endosperm modification.

Since PE is a major component of SW it is important to consider the potential influence of this on the malting process. It can be assumed that grain bulks with different PEs have an altered pore space distribution within the bulk of grains. Neethirajan et al. (2006) showed that different pore space distributions within the bulk

formed by cereals lead to an altered air flow through the bulk, in both the vertical and horizontal directions. This is likely to be extremely relevant to malting, where the first step in the process is steeping, which involves the soaking of grains in water. The barley grains imbibe water in this step increasing in moisture content and germination is initiated. Since PE will affect pore distribution, this could in turn influence the flow of water between grains. This will affect whether all grains in the bulk reach sufficient moisture content to germinate, impacting on steeping duration and efficiency. The same principles can be applied to kilning when hot air is passed through the malt, an irregular pore space distribution could lead to an unevenly kilned malt product.

The second major component of SW is grain density, the determinants of this were not investigated in this study. However, it is hypothesised that grain density, unlike PE is primarily influenced by grain composition and internal structure rather than morphological features of the grain (Walker and Panozzo, 2011). Aspects of grain composition that could influence density are: starch content, protein content, starch granule ratios, ratios of amylose and amylopectin, ratios of the different grain tissues and the internal packing of these within the grain (Walker and Panozzo, 2016). If grain density is positively influenced by a compositional aspect which is beneficial for malt quality, for example a high starch content, this would reinforce the value of SW as a grain quality measure. However, if grain density is increased by factors associated with a poor malt, for example a high protein content this would bring the value of this under question.

2.5 Conclusions

This study uncovers the contribution of the components PE and grain density to SW, and examines grain traits influencing these. When breeders target SW, this needs to

be done through the correct balance of density and PE relevant to the end-use. Knowledge of this is important so the malting industry can understand exactly what the effect of differing SWs and their components are likely to have upon the malting process. The work gives insight as to why grain bulks with similar SWs and hence similar market value grain could lead to different malting efficiencies, via altered PEs due to grain size. Therefore SW alone may not be a comprehensive standalone measure of grain quality for the malting industry.

2.6 Acknowledgements

2.7 References

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3 Chapter Three

Increased Grain Density of Spring Barley (*Hordeum vulgare* L.) is Associated with an Increase in Grain Nitrogen

Increased Grain Density of Spring Barley (*Hordeum vulgare* L.) is Associated with an Increase in Grain Nitrogen

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Chapter 3. *Increased Grain Density of Spring Barley (Hordeum vulgare L.) is Associated with an Increase in Grain Nitrogen*

Abstract

The quality of cereal grains are evaluated by different measures, in spring malting barley specific weight is one important measure. Increased specific weight is thought to be associated with a higher malt output, but this has not yet been proven. Therefore the value of specific weight as a malt quality indicator is disputed. Specific weight is the product of grain density and packing efficiency. We examined grain composition and density, to understand how specific weight relates to malt output. Our results show that both nitrogen content and the proportional volume of starch B-granules were positively correlated with grain density. An equation was built to predict grain density from grain nitrogen and the proportional volume of starch B-granules describing 47% of the observed variation in grain density. When validating the equation we found that starch B-granules were not as important for predicting density, but a model using nitrogen content alone was sufficient to estimate grain density. There is evidence that different genotypes and environments may require different coefficients for more precise prediction. These data show that nitrogen content is consistently correlated with grain density and hence specific weight. Therefore a high specific weight could be detrimental for some malting end-uses.

Keywords: Barley (*Hordeum vulgare* L.); specific weight; grain density; nitrogen content; starch; malting

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3.1 Introduction

Barley (*Hordeum vulgare* L.) is the main cereal used in the malting process, whereby grain undergoes steeping, germination and kilning to produce malt (Gupta et al., 2010). Steeping increases the moisture content of grains from 12% to greater than 40% (w/w), triggering germination and a cascade of physical and biochemical modifications within the endosperm (Briggs, 1998). These modifications include the accumulation of malt enzymes, cell wall degradation and physical changes such as softening of the grain (Briggs, 1998). Kilning arrests germination by drying the malt at elevated temperatures, stabilising the enzymes produced which are harnessed in downstream processes. In the UK, malt is primarily used in the brewing and whisky distilling industries, but it is also used in some food products and is an important export for the UK (Baik and Ullrich, 2008). Barley grain is graded on numerous quality criteria prior to acceptance for malt production. The strict criteria that have to be met by growers supplying for the malting industry result in a higher price for high quality barley. One of these grain quality criteria is specific weight (SW); one of the longest standing measures of grain quality. It is a measure of bulk density, that is, the weight of grain per unit volume (Briggs, 1998). A high SW is thought to be associated with higher malting efficiency and is therefore a breeding target. Our recent work has shown that the SW of barley grains is a product of two components: single grain density (SGD) and packing efficiency (PE) (Hoyle et al., 2018).

It is important to distinguish between bulk density, SGD and grain hardness because they are distinct measures. Bulk density describes the mass of grain in a given volume, whereas SGD describes the density of an individual barley grain. Grain hardness is harder to define in barley, however in wheat it is associated with milling energy. Hardness is not a measure or indicator of SGD, however in wheat it has been shown that soft and hard wheat cultivars have a large overlap in SGD (Dobraszczyk et al., 2002). The focus of this study is to dissect the SGD

component of SW further, to investigate how compositional variables that correlate with SGD could affect SW.

Links between SGD and barley grain composition have not previously been studied. The endosperm is the largest grain tissue comprised of two components, the aleurone and the starchy endosperm (Evers et al., 1999). The starchy endosperm forms the majority of this tissue, in which endosperm cells store nutrients which are mobilised upon the onset of germination to sustain the embryonic axis (Evers et al., 1999). Cell walls in the barley endosperm are abundant in mixed linkage β-glucans (Evers and Millar, 2002). The major constituents of barley grains are starch (60-80%), nitrogenous compounds (9-13%), lipids (1-2%) and water (10-15%) (Asare et al., 2011). Starch is composed of two different types of D-glucose polysaccharides; amylose and amylopectin (Jeon et al., 2010). Amylose is a linear polymer of 1,4-linked α-glucose residues with minor branching, whereas amylopectin is a highly branched polymer consisting of 1,6-linked α-glucose residues (Jeon et al., 2010). These two polysaccharides are stored in the form of semi-crystalline starch granules in the endosperm, in either A- or B-type granules. These granules differ in their size, shape and composition. The larger, biconvex A granules have a diameter of between 8 and 30 µm, whereas the smaller, spherical B granules have a diameter of less than 8 µm (Evers et al., 1999). The size distribution of barley starch granules exhibits a bimodal distribution distinguishing between the two granule types. The majority of nitrogenous compounds in barley grains are proteins, with hordeins being the most prevalent protein (Gupta et al., 2010).

Relationships between both grain physical characteristics, grain composition and malt quality parameters have long been studied (Agu et al., 2007). Physical characteristics that affect malt quality include grain size and size uniformity, weathering, and skinning (Fox, 2010). Compositional attributes such as starch content and composition are of high importance for

determining malt quality, with the ratio of amylose and amylopectin affecting starch gelatinisation properties (Fox, 2010). The gelatinisation of starch is important for malt quality because the rate of starch hydrolysis by malt enzymes post-mashing is increased once starch granules become soluble through gelatinisation (Macgregor et al., 2002). Both high amylose and waxy barley are associated with increased gelatinisation temperature, which means that during the mashing process a higher temperature has to be reached in order to ensure complete gelatinisation (Macgregor et al., 2002). Protein content is also important in regard to malt quality and is influenced by both growing conditions and genotype (Fox, 2010). A high protein content is considered detrimental for malting efficiency as it can reduce the proportion of starch in the endosperm. However, there must be sufficient amino acids present to sustain yeast, particularly for brewing (Fox, 2010).

It is important to characterise any correlations between SGD and grain composition, in order to determine whether increasing SGD and hence SW can either confer potential benefits, or detract from malting efficiency. If an increased SGD correlates with compositional characteristics thought to improve malt output, such as increased starch content or low protein content, this would provide evidence that increased SW truly is a good indicator that grain is of malting quality. However if an increased SGD correlates with traits that are detrimental for malting, such as an increased protein or a higher ratio of B starch granules, it could indicate that SW is unlikely to indicate whether grain is of high malting quality.

In this study the aims were (1) to examine correlations between quantitative changes in grain composition and SGD, (2) to build an equation to predict SGD from grain composition to understand the contributions of compositional aspects to SGD and (3) to test the accuracy and efficacy of the equation using a validation dataset.

3.2 Materials and Methods

3.2.1 Materials

Barley grains of five cultivars (Sienna, Laureate, Concerto, Olympus and Odyssey) from the Agriculture and Horticulture Development Board's (AHDB's) Recommended List (RL) 2016/17 were used in this study. These cultivars were selected due to their phenotypic range in grain size, SW and SGD (Hoyle et al., 2018). All cultivars were grown at AHDB's RL crop trials site in Docking, Norfolk under natural rainfall conditions in the 2016 season. Before analysis grain samples were cleaned using a 2.50 mm slotted sieve, with 19.05 mm long slots and shaken for 20 s. Barley grains from a separate sample of Sienna were used to validate the equation derived from the original five cultivars. This sample was a commercial bulk provided by Bairds Malt and grown during the 2017 season, which contains spring barley grown across Scotland.

3.2.2 Sampling

In order to obtain a representative sample of grains to analyse, 350 g grain samples were sequentially sieved into a range of size fractions using a stack of slotted 3.25, 3.00 and 2.75 mm sieves, with 19.05 mm long slots. The weight of grain in each size fraction designated; large (> 3.25 mm), medium (3.25 to 3.00 mm), small (3.00 to 2.75 mm) and very small (< 2.75 mm) was recorded using a Kern analytical balance PLJ 3500-2NM (accuracy ± 0.01 g). Three 100-grain samples were weighed from each size fraction, and the mean grain weight used to estimate the total number of grains in each fraction. A number of grains proportional to the total number of grains from each fraction were chosen at random, to give 300-grain samples which were representative of the total larger bulk sample, for each cultivar used in this study.

3.2.3 Grain Density and Sample Stratification

On each 300-grain sample, grains were individually weighed using a Mettler AE 160 electronic balance (Mettler-Toledo, accuracy ± 0.0001 g). The volume of individual grains was measured by placing them in a submersed, but suspended crucible in a beaker of water. The change in weight on the balance due to the buoyant force acting on the grain is equal to the weight of water displaced and hence the volume of the grain (Archimedes' principle) (Hughes, 2005). After five seconds the measurement was taken, then the grain removed from the water and patted dry on paper towel for later analysis. To create five density classes within each cultivar, grains were ordered by density. Density classes were created by grouping the 60 least dense grains and so on until the 60 most dense were left, creating 25 samples in total (**Figure 3-1 A**). In order to visualise the endosperm and in particular the starch granules within endosperms of different densities, scanning electron microscope (SEM) images were taken of five high density and five low density Laureate grains from the 60-grain sample (**Figure 3-1 B,C**).
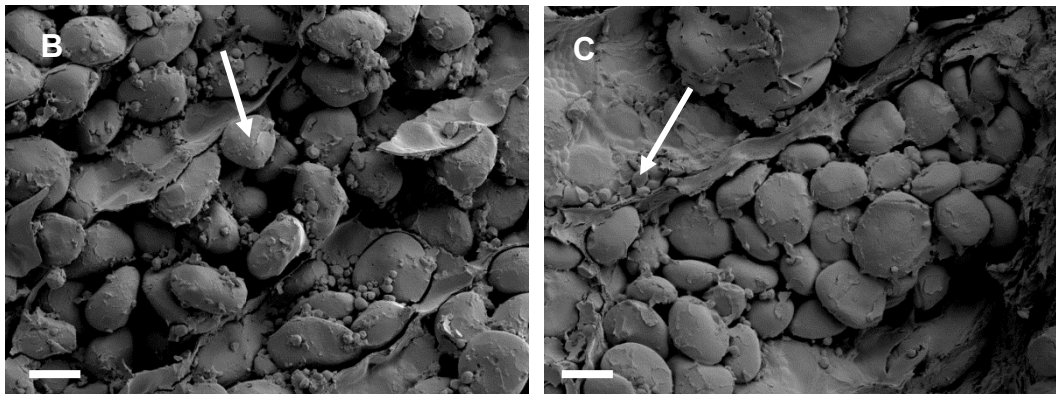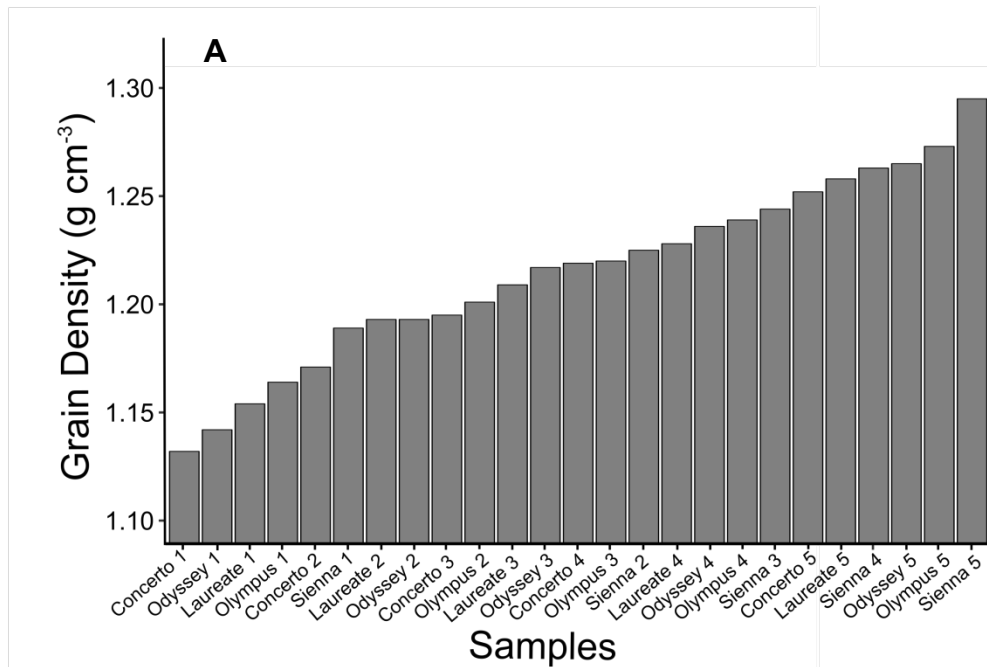
Figure 3-1 Range of grain densities created by stratifying grain samples (**A**) from five cultivars into five individual classes according to density. Concerto 1 referring to the least dense 60 grains of the 300 grain sample and Concerto 5 referring to the most dense 60 grains. Scanning electron micrographs from cracked endosperms of spring barley cultivar Laureate, (**B**) high density and (**C**) low density. Scale bar = 10 μm. The arrow in Fig 1B points to a large starch A-granule and the arrow in fig 1C points to a small starch B-granule.

3.2.4 Elemental and Starch Analyses

Twenty grains from each 60-grain sample were milled into a fine powder using a ball mill (Mixer Mill MM 200, Retsch, Germany) for compositional analyses. The proportion of carbon and nitrogen in the grain, typically referred to as carbon and nitrogen contents, were determined with a FLASH 2000 Organic Elemental Analyzer (Thermo Scientific). Total starch content and the ratio of amylose to amylopectin were measured on subsamples of the milled grain using Megazyme kits: Total Starch Assay Kit (K-TSTA-100A) and Amylose/Amylopectin Assay Kit (K-AMYL) (Megazyme Ltd. Ireland) using the assay procedures provided by the manufacturer. Starch analyses are reported as percentage content for amylose and amylopectin (w/w) and 'as is' basis (g/100g) for starch content.

3.2.5 Starch granule isolation and size distribution analysis

Starch was purified separately from three 10-grain subsamples of the 60-grain samples according to the "method 1" in Verhoeven et al. (2004) and then freeze-dried using an Alpha 1-4 LSCplus (Christ, Germany) overnight prior to analysis. A known mass of purified starch was dispersed in 100 ml of Isoton II Diluent (Beckman Coulter, United States). The size distribution of starch granules was determined with a Multisizer 4e Coulter Counter (Beckman Coulter) with a 70 µm aperture tube. The Multisizer measures the volume of each starch granule passing through its aperture between two electrodes using the Coulter Principle. In excess of 200,000 particles were measured per sample, and size frequency distributions were recorded in 400 logarithmically spaced bins between the diameter range of 1.4 µm to 42 µm. The number of granules passing through the aperture was counted and the surface area of these estimated by using the surface area of a sphere with the same measured volume. Therefore results of starch granule analysis include B-granule: number, volume and surface area. These are all reported as a percentage of the total for all measured granules. Consistent with previous studies (Chmelík et al., 2007), we used a threshold of 8 µm to distinguish between A- and B-type granules, as this threshold effectively approximated the minima

between the size distribution curves of the A- and B-type granules. We also tested an alternative method for estimating the proportion of A- and B-type granules, based on a mixed distribution curve-fitting method, similar to that described in Tanaka et al. (2017). However, the mixed distribution was not able to accurately fit our size distributions of barley starch granules, as there was very little overlap in the A- and B-type granule distributions.

3.2.6 Statistical Analysis

Data analysis was carried out in R software version 3.4.1 (R Core Team, 2019). Analysis of variance ($\alpha = 0.05$) was used to determine whether grain density class and cultivar had a significant effect on SGD, elemental analyses and starch analyses. Where a significant effect was indicated, a post-hoc Tukey's Honestly Significant Difference (HSD) ($\alpha = 0.05$) test was conducted to determine which samples differed from one another. This is indicated by different letters in the results table. A stepwise linear regression was performed in R using the 'olsrr' package to determine which variables significantly contributed to predicting SGD and therefore should be included in the equation (Hebbali, 2018). The response variable was SGD, and the dependent variables were: nitrogen, carbon, total starch, amylose, and B granule volume. Independent variables were selected based on p-value, the threshold for a variable to enter the equation was $P < 0.1$ and to exclude a variable from the equation was $P > 0.3$. The correlation between measured grain density and calculated grain density was determined using Pearson's product-moment in the R package "corrplot" (Wei and Simko, 2016).

3.3 Results

Single grain density and compositional variables including: nitrogen (N) content, carbon (C) content, total starch content, amylose/amylopectin ratio and starch B granule; number, volume and surface area were measured on the 25 samples created by stratifying 300 grains from

each cultivar into five density classes. The mean values of each sample are provided in **Table B-1** (see Appendix).

3.3.1   Effect of Single Grain Density on Grain Composition

Table 3-1 summarises the means and standard deviations of SGDs and compositional aspects of the five different density classes: very low, low, medium, high and very high. Stratifying samples by density created a range of 1.16 g cm^{-3} to 1.27 g cm^{-3}. No differences in C content were observed between the different density classes; this measure only had a small range of 39.85% to 40.23% from the medium and low density classes. Density had a significant effect on grain N content, with N content sequentially increasing with each density class. Nitrogen content of the very low and low class was 1.36% and 1.40%, respectively. These were both significantly ($P < 0.05$) lower than that of the very high class (1.53%). Starch content did not differ significantly among density classes. All starch contents were within the range from 58.62 g/100 g to 58.78 g/100 g. Amylose content was highest in the very low density class (20.76%) which was significantly greater ($P < 0.05$) than the high density class (16.98%). The inverse was the case for amylopectin content. No significant differences were observed in the three measures of B granule content, however the values increased sequentially from the very low density class to the very high density class as follows: B granule number 97.21% to 97.56%, B granule volume 20.20% to 23.55% and B granule surface area 54.79% to 59.05%.

Table 3-1 Grain density, elemental analysis and starch analyses on different density groups[a]

Density class	Grain density (g cm^{-3})	Nitrogen (%)	Carbon (%)	Total Starch (%)	Amylose (%)	Amylopectin (%)	B granule number (%)	B granule volume (%)	B granule surface area (%)
Very low	1.16±0.010^d	1.36±0.025^b	40.03±0.13^a	58.64±0.32^a	20.76±0.52^a	79.24±0.52^b	97.21±0.21^a	20.20±1.32^a	54.79±4.48^a
Low	1.20±0.009^c	1.40±0.012^b	40.23±0.07^a	58.69±0.07^a	18.57±1.04^{ab}	81.43±1.04^{ab}	97.29±0.26^a	22.02±1.44^a	56.88±4.81^a
Medium	1.22±0.008^{bc}	1.46±0.025^{ab}	39.85±0.20^a	58.78±0.26^a	18.34±0.96^{ab}	81.66±0.96^{ab}	97.44±0.16^a	22.40±1.31^a	57.76±3.83^a
High	1.24±0.007^{ab}	1.47±0.030^{ab}	40.14±0.13^a	58.75±0.62^a	16.98±0.45^b	83.02±0.45^a	97.47±0.22^a	23.09±1.52^a	58.58±4.64^a
Very High	1.27±0.007^a	1.53±0.046^a	39.92±0.14^a	58.62±0.41^a	19.40±0.82^{ab}	80.60±0.82^{ab}	97.56±0.12^a	23.55±0.98^a	59.05±2.60^a

[a] Data are reported on a wet weight basis and are means of five different cultivars ± standard error of the mean (n=5). When comparing mean values within a column those followed by different letters are significantly different from one another (p<0.05). B granule number is expressed as % of the total starch granule number, this is the same for B granule volume and surface area.

3.3.2 Effect of Cultivar on Grain Composition

Table 3-2 summarises the means and standard deviations of SGDs and compositional variables of the five spring barley cultivars; Sienna, Laureate, Concerto, Olympus and Odyssey. Mean SGD ranged from 1.24 g cm^{-3} for Sienna to 1.19 g cm^{-3} for Concerto, although no significant differences were observed among cultivars. No significant differences were observed in grain C or N contents among cultivars. Odyssey had both the lowest C and N contents at 39.85% and 1.41%, respectively. Sienna had the highest C content (40.22%), and Laureate the highest N content (1.50%). The total starch content of grains was highest in Sienna and Olympus which had 59.33 g/100 g and 59.17 g/100 g, respectively, both were significantly higher than Odyssey which had the lowest at 57.94 g/100 g ($P < 0.05$). The ratio between amylose and amylopectin did not differ significantly among the cultivars measured. The three measures of starch B granules; number, volume and surface area shown as a percentage of total granules, all showed similar patterns across the cultivars. Starch B granule number was highest in Laureate (97.75%) which was significantly higher ($P < 0.05$) than Odyssey (97.28%). Concerto's B granule number (96.75%) was significantly lower ($P < 0.05$) than the other four cultivars. Concerto had significantly lower B granule volume and surface area (17.86% and 51.15%, respectively) ($P < 0.05$) than the other four cultivars. Laureate had the highest B granule volume (24.27%) and surface area (60.51%), but this was only significantly higher than Concerto ($P < 0.05$).

Table 3-2 Grain density, elemental analysis and starch analyses on five spring barley cultivars[b]

Cultivar	Grain density (g cm^{-3})	Nitrogen (%)	Carbon (%)	Total Starch (%)	Amylose (%)	Amylopectin (%)	B granule number (%)	B granule volume (%)	B granule surface area (%)
Sienna	1.24±0.018[a]	1.42±0.030[a]	40.22±0.10[a]	59.33±0.47[a]	19.81±0.64[a]	80.19±0.64[a]	97.67±0.12[ab]	23.28±1.03[a]	59.37±1.36[a]
Laureate	1.21±0.017[a]	1.50±0.038[a]	39.88±0.14[a]	58.73±0.23[ab]	18.49±0.98[a]	81.51±0.98[a]	97.75±0.07[a]	24.27±0.78[a]	60.51±0.79[a]
Concerto	1.19±0.021[a]	1.42±0.017[a]	40.09±0.17[a]	58.30±0.18[ab]	20.65±0.64[a]	79.35±0.64[a]	96.75±0.16[c]	17.86±0.88[b]	51.15±1.39[b]
Olympus	1.22±0.018[a]	1.46±0.064[a]	40.13±0.13[a]	59.17±0.26[a]	17.35±0.88[a]	82.65±0.88[a]	97.53±0.07[ab]	24.16±0.88[a]	59.44±0.99[a]
Odyssey	1.21±0.021[a]	1.41±0.023[a]	39.85±0.14[a]	57.94±0.16[b]	17.74±0.78[a]	82.26±0.78[a]	97.28±0.05[b]	21.69±0.40[a]	56.59±0.53[a]

[b] Data are reported on a wet weight basis and are means of five different density grades per cultivar ± standard error of the mean (n=5). When comparing mean values within a column those followed by different letters are significantly different from one another (p<0.05). B granule number is expressed as % of the total starch granule number, this is the same for B granule volume and surface area.

3.3.3 Correlations Between Compositional Traits

The significance of correlations between SGD and different compositional variables were analysed and a matrix of the Pearson correlation coefficients (r) are given in **Table 3-3**. Corresponding p-values are in **Table B-2** (see Appendix).

The highly significant positive correlation between SGD and N content ($r = 0.61$, $P < 0.01$, **Figure 3-2A**.) highlights the effect of SGD on N content which was observed in *3.2*. In addition to this there is a significant correlation between SGD and B granule volume ($r = 0.55$, $P < 0.01$, **Figure 3-2D**.). These are the only two variables with which SGD is significantly correlated. Single grain density did not correlate with either C content or starch content (**Figure 3-2B**, **Figure 3-2C**). Alongside correlating with SGD, B granule volume positively correlated with N content ($r = 0.44$, $P < 0.05$), starch content ($r = 0.43$, $P < 0.05$) and was negatively correlated with amylose content ($r = -0.57$, $P < 0.01$).

Table 3-3 Correlation matrix of Pearson correlation coefficients (r) for grain density, elemental analysis and starch analyses.

	Grain density (g cm^{-3})	Nitrogen content (%)	Carbon content (%)	Starch content (%)	Amylose content (%)	B granule volume (%)	B granule number (%)	B granule surface area (%)
Grain density (g cm^{-3})	1	0.61**	-0.01	0.2	-0.31	0.55*	0.51**	0.55**
Nitrogen content (%)		1	0.1	0.09	-0.37	0.44*	0.34	0.41*
Carbon content (%)			1	0.19	0.05	-0.17	-0.19	-0.18
Starch content (%)				1	-0.12	0.43*	0.34	0.39
Amylose content (%)					1	-0.57**	0.37	-0.52**
B granule volume (%)						1	0.94***	0.99***
B granule number (%)							1	0.98***
B granule surface area (%)								1

"***", "**", "*" were significant at P < 0.001, P < 0.01 and P < 0.05 respectively.
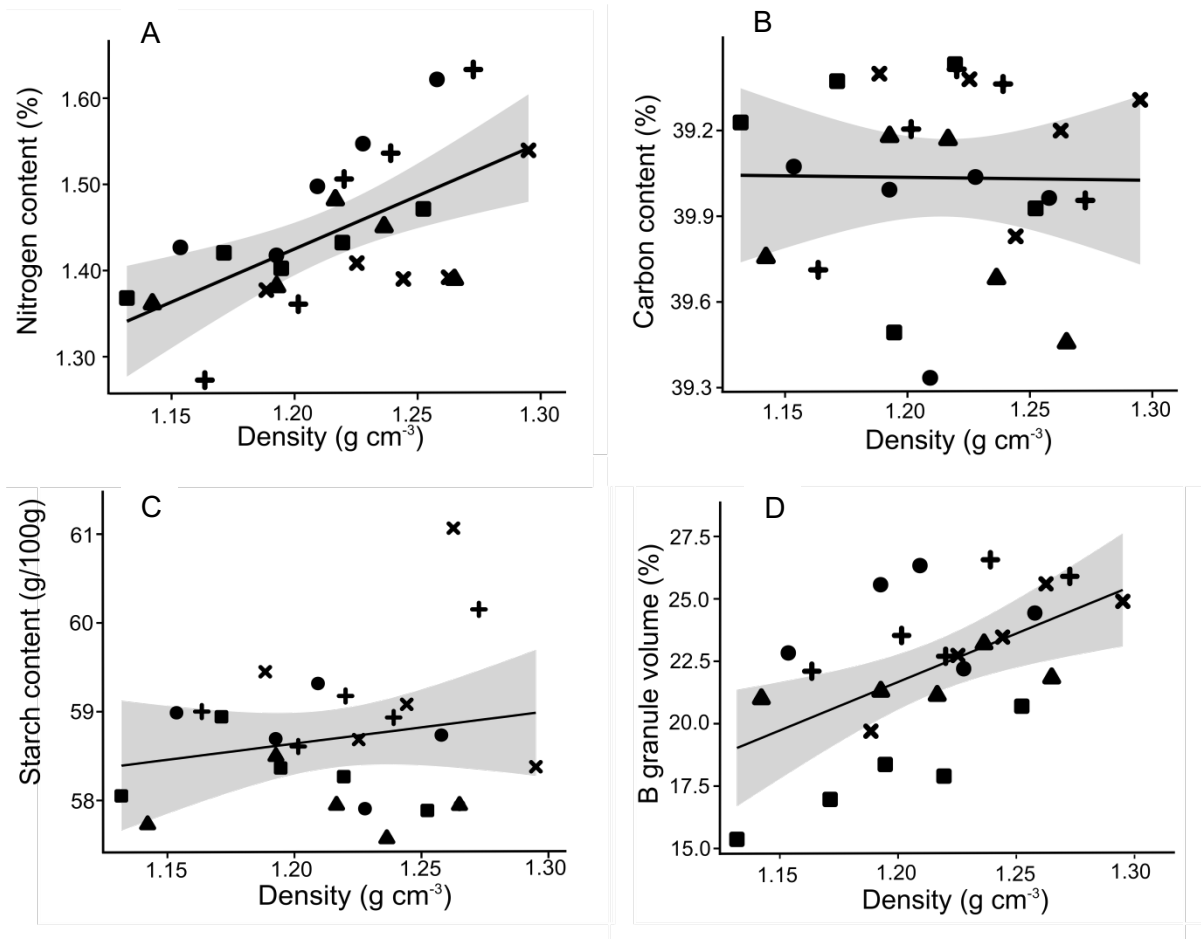
Figure 3-2 Regression analysis of grain density against grain constituents; (A) nitrogen content (r = 0.61, *P* = 0.001), (B) carbon content (r = 0.21, *P* = 0.948), (C) starch content (r = 0.06, *P* = 0.348) and (D) B granule volume (r = 0.53, *P* = 0.004). ■ Concerto; ●, Laureate; ▲ Odyssey; +, Olympus; ×, Sienna. Shaded areas represent the 95% confidence interval of the regression.

3.3.4 Predicting Single Grain Density from Compositional Traits

In order to determine the cumulative contribution of the independent variables to density (the dependent variable), a stepwise linear regression including all 25 grain samples was used. Independent variables which were calculated from one another (amylose/amylopectin) and those which displayed high levels of collinearity (B granule; volume, number and surface area) are represented only once by amylose and B granule volume, respectively. Stepwise regression analysis removed all independent variables apart from N content (%) and B granule volume (%). The independent variables removed were C content (%), amylose (%) and total starch (g/100 g). The predictive equation derived from this analysis was:

Density (g cm^{-3}) = 0.779 + 0.224*N + 0.005*B

N - Nitrogen Content (%)

B - Starch B granule volume (%)

Nitrogen content alone described 37.1% of the variation in SGD. The addition of B granule volume to the equation resulted in the r^2 value increasing from 0.371 to 0.473, with the final equation describing 47.3% of the variation in SGD. The relationship between measured grain density and the predicted grain density using this predictive equation on the original 25 samples was highly significant (r^2 = 0.473, *P* < 0.001, **Figure 3-3A**). Each cultivar is likely to have a slightly different slope as demonstrated in **Figure B-1** (see Appendix), therefore this predictive equation may need to be altered for highly accurate predictions to account for different genotypes.
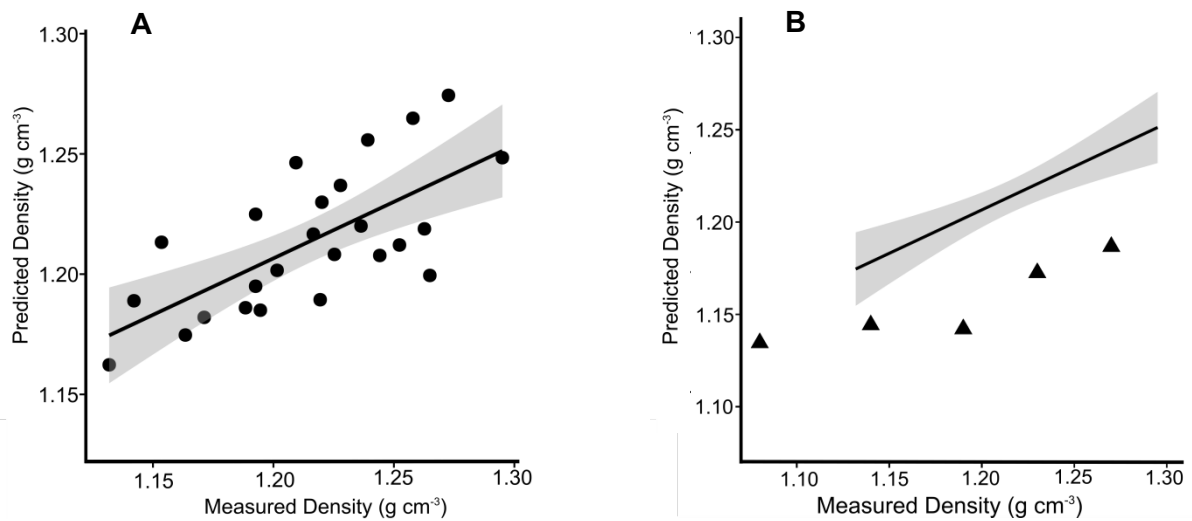
Figure 3-3 Scatter plots of measured grain density using Archimedes' Principle and predicted grain density using the predictive equation built in 3.3.4 for **(A)** the original 25 samples from five cultivars and **(B)** using N alone to predict the density of the validation five samples. The regression line (black) in both parts if formed from the original dataset, with the confidence interval of 95% shown by the grey shaded area.

3.3.5 Validation of the Density Equation

A separate sample of commercial barley grains from the cultivar Sienna was stratified in the same way to create five samples of differing densities to provide samples for equation validation. These were analysed for N content and starch B granule volume. The relationship between measured grain density and the predicted grain density (using the predictive model built in 3.3.4) of the validation sample was not significant (r = 0.83, P = 0.085). However when a model was built from the original data set using N content alone to predict density and applied to this validation set a significant positive correlation with measured grain density and predicted grain density was observed (r = 0.91, P < 0.05, **Figure 3-3B**). When comparing grain density with B granule volume and measured grain density, no significant correlations were observed.

3.4 Discussion

The value of SW as a measure of malting grain quality is disputed, therefore understanding what contributes to this measure is essential in order to determine whether SW could influence malting efficiency or productivity (Hoyle et al., 2018). Specific weight is one of the longest standing measures of grain quality, and is used across several cereals. In barley, this may be because it is easy to measure, the equipment is cheap and the results are straightforward to interpret, rather than because of its accuracy as a malt quality indicator (Manley et al., 2009). It is widely known that the composition of barley grains affects malt quality, which has been summarised by Fox (2010). Starch structure affects gelatinisation temperature and consequently hot water extract (Macgregor et al., 2002), high protein content correlates with reduced starch levels but low protein content is detrimental for yeast nutrition. However, how the composition of barley grains affects SGD, a component of SW (Hoyle et al., 2018), has not previously been studied. Linking this fills a gap in the knowledge between grain quality and malting. Furthermore, lessons could be applicable to other cereal species such as oats and

wheat which use SW as a measure of grain quality for alternative end-uses. If SGD also positively correlates with nitrogen content in wheat, this could reinforce the importance of SW as a quality measure in bread making, since both the quantity and quality of protein in wheat is important in this process (Johansson et al., 2001).

In this study we stratified grain samples from five spring barley cultivars into five different grain density classes, to create a large range in SGDs across 25 samples. Compositional analyses were performed on these samples to determine how compositional traits vary with SGD and to build a predictive equation to quantitatively link composition to SGD. We demonstrated that N content, which is often used as an estimate of protein content, is strongly correlated with SGD across the 25 samples. This is a novel finding since N or protein content have not previously been linked to the density of barley or other cereal grains. This link between protein content and one of the components of SW is an integral step to understanding how SW may affect malting, brewing and distilling. Generally, protein content is negatively correlated with available carbohydrates which reduces malt extract yield and is detrimental for malt quality (Agu, 2003; Peltonen et al., 1994). A high protein content can lead to low rates of modification, increased gelatinisation temperature and inadequate starch degradation through interfering with starch degradation enzymes and also enveloping starch granules in the endosperm (Yu et al., 2019). Therefore a high protein content can reduce the amount of fermentable sugars produced during mashing. However, too low a protein content could mean there are too few amino acids formed through proteolysis during mashing for yeast metabolism to occur (Gupta et al., 2010). Furthermore, through the analysis of a validation sample, N content also showed a positive correlation with SGD, demonstrating that this link between N and SGD is consistent for the samples tested.

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In addition to the relationship between N content and SGD in the original samples, starch B granule volume also showed a strong positive relationship with SGD. The conversion of starch into fermentable sugars is an integral part of brewing, therefore the rate of starch gelatinisation and hydrolysis are important factors to consider (Gupta et al., 2010). Barley starch A and B granules are different sizes and have an altered composition of polysaccharides, therefore they have distinctive physical and chemical properties (Jaiswal et al., 2014). Starch B granules have a lower proportion of amylose compared to A granules, as confirmed by the significant negative correlation observed between B granule volume and amylose in this study. At lower temperatures (35°C) the smaller starch B granules gelatinise more quickly than the larger A granules, but at higher temperatures more similar to that used in the mashing process (65°C) the opposite occurs (Gupta et al., 2010). Consequently the hydrolysis of starch B granules into soluble sugars during mashing occurs at a slower rate than A granules, which can cause problems in the brewing process (Macgregor and Ballance, 1980). Therefore in these samples an increased SGD is associated with potentially detrimental starch granule characteristics for malting. However when the validation sample was analysed this relationship between B granule volume and SGD was not observed. This demonstrates that this relationship does not always hold true across sites. The reason this relationship may not have held true may be due to the different environments this validation sample was grown in, since the ratio of A and B granules is affected by both the environment and genotype (Lindeboom et al., 2004). Temperature stress has been shown to reduce the size of A and B granules and the number of B granules (Tester, 1997). The synthesis of A granules starts soon after anthesis and B granule synthesis is initiated later such that throughout grain filling B granule number increases (Lindeboom et al., 2004).

No relationships were observed between SGD and both C content and total starch content of barley grains. It has been reported that starch content negatively correlates with protein content in barley grains. Therefore since N content positively correlates with SGD it might

have been expected that total starch content would display the opposite trend however, this was not the case. Other studies have also shown that starch content does not always correlate with protein content (Yu et al., 2019, 2017).

The equation derived to predict SGD from grain composition is the first of its kind and went some way to accurately predicting SGD through using just nitrogen content and B granule volume. The aim of this equation was to better understand the basis of barley SGD and therefore this component of SW. To test both the accuracy and efficacy of this equation at predicting density for samples from different origins, the equation was applied to a validation sample. The equation generally under-estimated density for samples with a higher measured density, the gradient of this was not parallel with the original dataset. However when the model was built from the original dataset using N alone to predict density and applied to this validation dataset improved predictions were made. Different environmental conditions are known to have large effects on starch granule composition, functionality and proportions which could explain why validating this equation starch granules were less important. This validation sample was from the cultivar Sienna, which was one of the five cultivars used to build the predictive equation. Therefore although the predictive equation built in this study was useful to predict differing grain densities from one site and year, other variables may need to be included or excluded to successfully predict densities when plants are grown in differing environmental conditions. Other variables may include the proportion of husk to endosperm, cell wall composition and the internal structure created by these cell walls. The density value used for each density class was the mean of the 60-grain sample, however with compositional analyses requiring a different subset of these 60 grains, the full 60 grains could not be used for each analysis. Therefore this 60-grain sample had to be subsampled for each method, despite the subsamples all having similar densities this could have potentially introduced some error since each subsample could have had a slightly different composition.

Since the predictive equation described 47% of the observed variations in SGD, additional grain characteristics need to be measured to fully describe SGD. Further studies could move away from composition and examine the internal architecture of the endosperm. These would investigate how the endosperm cell architecture influences density or endosperm porosity. Porosity is known to affect grain density and hardness in wheat, so is therefore likely to influence barley SGD (Dobraszczyk et al., 2002). Grain hardness has previously been shown to have a relationship with malt quality. Therefore this could provide further links between characteristics affecting SW through SGD and ultimately influencing malt quality (Psota et al., 2007). In addition to porosity, the proportion of husk tissues to endosperm may also influence SGD and SW. In oats it has been demonstrated that the density of groat alone is greater than that of the oat kernel, implying the hull negatively contributed to SGD (Doehlert et al., 2009).

This study demonstrated links between grain composition and SGD, one of the components of SW. We have shown that grain N content, which can be potentially detrimental to malt quality, shows robust correlations with SGD in spring barley. Therefore when using SW as an indicator of malt quality it is important to determine how this SW has been achieved. If a high SW has been achieved due to an elevated N content and B granule volume this does not necessarily mean this grain is of the highest value for malting and other downstream uses. Therefore a more detailed understanding into how SGD or PE has resulted in a SW value needs to be known to link SW through to malt quality. It is possible that in the future, SW as a measure could be altered or replaced to account for these problems, a potentially improved measure of quality could be starch content (and composition of starch) per volume of grain.

3.5 Acknowledgements

3.6 References

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4 Chapter Four

Specific Weight of Spring Barley is Maintained Under Post-anthesis Water Stress

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Specific Weight of Spring Barley is Maintained Under Post-Anthesis Water Stress

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Abstract

Specific weight (SW) is a long established grain measure used as a malting quality specification in barley, with an increased SW thought to result in a higher malt output. Specific weight is a product of grain density and packing efficiency, however the grain physical and compositional characteristics that affect these are not yet established. We investigated the effect of moderate but prolonged post-anthesis water stress on cultivars with a range of grain characteristics and SWs. Water stress was chosen to influence these grain characteristics through decreased photosynthetic capacity. We examined relationships amongst different grain parameters, specifically how these correlate with SW and its components. We demonstrated that SW was maintained under water stress conditions through compensatory mechanisms such as increased tiller mortality which preserved grain physical parameters. However, water stress significantly affected plant development by reducing: grain filling duration, plant biomass, ear length, ear number and yield. Grain composition was also altered, with reduced carbon:nitrogen ratio in water stressed plants. This work shows that although SW can be conserved under water stressed conditions, grain composition and plant development are altered to balance source/sink availability. This could result in bulks of malting grain with the same SW having different malt outputs.

4.1 Introduction

Barley (*Hordeum vulgare* L.) is an important cereal crop worldwide. In 2017 a total of 147 million tonnes were harvested globally and in the UK 7 million tonnes were harvested from 1.2 million hectares (FAOSTAT, 2017). The majority of this is grown for feed and malting. Barley that is grown for malting is required to attain certain grain quality specifications and if these are achieved a premium is paid over barley destined for feed. One of these quality specifications is specific weight (SW), a measure of the weight of grain per unit volume, and is measured in kilograms per hectolitre (kg hl^{-1}). Specific weight is an established measure used throughout the cereal sector when grading quality. A high SW is thought to be indicative of higher quality grain which is associated with a high starch content, enhanced malt quality and/or malting efficiency. Therefore minimum SWs are specified in a contract between farmers and end users. Consequently farmers often strive to select cultivars with an inherently high SW and employ agronomic techniques such as cultivar selection and fertiliser regimes to keep SW high.

Specific weight is a complex quality trait determined by two components: grain density (GD) and packing efficiency (PE) of the grain (Hoyle et al., 2018). There is limited information concerning the grain parameters which determine these components. It is hypothesised that grain composition and internal structure influence GD, and grain morphology determines PE. By weight, barley grains are composed of 60 to 80% starch, 9 to 13% protein, 10 to 15 % water and 1 to 2 % lipids (Asare et al., 2011). It is these constituents that are thought to influence GD, and they are known to have impacts on the malting process and product yield. It is not only the absolute amount of these constituents that is thought to be important in malting but also the composition or fine structures of these molecules. For example, both the composition of starch in terms of the proportion of the polymers amylose and amylopectin,

and ratios of A and B starch granules, impact the fermentable sugars produced in the malting process (Yu et al., 2019).

Previous work has shown that within a cultivar if grains are graded by GD, there is no relationship between starch content and GD (Hoyle et al., 2019). Despite starch content not correlating with GD, there is evidence that starch composition may influence GD with the proportion of starch B granules positively correlating with GD (Hoyle et al., 2019). Furthermore, higher density grains have an increased nitrogen (N) content and this has been shown to explain nearly 50% of the observed variation in GD. An excessively high N content is undesirable for many malting uses (Hoyle et al., 2019). This highlights the potential role of N content in increasing GD and consequently SW. Furthermore longer, deeper grains have a reduced PE, resulting in a reduced SW for these larger grains (Hoyle et al., 2018).

The growth of barley plants and consequently the quality of grain produced by these plants is known to be influenced by an array of environmental conditions. High night time temperature after anthesis reduced grain weight and the duration of grain filling in barley (García et al., 2016). Shading of barley was found to reduce the rate of grain filling, but not affect the duration of grain filling (Kennedy et al., 2018). Grain weight and yield was reduced by a combination of post-anthesis water deficit and heat stress (Macnicol et al., 1993). Post-anthesis water stress has been shown to decrease grain width, but not grain length indicating that a reduction in grain filling affects grain width more than length (Afshari-Behbahanizadeh et al., 2016). Prolonged water stress throughout the grain filling period reduces the length of grain filling and yield (Samarh, 2005). Increased tiller mortality has been shown to be a significant contributor to the reduction in yield in water stressed plants through reducing total grain number (Samarh,

2005). These stresses are likely to influence grain development and consequently the PE of the grain.

Alongside physical characteristics, chemical and biochemical processes are also impacted by water stress. Grain starch content has been shown to be lower under water stressed conditions, negatively influencing yield (Thitisaksakul et al., 2012). Water deficit has also been shown to influence starch accumulation, composition, ultrastructure and functionality (Beckles and Thitisaksakul, 2014). Barley starch composition is thought to be more resilient to water stress than other cereals, with changes only noticeable after a more severe stress (Beckles and Thitisaksakul, 2014). Therefore water stress is likely to change grain composition, and consequently GD. In general water deficit throughout cereal development tends to result in a higher proportion of A-type starch granules, however the timing of the stress alters its effects, making predictions of the stress effect on malting quality difficult.

In order to enhance our current understanding of how changing plant growth and grain parameters influence SW, a stress known to have relevant physiological consequences to grain composition, water stress, was used as a tool to impose changes in plant development. The effect of water stress on SW has not been studied before, and understanding the mechanisms through which SW is achieved under changing environmental conditions is especially relevant due to climate change. Also quantifying the outcome of a water stress on both components of SW and cultivars with varying SWs is necessary to understand how changes in SW relate to grain quality for different markets. The aims of this study were to investigate the effect of moderate but prolonged water stress during grain filling on the grain quality measure SW, and the mechanisms influencing this measure. This was achieved by the following objectives: i) establish how water stress modified plant development, yield

components and grain composition that impact on SW, ii) evaluate changes in SW according to its components and traits which affect them and iii) investigate associations between grain parameters and the components of SW.

4.2   Materials and Methods

4.2.1   Plant Material and Growth

Three spring malting barley cultivars (Octavia, Concerto and Sienna) were used in this study were selected due to their different SWs. According to the Agriculture and Horticulture Development Board's (AHDB's) recommended list 2016; Octavia is a low SW cultivar, Concerto intermediate and Sienna high with reported SWs of 66.7, 68.8 and 70.7 kg hl^{-1} respectively. Seed was sourced from the AHDB and had been grown in the 2016 recommended list trial in Docking, Norfolk, UK under natural rainfall conditions. Experimental plants were grown under glasshouse conditions at Scotland's Rural College, Edinburgh from November 2016 to June 2017, in triplicate experiments as described below. The three experiments were sown on 21st November 2016, 18th January 2017 and 1st March 2017 respectively. From November until the end of April, plants were grown in a heated glasshouse (min temperature 16˚C). When natural daylight hours were insufficient light was supplemented artificially using 400 W sodium lights to give 16 h days with a photosynthetically active radiation at plant ear level of 150 µM m^{-2} s^{-1}. From 1st May plants were moved into an unheated glasshouse with no supplementary light. Seven grains of each cultivar were sown into each of 10 separate 5 L pots and grown in Levington's Advance M3 High Nutrient Potting compost containing ratios of 204 N, 104 P and 339 K (Levington Horticulture, Ipswich, UK). Five of these pots were allocated to a water stress treatment and five to a well-watered control treatment for each cultivar. There were therefore 30 pots in total. The five pots per treatment were expected to yield sufficient grain for one measurement of SW using a scaled down method comparable to industry standards (Hoyle et al., 2018). A complete randomized block

design with five blocks was used, with each cultivar: treatment combination represented once in each block and randomly assigned a position. The experiment was conducted three times in order to obtain replication with pot order randomly re-assigned in each block for each repetition.

4.2.2 Water Stress Treatment

All plants were grown under the same non-stressed conditions and watered daily until half of the main shoots in a pot reached anthesis, defined as growth stage 61 (Tottman, 1987). At this point pots were watered to field capacity and allowed to drain overnight and after which differential treatments were applied. Following anthesis, soil moisture readings were taken at least 6 days a week using a SM150 dielectric soil moisture sensor (Delta-T Devices Ltd.) with an attached HH150 hand moisture meter. Soil moistures were recorded as electrical conductivity (mV) from an average of three readings per pot and the manufacturers calibration used to equate this to volumetric water content. If a daily readings for volumetric water content was below 21% for well-watered pot, or below 10% for a water stressed pot, enough water was added to increase soil moisture above these threshold values which was determined by using the soil moisture sensor. In addition, at least every three days chlorophyll readings were taken using a SPAD-502 chlorophyll meter (Minolta, Japan). Readings were taken a third of the way up the penultimate leaf away from the main stem, described as leaf 2 (AHDB, 2015). For each pot the average of three readings from three labelled leaves was recorded. The experiment proceeded in this way until maximum grain dry weight had been reached for at least half of the main shoots in a pot, defined as growth stage 87 by Zodak's decimal code (Tottman, 1987). At this point all watering was stopped and plants were allowed to dry out prior to harvest.

4.2.3 Plant Growth Measurements

Plants were hand threshed and numerous measurements taken per pot. These were grain weight (± 0.0001 g), ear number per pot, ear length (± 1 mm), grain number per pot, grain number per ear, spike fertility which is measured as a proportion of total florets to grains, days of grain fill, plant biomass (± 0.01 g) and grain yield (± 0.0001 g). Grain moisture per pot was estimated by drying two centrally located grains in an ear from each pot in an oven at 130˚C for 20 h, and calculating the percentage weight loss from wet to dry. To enable harvest index to be calculated, shoots were dried at 70˚C for 48 h and weighed to give dry shoot biomass. Harvest index was then calculated as the ratio of harvested dry grain to total above ground shoot dry biomass.

4.2.4 Grain Sampling

Grain from each treatment and cultivar combination in each of the five blocks was pooled to give grain samples large enough to make SW measurements. Grain samples were cleaned by screening over a slotted 2.25 mm sieve with 19.05 mm long slots. SW was measured on this pooled grain using a scaled down published method which corresponds to the industry standard method (Hoyle et al., 2018). For further analysis to be conducted on grain samples from each cultivar and treatment combination a representative sample of grain was obtained by sequentially sieving each sample into size fractions using a stack of slotted 3.25, 3.00, 2.75 and 2.50 mm sieves, with 19.05 mm long slots. The weight of grain retained by each sieve fraction: extra-large (> 3.25 mm), large (3.25 to 3.00 mm), medium (3.00 to 2.75 mm), small (2.75 to 2.50 mm) and extra small (2.50 to 2.25 mm) was weighed (accuracy ± 0.01 g). Three 100-grain samples were weighed from each fraction to estimate the mean grain weight in each fraction. This was used to estimate the total number of grains in each fraction and a number proportional to the total number of grains from each fraction were chosen at random, to give two separate 100-grain samples with grain sizes representative of the original bulk.

4.2.5 Grain Morphometrics and Specific Weight Components

On the first 100-grain sample each grain was weighed and the grain dimensions length, width and depth were measured using a hand-held digital caliper (± 0.01 mm). These grains were placed onto an Epson Expression 836XL flatbed scanner alongside a ruler for scale. The 2-D area of the grains was estimated through image analysis in ImageJ (National Institutes of Health, USA, https://imagej.nih.gov/ij/). Circularity was calculated in ImageJ as $4\pi(area/perimeter^2)$. A value of 1.0 represents a perfect circle, the closer to 0.0 the more the shape represents an elongated polygon. Grain volume was measured on this grain sample using Archimedes' principle with the weight of water being displaced by a grain being equal to the volume of grain (± 0.0001 g) (Hughes, 2005). Grains were individually submerged into a crucible which was suspended in a beaker water, and the change in weight on the balance recorded after five seconds, grains were then removed and patted dry with paper towel.

4.2.6 Compositional Analysis

The second 100-grain sample was milled into a fine powder using a ball mill (Mixer Mill MM 200, Retsch, Germany). A FLASH 2000 Organic Elemental Analyzer (Thermo Scientific) was used to determine the proportion of carbon and nitrogen in the grain, usually referred to as the carbon and nitrogen content. Total starch content and the ratio of amylose and amylopectin were measured using Megazyme kits as previously described (Gibson et al., 1997; McCleary and Codd, 1997).

4.2.7 Data Analysis

All data were analysed in the open-source R software using version 3.4.1 (R Core Team, 2017). Linear mixed-effects model (LMM) analysis was used via the restricted maximum

likelihood algorithm (REML) in the 'lme4' package (Bates et al., 2015). An LMM was fitted for each measured trait in the experiment, listed as plant/grain parameters in **Table 4-1**. Cultivar, water stress treatment and their interaction were fixed effects and the replicate number of the experiment was the random effect. Model fits were compared by analysis of variance (ANOVA) on hierarchical models and non-significant variables (α = 0.05) were dropped sequentially until a minimally adequate model was reached for each parameter. The 'emmeans' package was then used to calculate 95% confidence intervals to determine significant differences among samples (Lenth, 2018). Only the explanatory variables required by the minimally adequate model were tested (**Table 4-1**), therefore letters of significance are the same within either treatment or cultivar in the cases where the interaction term was not significant, and only one of the individual explanatory variables were significant.

4.3 Results

4.3.1 Quantification of water stress

To record the effect of the water stress treatment on plant health, SPAD readings were taken every day or two from anthesis. These demonstrated that the water stress treatment resulted in a quickening in leaf senescence evidenced by a faster decline in leaf greenness. This indicated that the water stress treatment successfully reduced the total duration of photosynthesis in these plants (**Figure 4-1**). The mean volumetric water content (v/v) of pots over the grain filling period under well-watered and water stressed treatments showed that the treatment imposed significantly reduced water content from 23.27% in well-watered pots to 12.94% in water stressed pots ($P < 0.001$, **Figure 4-1**).
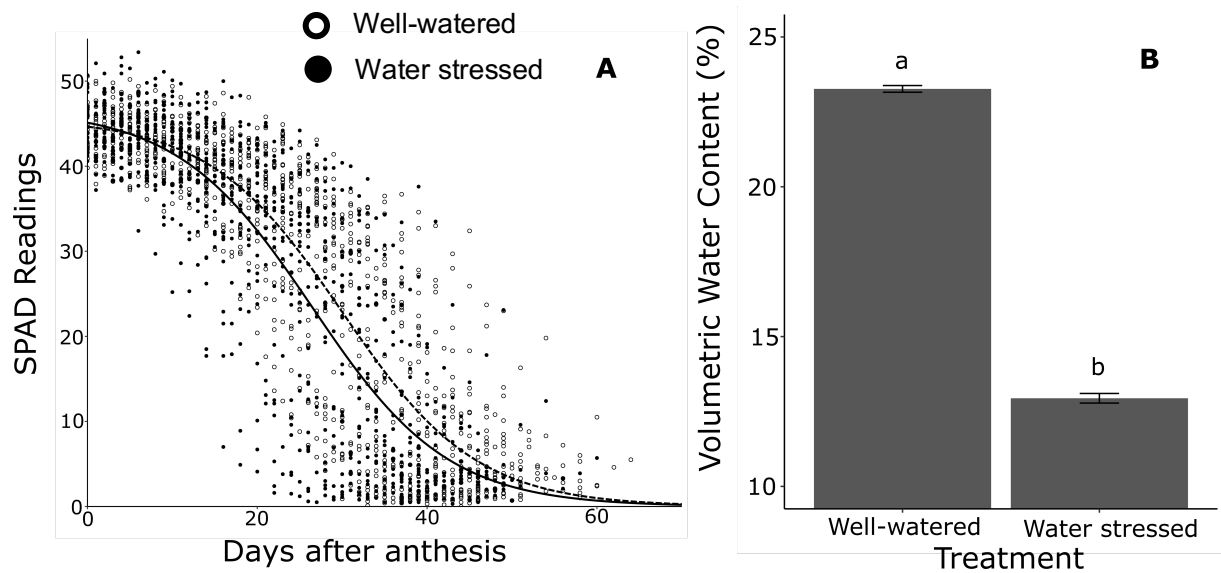
Figure 4-1 (A) The relationship between SPAD values and days after anthesis for experimental plants grown under well-watered (dashed line) and water stressed conditions (solid line). (B) The mean volumetric water content (v/v) of pots over the grain-filling period under well-watered and water stressed treatments.

4.3.2   The effect of water stress on plant development

Plant growth parameters including ear number, ear length, grain numbers, biomass, fertility and length of grain fill were measured on the three cultivars Octavia, Concerto and Sienna under well-watered and water stressed conditions. Mean values across the three reps for these plant growth parameters are provided in **Table 4-1** with levels of significance in **Table C-1** (see Appendix). The significant effects of water stress on plant growth parameters on a per pot basis with seven plants in each pot are summarised in **Figure 4-2**. The implementation of a prolonged water stress treatment decreased a number of plant growth parameters including: ear number from 30 to 22 ($P < 0.001$, **Figure 4-2A**), grain number from 529 to 391 ($P < 0.001$, **Figure 4-2B**), plant biomass from 26.25 g to 23.56 g ($P < 0.001$, **Figure 4-2C**), grain yield from 23.36 g to 18.26 g ($P < 0.001$, **Figure 4-2D**), harvest index from 0.43 to 0.40 ($P < 0.01$, **Figure 4-2E**) and the length of grain fill from 51 days to 45 days ($P < 0.001$, **Figure 4-2F**). The only plant growth parameter which increased with water stress was ear length which increased from 69.17 mm to 72.45 mm ($P < 0.01$, **Figure 4-2G**). There was no evidence that water stress had an effect on grain weight with well-watered plants having a mean grain weight of 47.13 mg and water stressed plants 47.64 mg. There was a significant interaction between cultivar × treatment for fertility, with fertility reduced under water stress in Octavia and Sienna but not Concerto ($P < 0.05$, **Table 4-1**).

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Table 4-1 Summary of mean values ± standard deviations for plant growth and grain parameters across three reps for the three cultivars and two treatment levels used in this study[a] (n=3).

Plant/Grain Parameters	Octavia		Concerto		Sienna	
	Well-watered	Water stress	Well-watered	Water stress	Well-watered	Water stress
Ear and grain traits						
Grain Weight (mg)	39.23 ± 0.61a	39.84 ± 4.32a	40.17 ± 3.49a	39.90 ± 0.81a	39.90 ± 3.42a	39.93 ± 2.58a
Ear Number per pot	29.67 ± 6.91a	22.73 ± 8.76b	31.60 ± 6.05a	20.47 ± 4.12b	28.40 ± 8.77a	22.80 ± 5.35b
Ear Length (mm)	73.42 ± 4.07ab	75.68 ± 4.48a	66.57 ± 6.96c	73.22 ± 7.70bc	67.51 ± 6.28c	68.44 ± 4.51bc
Grains per pot	510.67 ± 175.74a	374.33 ± 152.49b	534.53 ± 135.16a	387.07 ± 98.84b	542.27 ± 152.68a	412.73 ± 102.50b
Grains per ear	16.99 ± 2.46bc	16.40 ± 1.55c	16.83 ± 2.12bc	18.90 ± 2.67ab	19.41 ± 2.39a	18.12 ± 1.69abc
Fertility	0.81 ± 0.03bc	0.79 ± 0.03c	0.82 ± 0.03abc	0.85 ± 0.03ab	0.86 ± 0.03a	0.82 ± 0.03abc
Days Grain Fill	49.93 ± 4.06b	44.40 ± 5.22d	54.40 ± 4.85a	46.73 ± 6.15bc	50.13 ± 3.81ab	44.40 ± 6.01cd
Biomass partitioning						
Vegetative Dry Biomass (g pot^{-1})	24.80 ± 3.96b	22.26 ± 3.53cd	24.68 ± 3.71bc	21.93 ± 2.85d	29.26 ± 4.56a	26.47 ± 3.67b
Grain Yield (g pot^{-1})	21.81 ± 9.19a	17.07 ± 6.99b	23.51 ± 5.37a	18.42 ± 4.82b	24.76 ± 6.60a	19.27 ± 6.04b
Harvest Index	0.42 ± 0.06ab	0.39 ± 0.08b	0.45 ± 0.05a	0.42 ± 0.05ab	0.42 ± 0.05ab	0.38 ± 0.05b
Soil Moisture						
Volumetric water content (%)	23.51 ± 0.60a	13.13 ± 1.18b	22.99 ± 0.81a	12.65 ± 0.77b	23.3 ± 0.78a	13.04 ± 1.23b
Mean theta (mV)	220.77 ± 7.22a	114.72 ± 10.02b	214.61 ± 9.60a	110.55 ± 6.52b	218.27 ± 9.43a	113.93 ± 10.54b
Grain Size Classes						
>3.25 mm	8.29 ± 3.72a	5.30 ± 3.78a	5.96 ± 3.80a	5.95 ± 1.25a	8.94 ± 5.36a	8.33 ± 3.29a
3.00-3.25 mm	20.45 ± 4.93a	18.90 ± 10.89a	30.08 ± 21.33a	33.84 ± 12.90a	25.43 ± 10.11a	22.30 ± 8.90a
2.75-3.00 mm	27.56 ± 6.78a	35.62 ± 9.02a	31.09 ± 9.56a	34.30 ± 3.49a	30.15 ± 2.53a	26.36 ± 7.78a
2.50-2.75 mm	24.70 ± 3.99a	25.92 ± 7.40a	17.36 ± 9.91a	17.38 ± 9.51a	21.57 ± 6.84a	24.52 ± 8.01a
2.25-2.50 mm	18.99 ± 8.30a	14.26 ± 8.37a	15.52 ± 18.15a	8.53 ± 5.23a	13.91 ± 11.13a	18.48 ± 8.78a
Screenings	8.06 ± 5.46a	3.45 ± 2.18a	6.65 ± 9.33a	2.34 ± 1.13a	5.49 ± 6.53a	5.33 ± 2.89a
Grain Dimensions						
Length (mm)	9.09 ± 0.06a	9.08 ± 0.08a	8.77 ± 0.34b	8.80 ± 0.40b	8.96 ± 0.36ab	8.92 ± 0.36ab
Width (mm)	3.60 ± 0.08a	3.61 ± 0.10a	3.60 ± 0.14a	3.74 ± 0.04a	3.68 ± 0.12a	3.66 ± 0.09a

Depth (mm)	2.86 ± 0.06a	2.88 ± 0.14a	2.87 ± 0.15a	2.98 ± 0.07a	2.93 ± 0.16a	2.90 ± 0.10a
2D area (mm^2)	24.20 ± 0.27a	24.25 ± 0.82a	23.04 ± 0.99a	24.08 ± 1.11a	24.27 ± 0.29a	23.72 ± 0.17a
Circularity	0.56 ± 0.01b	0.57 ± 0.01b	0.59 ± 0.03a	0.59 ± 0.03a	0.59 ± 0.03ab	0.58 ± 0.02ab
Specific weight and components						
Specific Weight (kg hl^{-1})	62.73 ± 2.10b	63.76 ± 3.01b	64.12 ± 2.71ab	65.75 ± 1.57ab	66.86 ± 1.91a	66.69 ± 2.52a
Packing Efficiency (%)	52.84 ± 1.95a	51.95 ± 1.93a	51.26 ± 0.09a	52.96 ± 1.21a	52.72 ± 2.85a	53.08 ± 0.80a
Grain Density (g cm^{-3})	1.17 ± 0.02b	1.21 ± 0.02b	1.23 ± 0.05ab	1.25 ± 0.02ab	1.28 ± 0.05a	1.25 ± 0.05a
Composition						
Total starch	53.80 ± 0.14a	52.89 ± 1.00a	52.96 ± 2.04a	52.88 ± 0.90a	54.95 ± 2.17a	54.46 ± 2.35a
Amylose (%)	20.17 ± 1.66a	19.16 ± 0.86a	19.24 ± 0.95a	19.40 ± 2.03a	18.68 ± 1.32a	21.05 ± 0.77a
Carbon (%)	39.23 ± 0.89a	39.84 ± 0.02a	40.17 ± 0.85a	39.90 ± 0.12a	39.90 ± 0.04a	39.93 ± 0.15a
Nitrogen (%)	1.67 ± 0.23a	1.84 ± 0.04a	1.81 ± 0.32a	1.90 ± 0.12a	1.61 ± 0.17a	1.76 ± 0.26a
C:N	23.80 ± 2.87a	21.70 ± 0.50b	22.62 ± 3.56a	20.99 ± 1.21b	24.92 ± 2.61a	23.00 ± 3.11b

[a] Results which share a letter in a given row are not significantly different from one another after comparison of 95% confidence intervals of the linear mixed model.

4.3.3   The effect of water stress on grain parameters

Detailed grain parameters including grain size fraction distribution, screenings, dimensions, 2-D area, circularity, SW, PE, GD and composition were measured on the three cultivars Octavia, Concerto and Sienna under well-watered and water stressed conditions. Mean values across the three reps for these grain parameters are provided in **Table 4-1** with levels of significance in **Table C-2** (see Appendix).

Figure 4-2 shows that water stress had a significant effect on composition in terms of both nitrogen content and the ratio of carbon to nitrogen, these are summarised in **Figure 4-2**. However similarly to the effect of cultivar on harvest index, a post hoc multiple comparison test could not distinguish between N content in well-watered (1.70 %) and water stressed (1.83 %) grain. Well-watered plants did however have a significantly higher ($P < 0.05$, **Figure 4-2H**) carbon to nitrogen ratio with a ratio of 23.78 to a ratio of 21.90 for water stressed grains. Water stress did not have a significant effect on many grain parameters including size classes, dimensions, SW and its components.
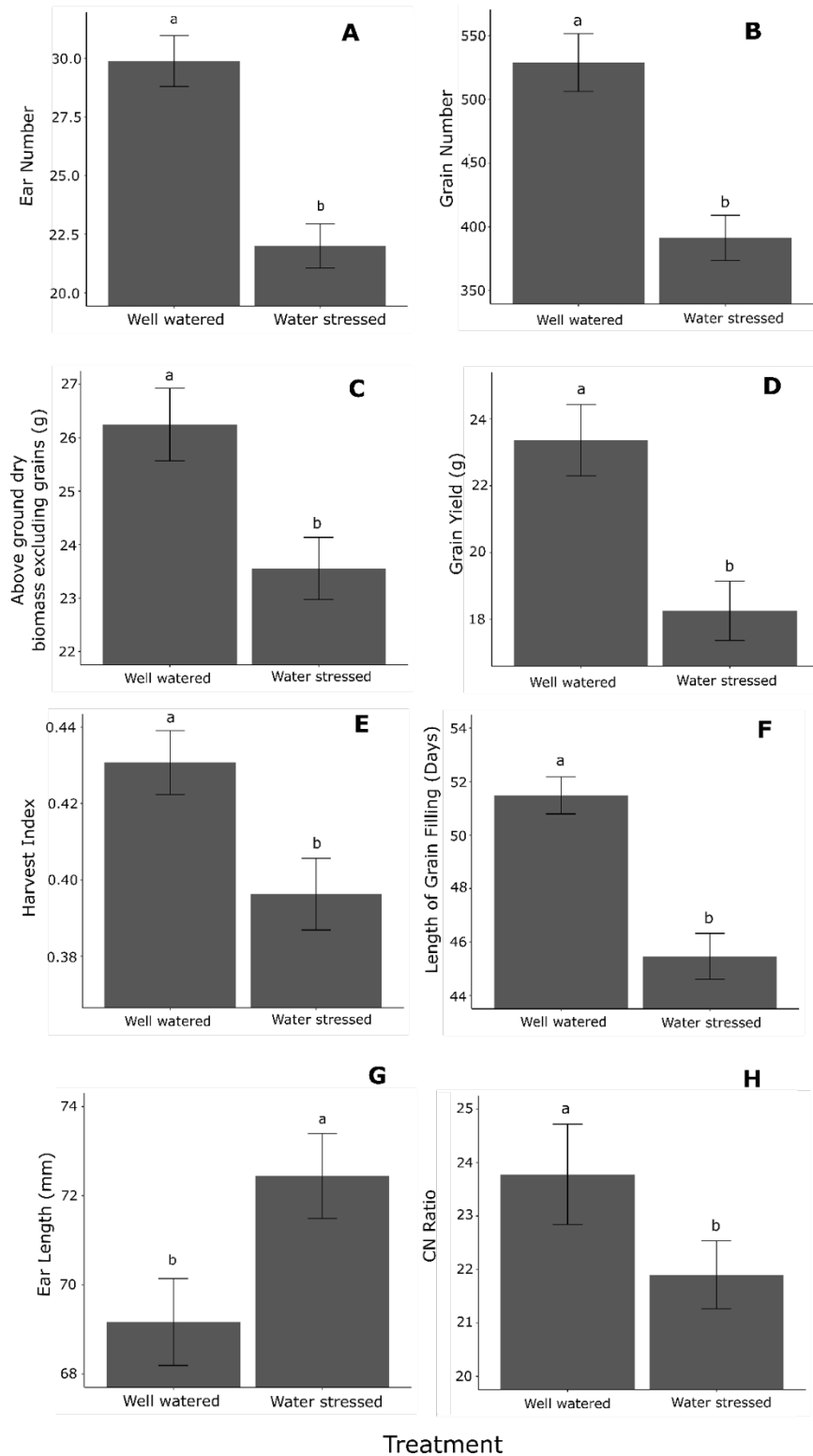
Figure 4-2 The significant effects of water stress on plant growth parameters (n=9): (**A**) ear number, (**B**) grain number, (**C**) above ground biomass excluding grains, (**D**) grain yield, (**E**) harvest index, (**F**) length of grain filling, (**G**) ear length and grain parameter (**H**) C:N ratio. Significant differences are indicated by different letters above bars (P < 0.05). Bars are the standard error of the means.

4.3.4   The effect of cultivar on plant development

The significant plant growth parameter differences due to cultivar on a per pot basis are summarised in **Figure 4-3**. The ear length of Octavia was significantly longer than both other cultivars with a length of 74.55 mm and lengths of 69.89 mm ($P < 0.01$, **Figure 4-3A**) and 67.97 mm ($P < 0.001$, **Figure 4-3A**) for Concerto and Sienna respectively. Sienna had a significantly greater dry plant biomass with 27.87 g than Concerto 23.30 g ($P < 0.001$, **Figure 4-3B**) and Octavia 23.53 g ($P < 0.001$). The length of grain filling was longest for Concerto with 51 days, which was significantly longer than that of Octavia with 47 days ($P < 0.05$, **Figure 4-3C**) but not significantly different from Sienna with 48 days. The length of grain fill was not significantly different between Sienna and Octavia. Linear mixed model analysis showed a significant effect of cultivar on harvest index, however no significant differences were displayed when multiple pairwise comparison tests were made. There was no evidence that the different cultivars had an effect on grain weight, ear number, grain number or grain yield.
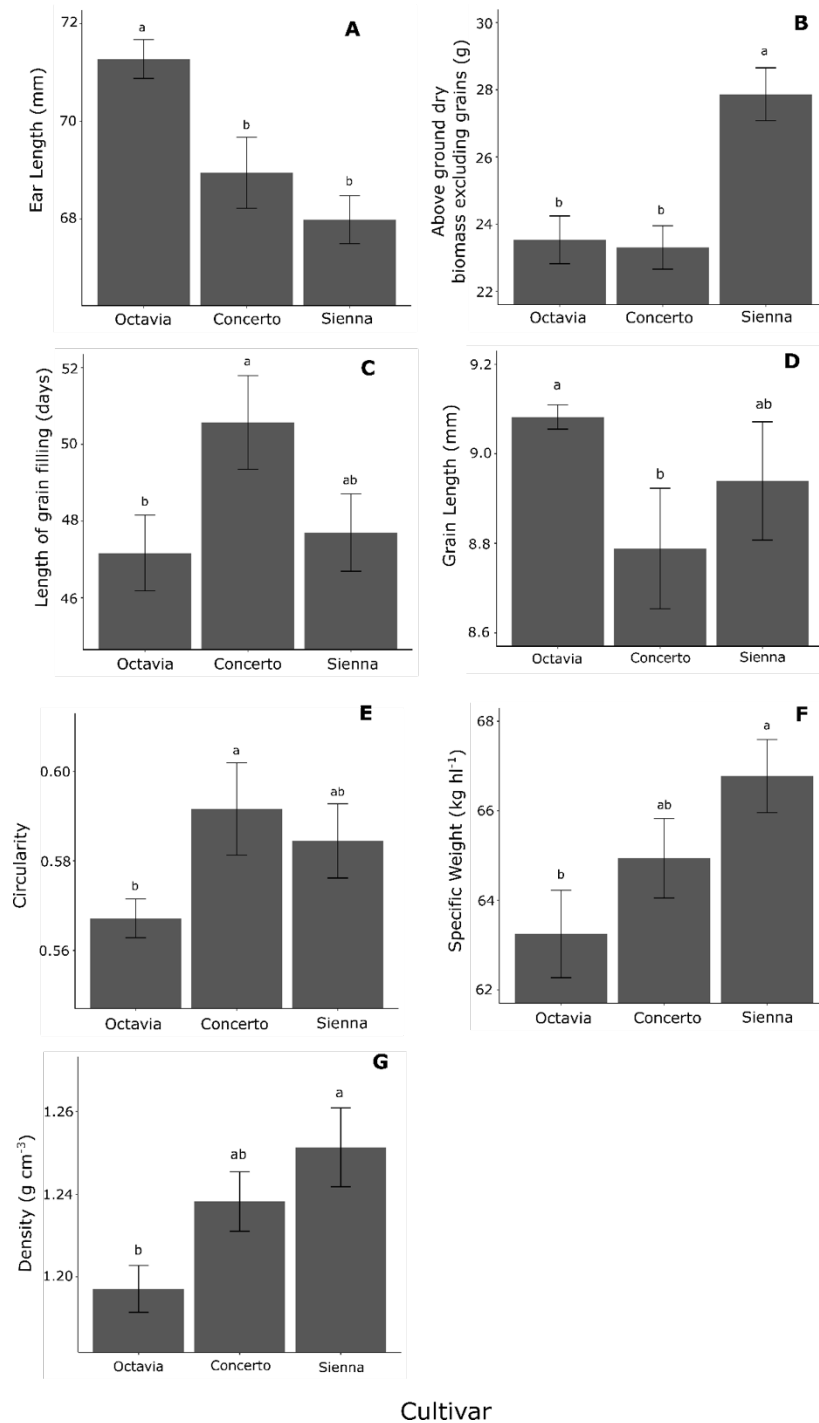
Figure 4-3 The significant effects of cultivar on plant growth parameters (n=6) (**A**) ear length, (**B**) above ground dry biomass excluding grains, (**C**) length of grain filling and grain parameters: (**D**) grain length, (**E**) circularity, (**F**) SW and (**H**) GD. Significant differences are indicated by different letters above bars (*P* < 0.05). Bars represent the means of stressed and unstressed pots, with arrows representing the standard error of the means.

4.3.5 The effect of cultivar on grain parameters

The significant effects of cultivar on grain parameters on a per pot basis are summarised in **Figure 4-3**. In terms of dimensions and morphology, cultivar had a significant effect on grain length with Octavia 9.08 mm being significantly longer than Concerto 8.79 mm ($P < 0.05$, **Figure 4-3D**), but Sienna's grain length 8.94 mm was not different to Octavia or Concerto. Cultivar had a significant effect on grain circularity with Concerto having the highest circularity 0.59 which is significantly higher than Octavia 0.57 ($P < 0.01$, **Figure 4-3E**). Sienna has a circularity of 0.58, but this did not differ significantly from the other two cultivars. Specific weight was significantly affected by cultivar, Sienna having the highest SW with 66.78 kg hl^{-1} which is significantly greater than Octavia with 63.25 kg hl^{-1} ($P < 0.001$, **Figure 4-3F**). The SW of Concerto, 64.94 kg hl^{-1} was not significantly different to the other two cultivars. Packing efficiency, one component of SW was not affected by cultivar, but GD (the other component of SW) was. Grain density was highest in Sienna with 1.26 g cm^{-3}, which was significantly higher than Octavia with 1.19 g cm^{-3} ($P < 0.01$, **Figure 4-3G**). The GD of Concerto was 1.24 g cm^{-3} which was not significantly different from the other two cultivars. Cultivar had no effect on size fraction distributions.

4.3.6 Correlations with components of specific weight

The significance of correlations between grain parameters were analysed and a matrix of the Pearson correlation coefficients (r) are provided in **Table 4-2** and the corresponding p-values are in **Table C-3** (see Appendix). Grain weight was shown to be strongly positively correlated with width (r = 0.89, $P < 0.01$) and depth (r = 0.84, $P < 0.01$) but not length. Grain length was negatively correlated with grain dimensions width (r = -0.51, $P < 0.05$) and depth (r = -0.53, $P < 0.05$). Grain width and depth are highly positively correlated with each other (r = 0.95, $P < 0.001$). This demonstrates that grain width and depth are tightly correlated with each other, but grain length is not correlated with either. Grain 2-D area is positively correlated with length (r = 0.7, $P < 0.01$) and has a slightly weaker but still positive correlation with volume (r = 0.48,

P < 0.05), but with neither width nor depth. Grain perimeter was positively correlated with length (r = 0.91, *P* < 0.01) and negatively so with depth (r = -0.53, *P* < 0.05). The strong positive correlation between circularity and grain weight (r = 0.64, *P* < 0.01) highlights that 'plumper' grains weigh more. Longer grains tend to be less dense (r = -0.56, *P* < 0.05), this could be a result of the husk not filling entirely at the extremity of the grain.

Table 4-2 Correlation matrix of Pearson correlation coefficients (r) for grain characteristics.

	Grain weight (mg)	Length (mm)	Width (mm)	Depth (mm)	Volume (mm³)	Area (mm²)	Perimeter (mm)	Circularity	Grain Density (g cm⁻³)	Packing Efficiency (%)	Specific Weight (kg hl⁻¹)	Nitrogen Content (%)	Carbon Content (%)	Starch Content (%)
Grain weight (mg)	1	-0.33	0.89**	0.84**	0.83*	0.32	-0.24	0.64**	0.48*	0.62**	0.84**	-0.47	-0.18	0.68**
Length (mm)		1	-0.51*	-0.53*	-0.03	0.7**	0.91**	-0.8*	-0.56*	-0.08	-0.65**	0.35	0.04	-0.30
Width (mm)			1	0.95***	0.79	0.08	-0.46	0.78***	0.36	0.57*	0.76***	-0.55*	-0.38	0.62**
Depth (mm)				1	0.79	-0.02	-0.53*	0.8*	0.27	0.62**	0.76***	-0.49*	-0.34	0.59**
Volume (mm³)					1	0.48*	-0.02	0.44	-0.09	0.81*	0.53*	-0.43	-0.31	0.63**
Area (mm²)						1	0.80	-0.38	-0.21	0.37	-0.09	0.13	0.02	0.05
Perimeter (mm)							1	-0.86	-0.43	-0.03	-0.55*	0.42	0.10	-0.30
Circularity								1	0.47	0.34	0.76***	-0.53*	-0.14	0.5*
Grain Density (g cm⁻³)									1	-0.17	0.67**	-0.18	0.13	0.23
Packing Efficiency (%)										1	0.53*	-0.24	-0.20	0.48*
Specific Weight (kg hl⁻¹)											1	-0.41	-0.04	0.64**
Nitrogen Content (%)												1	0.68**	-0.76***
Carbon Content (%)													1	-0.28
Starch Content (%)														1

"***", "**", "*" were significant at *P* < 0.001, *P* < 0.01 and *P* < 0.05 respectively.

A highly significant relationship between SW and the product of its components (r = 0.94, *P* < 0.001, **Figure 4-4**) PE and GD was observed. Grain PE positively correlated with grain weight (r = 0.62, *P* < 0.01) and depth (r = 0.62, *P* < 0.01). Specific weight correlates with all grain dimensions, positively so with width (r = 0.76, *P* < 0.001) and depth (r = 0.76, *P* < 0.001), negatively so with length (r = -0.65, *P* < 0.01). Specific weight also positively correlates with grain volume (r = 0.53, *P* < 0.05) and circularity (r = 0.76, *P* < 0.001).

Nitrogen content is negatively correlated with grain width (r = -0.55, *P* < 0.05), grain depth (r = -0.49, *P* < 0.05) and circularity (r = -0.53, *P* < 0.05) highlighting than thinner more needle-like grains have higher nitrogen contents. Carbon content has a strong positive correlation with nitrogen content (r = 0.68, *P* < 0.01). Starch content was positively correlated with numerous grain parameters including: grain weight (r = 0.68, *P* < 0.01), width (r = 0.62, *P* < 0.01), depth (r = 0.59, *P* < 0.01), volume (r = 0.63, *P* < 0.01), PE (r = 0.48, *P* < 0.05) and SW (r = 0.76, *P* < 0.01). The only other variable that starch content correlates with is nitrogen content, with which it has a strong negative relationship (r = -0.76, *P* < 0.001). Amylose and amylopectin had no significant correlations with any other variables.
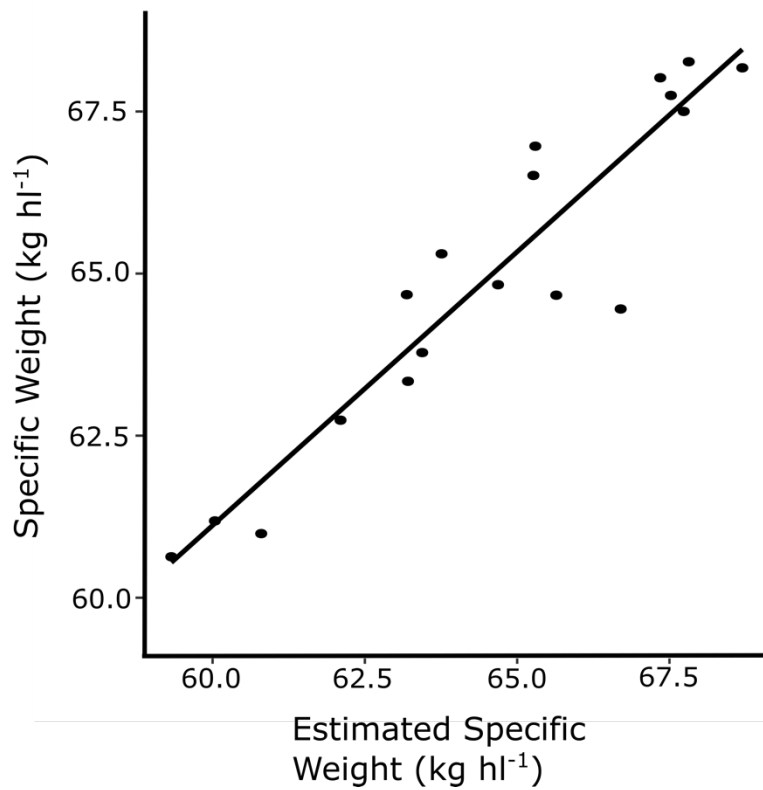
Figure 4-4 Specific weight of 18 barley grain samples plotted against the product of

packing efficiency and grain density. The linear relationship is shown by the solid black

line which has the equation, y = 0.84× + 10.15 (r^2 = 0.88, *P*<0.001).

4.4 Discussion

Specific weight is an established measure of grain quality across cereals. Although we understand that SW is influenced by its components GD and PE, little is known about how pre-anthesis plant development and post-anthesis grain development combine to determine the SW of a sample. In this study, the development of barley plants was significantly altered by water stress. Through investigating the effect of this stress on grain development we have enhanced the understanding of which grain parameters influence SW and how plants compensate to maintain certain characteristics under suboptimal conditions.

Interestingly SW was maintained under water stressed conditions, however many aspects of plant development were altered by water stress. A major effect of water stress on plant development was a reduction in ear number. Lower ear numbers had consequential effects on total grain number and yield, this is consistent with previous studies and is an important contributor to the reduction in yield experienced by water stressed plants (González et al., 1999; Samarh, 2005). The ears that were left on water stressed plants were longer, implying that tillers rather than primary shoots had an increased mortality. Plants were treated equally until anthesis, so this reduction in ear number but increase in ear length demonstrates a post-anthesis compensatory mechanism where tillers are aborted under water stressed conditions. This is hypothesised to occur so plants maintain grain weight when reduced photoassimilates are available under stressed conditions as a result of compromised photosynthesis. This response would be beneficial for plant progeny with more carbohydrates being available to fewer plant embryos instead of spreading resources more thinly across many embryos. Water stress also resulted in a shortening of grain filling by six days and a reduction in above ground leafy biomass, both expected effects of water stress on cereal development (Gooding et al., 2003; Samarh, 2005). Yield and above ground leafy biomass did not decrease by the same proportions because water stress reduced harvest index, demonstrating a reduction in

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reproductive efficiency as a result of stress. Similarly, harvest index has been shown to be lowered in rice when subject to a shading treatment, although unlike water deficit the shade treatment lengthened the grain filling period highlighting the contrasting effects on development of different stresses (Liu et al., 2019). A restricted water supply is known to cause osmotic stress in plant cells and consequently cell damage contributing to premature leaf senescence, whereas plants under shaded conditions receive less photosynthetically active radiation reducing plant growth and the rate of grain filling.

Alongside water stress, different cultivars also resulted in significant differences in plant development. Cultivars varied in ear length, biomass and the length of grain fill. Although studying plant development is important to understand the effects of water stress and cultivar, it is how development changes grain parameters that is of most importance to enhance the understanding of SW. Grain morphology was influenced by cultivar but not treatment with Octavia having longer, less circular grains in comparison to Concerto. The existence of a cultivar effect on grain morphology is consistent across different cereal species demonstrating the genetic basis of grain dimensions which is being controlled by multiple genes known as quantitative trait loci (QTLs) (Huang et al., 2013). The SWs of cultivars followed the same rank order as parent material, highlighting the strong and consistent genetic influence on SW. Quantitative trait loci have been detected which are associated with grain size in barley, with one of the components of SW being PE which is highly influenced by grain dimensions it is likely that there are QTLs in barley which partly control SW (Walker et al., 2013). Therefore with SW remaining a breeding target, research into discovering these related QTLs would provide a useful molecular genetic basis for improving this measure of grain quality. The same rank order was exhibited by GD for the three cultivars, this and how GD correlates more strongly with SW than PE suggests that GD may contribute more proportionally to SW.

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Previous work has shown that GD contributed to 48.5% of the variation in SW and PE to 36.5% further highlighting a slight dominance in GD over PE in determining SW (Hoyle et al., 2018).

Alongside plant development water stress also influenced grain composition with a reduction in the C:N and increase in N content by 0.13%. Grain composition is thought to be impacted by environmental conditions to a greater extent than morphology, despite this, QTLs have been identified for grain protein content. With nitrogen content known to significantly affect GD it is likely these QTLs are also related to SW, which could contribute to molecular breeding. Furthermore this work has emphasised that grain morphology and composition are related so should be discussed in tandem (Mather et al., 1997). Strong correlations between starch content and grain width, depth and volume indicate that either starch accumulation in grains can result in these plumper grains, or plumper grains facilitate an enhanced storage of photoassimilates. Numerous genes have been identified in rice which are associated with grain shape: *GRAIN SIZE 3* is a QTL for rice grain length and weight, GRAIN WIDTH 2 and GRAIN WIDTH 8 are QTLs for width and weight (Huang et al., 2013).

Grain weight was not affected by the water stress treatment, this was an unexpected result. However this could be explained by the reduction in ear number, so despite having reduced photosynthetic assimilates due to the stress, the plant total sink size was reduced so the same grain weight could be attained across fewer grains. Previous studies have shown that despite a reduced grain filling period as a result of water stress in cereals, the rate of grain filling in this period was enhanced due to increased remobilization of carbohydrates from the stem providing evidence to support this hypothesis (Zhang et al., 1998). On the other hand when a water deficit stress was imposed at pre-anthesis grain yield, number and weight were all reduced (Al-Ajlouni et al., 2016). This implies that when water stress occurs post-anthesis,

plants are able to compensate by utilising more carbohydrate reserves to maintain grain weight than under non stressed conditions. However when this stress occurs pre-anthesis grain weight is sink-limited, this provides further evidence to support the hypothesis that grain weight is set pre-anthesis for cereals (Millet, 1986). Interestingly, cultivars which exhibit "stay-green" characteristics under water stressed conditions can buffer against the detrimental effects of the stress. In comparison to non-"stay-green" cultivars they maintain grain quality under stress in terms of lower grain N content and similar starch molecular structures as non-stressed plants (Gous et al., 2013).

Through a correlation analysis it was possible to investigate which grain parameters correlated with SW and its components. The results reinforced previous studies which have shown that SW is determined by PE and GD showing a very strong relationship between the product of these and SW (Hoyle et al., 2018). This also showed that the relationship between components and SW remains accurate across well-watered and water stressed conditions. Interestingly wider and deeper grains have a higher PE, indicating plumper grains can pack more efficiently, this has previously been associated with SW but not PE (Atkinson and Kettlewell, 2008). A new finding from this work is that GD and grain length are negatively correlated with each other, further work would need to be done to determine why this is. However it could be a result of these longer grains having a greater capacity to store photoassimilate, but have become source limited which has meant they have not filled densely with starch. This is highlighted by the positive correlation between grain width, depth and starch content, but the negative correlation with length. The only compositional aspect which correlated with SW was starch content, this provides evidence for the traditional opinion that SW is associated with an increased starch content in the grain. The multifaceted nature of how many grain parameters influence SW is apparent in this analysis with SW significantly correlating with 10 grain parameters.

In conclusion, barley SW can be maintained in response to water stress by compensatory response mechanisms. However despite SW being maintained this does not necessarily mean the grain from water stressed plants is of the same quality. This was demonstrated by the decrease C:N and increased N content in grains from water stressed plants, despite there being no difference in SW. The observed increase in N content from 1.70% to 1.83% under water stressed conditions is an appreciable difference in the malting industry. For example according to the Maltsters Association of Great Britain this increase would result in this grain being rejected for the brewing industry with targets set of 1.60% to 1.75%. Therefore this work has continued to highlight the complexity of SW and its use as a malting quality criterion.

4.5 Acknowledgements

4.6 References

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5 Chapter Five

Relationship Between Specific Weight of Spring Barley and Malt Quality

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Relationship Between Specific Weight of Spring Barley and Malt Quality

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Abstract

The assessment of malting barley to determine if it meets grain quality requirements is an integral step in ensuring an efficient malting process and a good quality malt output. Specific weight (SW) is an industry standard criterion, however links between SW and malting are not well understood. In this study the effect of a changing SW on malting was investigated. Samples were manipulated according to both grain size and weight, creating grain fractions with a range in SW. Prior to malting, grain quality traits were measured, and after malting, malt quality traits were examined. Increased SW resulted in a reduced number of whole corns in malt, implying increased levels of modification. Specific weight correlated with both hot water malt extract (r=0.82, *P*<0.01) and predicted spirit yield (r=0.84, *P*<0.01), this highlights an increased malt output. Furthermore peak gelatinisation temperature of extracted starch from the malt correlated with both SW (r=0.69, *P*<0.05) and grain density (r=0.65, *P*<0.05). This could benefit malt efficiency by increased conversion of starch to fermentable sugars, but with the same energy input. The changes in SW and consequently malt output in this study are a result of changing grain density rather than packing efficiency.

Keywords: Barley (*Hordeum vulgare* L.); grain size; malt quality, specific weight, grain density

5.1 Introduction

Barley (*Hordeum vulgare* L.) is an ancient cereal crop; it was domesticated in the fertile crescent 10,000 years ago and has remained an important crop ever since. In 2018 the global harvest of barley was 141 Mt, placing it fourth in terms of crop production worldwide (FAOSTAT, 2020). The primary use for barley is as a livestock feed which accounts for roughly two thirds of its usage, one third is used for malting and 2% is used directly for human consumption (Baik and Ullrich, 2008). However in Scotland, barley is the main cereal crop grown, accounting for 68% of the total area of cereal grown in 2019, of this, 80% is planted with spring barley (The Scottish Government, 2019).

Barley is the preferred cereal crop for the malting industry. Its physical, physiological and biochemical characteristics are well suited to malting and downstream processes such as brewing and whisky distilling. The barley-malt-whisky supply chain forms an important part of the Scottish economy (Gupta et al., 2010). The key difference between barley destined for malt or feed are the quality requirements which the grain has to meet to be accepted for malting. Quality requirements include cultivar selection, germination rate, protein content, moisture content, grain uniformity, specific weight (SW), quantity of screenings and levels of damaged grains (caused by disease, mechanical damage or weathering) (Brewing and Malting Barley Research Institute (BMBRI), 2010). These requirements are decided upon in contracts between growers and maltsters, if met a premium is paid for the grain. It is understood that these traits are directly related to the processing efficiency and/or malt output. Despite SW being used as a grain quality trait in malting for many years, direct links between this trait and malt quality are not well understood. However, SW remains a breeding target, which is routinely measured and listed alongside screenings and nitrogen content in the Agriculture and Horticulture Development Board's (AHDB) Recommended List (RL), as one of the few grain quality traits for spring barley.

Chapter 5. *Relationship Between Specific Weight of Spring Barley and Malt Quality*

Specific weight is the mass of grain per unit volume and is measured in kilograms per hectolitre (kg hl^{-1}). It is also referred to as test weight, bushel weight and hectolitre mass in the literature. Specific weight is quick and easy to measure during the intake of barley at a maltings, in comparison to other measures of grain quality, such as starch content. Specific weight is measured using either a chondrometer or devices calibrated against this instrument (Manley et al., 2009). Previous work to enhance the understanding of SW has demonstrated that this trait is a product of barley grain density (GD) and its packing efficiency (PE) (Hoyle et al., 2018). These two components of SW can change independently, therefore both need to be considered jointly in future studies on SW. Grain density is thought to be determined by grain composition and the internal architecture of the grain. A previous study showed that when a grain sample is stratified by ascending GD, this is associated with an increase in grain nitrogen (Hoyle et al., 2019). However, when different cultivars without this stratification process are compared, the relationship between GD and grain nitrogen is not maintained. This demonstrates that even though within a cultivar this relationship is maintained, it is not maintained across different cultivars (Hoyle et al., 2019). The PE of grains on the other hand is thought to be determined by a combination of the following parameters: grain dimensions, ratios of these dimensions, grain shape, uniformity of these within a sample and also surface textures (Hoyle et al., 2018).

Malting is the controlled germination of cereal grain which takes place in three stages i) steeping, ii) germination and iii) kilning. During germination the starchy endosperm undergoes modification, a key step in achieving good malt quality. Modification of the endosperm is a result of the activity of enzymes primarily produced within the aleurone layer (Palmer, 2017). Modification involves the breakdown of both cell walls by hydrolytic enzymes such as β-glucanases and pentosanases, and hordein proteins by proteolytic enzymes, into soluble

peptides and associated amino acids (Baxter, 1981; Palmer, 1993). Modification is an essential part of malting, which makes starch stored within endosperm cells available for later gelatinisation during mashing, and also releasing nutrients which are metabolised by yeast. Diastase enzymes (e.g. α-amylase, β-amylase and limit dextrinase) are also produced during endosperm modification, these are later utilised in mashing to convert the dissolved starch into numerous sugars including: maltose, sucrose, dextrins and glucose. The power of these enzymes to breakdown starch is referred to as the diastatic power (DP), a malt quality parameter.

A range of additional quality assessments are carried out on malted grain. Hot water extract (HWE) is an important malt quality parameter which measures the amount of dissolved solids within the wort, the sugary liquid created by the mashing of ground malt (grist) and hot water (Briggs, 1998). These dissolved solids are primarily fermentable sugars but also consist of nitrogenous compounds and polyphenols. Mashing is typically carried out at 65°C, which is just higher than the typical gelatinisation temperature of barley starch 62°C (Macgregor et al., 2002). This is an integral step in both beer and whisky production, which gelatinises complex starch into simpler fermentable sugars, which can then be utilised by yeast. Starch gelatinisation occurs over a range of temperatures, exhibiting a normal distribution. The temperature at which barley starch begins to gelatinise, the peak of its gelatinisation and also the conclusion of gelatinisation, are influenced by both barley genotype and environmental growth conditions (Tester, 1997). Therefore these gelatinisation properties of starch show seasonal variation and are also be considered malt quality parameters. The SW of barley adjuncts (additional unmalted grains) have previously been reported to show a positive correlation with HWE, but this has not been demonstrated for the SW of the malting barley itself (Agu, 2008). Specific weight is thought to be associated with a higher starch content. Therefore this positive relationship is predicted to be maintained with malting barley SW

because of an increased amount of starch potentially contributing to more dissolved sugars in the wort, and hence a higher HWE. Predicted spirit yield (PSY) is another malt quality parameter and is calculated using the fermentable extract, but does not include unfermentable dissolved solids, such as complex sugars. Therefore PSY is influenced by the total quantity of dissolved solids but also the levels of gelatinisation of the starch. The interaction between SW and PSY has not been established, but a positive relationship is expected due to higher SW barley samples being associated with elevated levels of starch. To understand links between SW and malting output or efficiency, the influence of GD and PE have to be considered, as grain composition and grain packing within the bulk both influence malting.

The primary focus of this work is to study the effects of SW and its components GD and PE on the malting process. This will be addressed through the following aims: (1) alter SW and its components through the manipulation of grain size and grain weight, (2) determine the malting quality of grain samples with different SWs and/or components and (3) examine correlations between grain parameters and malt quality parameters to establish links between SW and malt quality. This work should help provide improved information about how SW can be used in the grading of malting barley and the impact it has on the malting process.

5.2 Materials and Methods

5.2.1 Plant Material and Sample Preparation

Commercial spring barley (*Hordeum vulgare* L.) samples were obtained from Bairds Malt (Witham, UK); 20 kg of the cultivar Concerto and 5 kg of the cultivar Sienna. The samples were harvested from across Scotland in the 2018 season. Samples were cleaned over a 2.25 mm slotted sieve with 19.05 mm long slots to remove screenings. Sienna was used as received with no further selection for different grain sizes. Concerto was used both as

received, and also after sorting based on both size and weight as described in the following sentences, in order to create fractions of grain with different SWs. Firstly, 1.5 kg of Concerto was removed for the "as received" fraction to maintain its natural grain size distribution. The remaining 18.5 kg of Concerto grain was sequentially sieved over 2.25, 2.50, 2.75, 3.00 and 3.25 mm wide slotted sieves with 19.05 mm long slots in order to sort the grain based on size. Grains retained by these sieves were labelled as size fractions A, B, C, D and E respectively. Additional fractions were then created by separating fractions B and D into two; first the mean grain weight of fractions B and D were measured, then grains were sorted individually based on whether their weight was above or below the mean weight of the corresponding fraction. The mean grain weight was calculated from three separate 100-grain subsamples from fractions B and D (Mettler AE 160 electronic balance, Mettler-Toledo, accuracy ± 0.0001 g), giving mean individual grain weights of 35.50 and 49.99 mg for fractions B and D, respectively. Fraction B1 contained grains weighing less than 35.50 mg, and fraction B2 contained grains weighing more than that weight. Fraction D1 contained grains that weighed less than 49.99 mg, and fraction D2 contained grains that weighed more than that weight. This resulted in the production of the 10 fractions listed in **Table 5-1**.

Table 5-1 Descriptors of sample fractions for miromalting.

Cultivar	Fraction	Size (mm)	Weight selected by (mg)	Contribution to mix (%)[a]
Concerto	A	2.25 to 2.50		5.5
Concerto	B1	2.50 to 2.75	≤35.50	14.5
Concerto	B2	2.50 to 2.75	>35.50	
Concerto	C	2.75 to 3.00		26.4
Concerto	D1	3.00 to 3.25	≤49.99	
Concerto	D	3.00 to 3.25		35.5
Concerto	D2	3.00 to 3.25	>49.99	
Concerto	E	>3.25		9.1
Concerto	Mix	Mix		100
Sienna	Mix	Mix		100

[a]% Contribution is by fraction weight to show the relative contribution of each fraction to the natural mix

5.2.2 Grain Analyses

Specific weight of each fraction was measured using a scaled down method in a 25 ml measuring cylinder which was previously shown to be representative of the industry standard (Hoyle et al., 2018). Two 100-grain samples were removed from each sample. One of these samples was milled into a fine flour using a ball mill (Mixer Mill MM 200, Retsch, Germany). This flour was used to determine the proportion of carbon (C) and nitrogen (N) in the grain with a FLASH 2000 Organic Elemental Analyser (Thermo Scientific). Using the other 100-grain sample, grains were individually weighed on a Mettler AE 160 electronic balance. Grain volume was also measured on these 100-grain samples according Archimedes' principle using a previously described technique, and from this GD was calculated (Hoyle et al., 2019). Packing efficiency was then calculated using the same method as previously described (Hoyle et al., 2018).

5.2.3 Micromalting

Laboratory micromalting and malt analyses were performed using equipment at the Scotch Whisky Research Institute (SWRI, Roberston Trust Building, Research Avenue North, Riccarton). Five hundred grams of grain was used for each micromalting run, from each of the 10 fractions after SW and grain analyses were measured. The micromalting was performed in three runs for each fraction of grains. Micromalting was carried out in a Curio Malting (Milton Keynes, UK) MMSG Steep and Germinator 4 tank system, each tank containing space for four grain samples. In each run the position of the different fractions of grain samples both within the tanks and across tanks was randomly allocated. The same micromalting regime was used for all batches, which consisted of a first steep for 8 h at 17°C, 16 h of air rest at 17°C, a second steep for 24 h at 17°C and finally 96 h of germination at 17°C. Malt was then kilned in a MMK four unit kiln (Curio Malting) at 55°C for 16 h, then 75°C for 10 h. This was followed by

deculming over a 2.2 mm sieve for two minutes. This created a total of 30 malt samples for malt analyses. Prior to analysis samples were stored in sealed bags to preserve their integrity.

5.2.4 Malt Analyses

5.2.4.1 Moisture and Nitrogen Analysis

Malt samples were first analysed by NIR using an Infratec 1241 Grain Analyser instrument (Foss Analytics, UK). From this, malt moisture, total N and soluble N were determined using a barley malt specific calibration based on data from spectral libraries, pairing NIR and laboratory based techniques.

5.2.4.2 Friability and Homogeneity

A subsample of malt (50 g) was loaded into a Friabilimeter (Pfeuffer, Germany) and the machine ran for 8 minutes. The material retained by the drum was weighed (accuracy ± 0.01 g) and friability (%) assessed (Baxter and O'Farrell, 1983). The non–friable fraction was then shaken over a 2.2 mm slotted sieve until no more material would pass through. Material retained by the sieve was weighed (accuracy ± 0.01 g) and homogeneity (%) calculated (Baxter and O'Farrell, 1983). Any remaining whole grains were then counted and weighed (accuracy ± 0.01 g) and recorded as the number of whole corns (Wc) and weight of whole corns.

5.2.4.3 Viscosity

The viscosity of samples was also measured using a Newport Scientific Rapid Visco Analyser (RVA). Malt was milled to 0.2 mm and then 0.1 mm to ensure a fine grind using a Bühler Miag disc mill. Approximately 9.3 g of this was adjusted for moisture in accordance with the manufacturer's instructions and was mixed with approximately 18.7 g of water and processed in the RVA, using a previously described malted barley specific 30 minute program (Agu et

al., 2007). Three variables from the RVA were analysed: i) peak temperature, which is the temperature at which peak viscosity was reached for the sample, ii) pasting temperature, which is the temperature at which the viscosity starts to increase and iii) pasting time, the time to peak viscosity.

5.2.4.4 *Hot Water Extract and Predicted Spirit Yield*

To determine HWE and PSY 50 g of malt was milled to 0.7 mm and then mashed for 1 h in 360 ml of water at 65°C using the Mash Bath – R8 (1-CUBE, Czech Republic). Samples were gradually cooled over a 20 minute period to 20°C and held at this temperature for 10 minutes. Samples were then made up to 450 g with water and shaken for 4 to 5 minutes, followed by filtering using Ederol 12 folded filter paper (Rudebeck). The density of 50 ml of the filtered wort was measured using a Paar DMA 5000 density meter (Anton Paar Ltd, UK). From this the HWE was calculated. A 200 ml volume of wort was then pitched with 1.00 g of yeast, and the 44 hour fermentation carried out in a water bath at 33°C. This wash was then filtered using Whatman 2V folded filter papers and the density of the solution collected was measured with an Anton Paar 5000 density meter. Using this value and the previously measured wort density the PSY was calculated.

5.2.5 **Statistical Analysis**

All data analysis was carried out in R software version 3.6.1 (R Core Team, 2019). Data were analysed by using analysis of variance (ANOVA) ($\alpha = 0.05$) using linear models to determine whether grain fraction had a significant effect on either grain parameters or malt quality parameters. Where a significant effect was indicated by the ANOVA, a post-hoc Tukey's Honestly Significant Difference (HSD) ($\alpha = 0.05$) test was used to show which fractions differed from each other in the parameters measured. Pearson product-moment correlation

coefficients were calculated between all variables measured in this study to produce a matrix using the 'corrplot' package (Wei and Simko, 2016). Principal component analysis (PCA) was used with mean values for Wc, SW, PSY, HWE and homogeneity. Plots of scores were created using the 'factoextra' package (Kassambara and Mundt, 2019) to investigate the relationship between grain fractions and grain characteristics and malt parameters.

5.3 Results

5.3.1 Grain Parameters

Prior to malting, grain parameters including weight, volume, density, SW, C content, N content and C:N were measured on ten fractions across three micromalting repetitions. The mean values of each fraction, and significant differences among fractions for these parameters, are displayed in **Table 5-2**.

Table 5-2 Mean values[a] for grain parameters measured on the ten grain fractions[b] used in this study.

Fraction	Weight (mg)	Volume (mm^3)	Density (g cm^{-3})	Packing Efficiency (%)	Specific Weight (kg hl^{-1})	Carbon (%)	Nitrogen (%)	C:N
A	29.06±0.76h	26.66±0.18h	1.09±0.02a	54.91±0.42b	58.97±0.96d	40.05±0.14a	1.41±0.03a	28.37±0.82a
B1	32.54±0.28g	30.63±1.42g	1.10±0.01a	57.52±2.66ab	60.82±0.18d	39.86±0.28a	1.32±0.02ab	30.19±0.49a
B2	39.08±0.62f	34.18±0.44f	1.15±0.03a	56.57±1.16ab	64.97±0.68bc	39.66±0.32a	1.33±0.04a	29.88±1.16a
C	43.04±0.38e	37.72±0.28e	1.11±0.06a	56.91±0.71ab	64.73±0.51c	39.94±0.03a	1.35±0.03a	29.68±0.34a
D1	46.63±0.22d	41.02±0.92d	1.14±0.02a	56.77±1.20ab	64.02±0.79c	39.76±0.22a	1.35±0.05a	29.50±0.79a
D	50.27±0.22c	44.01±0.65c	1.14±0.02a	56.96±0.89ab	65.25±0.75abc	39.51±0.45a	1.34±0.05a	29.41±0.60a
D2	53.95±0.09b	46.22±0.07b	1.17±0.00a	56.87±0.60ab	66.98±0.32a	39.69±0.39a	1.40±0.10a	28.51±1.27a
E	57.94±0.54a	50.87±0.63a	1.14±0.01a	57.29±1.09ab	65.80±0.33abc	39.52±0.22a	1.35±0.01a	29.32±0.20a
Concerto Mix	45.83±2.32de	40.73±1.88c	1.15±0.03a	59.12±0.47a	64.02±0.47c	39.85±0.21a	1.34±0.02a	29.77±0.34a
Sienna Mix	53.65±1.81b	45.21±1.31b	1.17±0.02a	59.30±0.98a	66.83±0.98ab	39.32±0.35a	1.23±0.08b	32.02±1.50a

[a] Mean values (n=3) are expressed as mean ± standard deviation.

[b] Fractions which do not share a letter for each of the measured parameters are significantly different from one another.

As expected, in fractions with increasing grain size, grain weight and grain volume increased from 29.06 mg and 26.66 mm^3 in fraction A to 57.94 mg and 50.87 mm^3 in fraction E. Significant differences were also observed between the two mixed fractions with Concerto Mix having a mean grain weight of 45.83 mg and volume of 40.73 mm^3, compared to Sienna Mix having a mean grain weight of 53.65 mg and volume of 45.21 mm^3. Grain density ranged from fraction A with 1.09 g cm^{-3} to fraction D2 and Sienna Mix both with densities of 1.17 g cm^{-3}, however this difference was not significant. Through sequential sieving and creating these fractions SW was significantly affected (**Figure 5-1A**). Fractions A and B1 were significantly lower than all other fractions, with SWs of 58.97 and 60.82 kg hl^{-1}, respectively. Fraction D2 had the highest SW with 66.98 kg hl-1 which was significantly higher than Concerto Mix, fraction D1, C, B2, B1 and A. Both mixed fractions, Concerto and Sienna had the highest packing efficiencies of 59.12 and 59.30% respectively. These were significantly higher than fraction A with 54.91%. No significant differences were observed between fractions for C content or C:N. Nitrogen content was lowest in the Sienna Mix fraction with 1.23%, this was significantly lower than all other fractions excluding fraction B1.
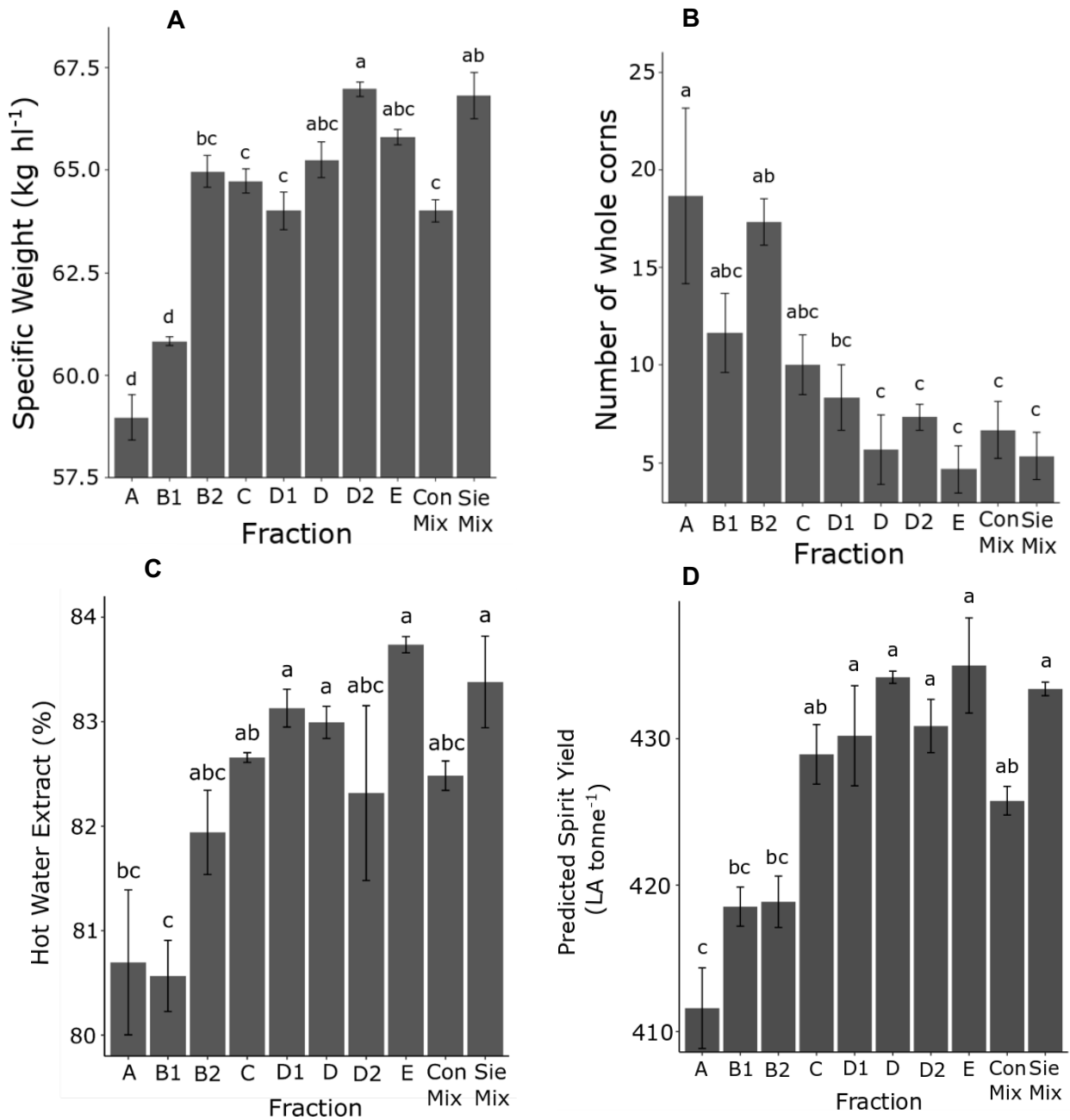
Figure 5-1 Mean values (n=3) of (A) whole corns, (B) specific weight, (C) hot water extract and (D) predicted spirit yield. Error bars represent ± standard error of the mean (n = 3). Grain fractions with different letters are significantly different at P < 0.05.

5.3.2 Malt quality parameters

Malt quality parameters including PSY, HWE, friability, homogeneity and nitrogen were measured on ten fractions across three micromalting repetitions. The mean values of each fraction, and significant differences among fractions for these parameters, are displayed in **Table 5-3**.

Table 5-3 Mean values[a] for malt and starch quality parameters measured on the ten grain fractions[b] used in this study.

	Malt									Starch	
Fraction	Soluble Nitrogen (%)	Total Nitrogen (%)	Soluble Nitrogen Ratio (%)	Friability (%)	Homogeneity (%)	Number of Whole Corns	Moisture (%)	Predicted Spirit Yield (LA/tonne)	Hot Water Extract (%)	Peak Gelatinisation Temperature (ºC)	Onset of Gelatinisation Temperature (ºC)
A	0.590±0.02	1.40±0.03	42.15±1.25	89.17±3.10	98.71±0.61	18.7±7.8a	5.93±0.81	411.6±4.77c	80.70±1.20bc	61.17±0.38	54.77±0.29
B1	0.597±0.02	1.32±0.02	45.11±2.25	92.43±3.72	99.07±0.41	11.7±3.5abc	6.27±0.65	418.5±2.32bc	80.57±0.59c	61.03±0.63	54.82±1.09
B2	0.600±0.02	1.39±0.02	43.16±1.01	90.09±2.62	98.43±0.20	17.3±2.1ab	5.60±1.04	418.9±3.04bc	81.94±0.70abc	61.02±0.46	55.70±2.26
C	0.587±0.02	1.37±0.04	42.82±0.54	93.99±2.95	99.07±0.27	10.0±2.6abc	5.67±0.74	428.9±3.52ab	82.99±0.27ab	60.87±0.45	56.70±0.75
D1	0.600±0.02	1.35±0.05	44.34±0.76	93.47±3.41	99.17±0.19	8.3±2.9bc	6.07±0.76	430.2±5.92a	83.13±0.31a	60.95±0.35	57.38±0.84
D	0.610±0.02	1.34±0.04	45.55±2.01	94.89±3.49	99.29±0.45	5.7±3.1c	5.87±0.70	434.2±0.72a	82.99±0.27a	60.38±0.03	56.47±0.73
D2	0.597±0.02	1.40±0.04	42.66±2.15	91.80±2.67	99.16±0.12	7.3±1.2c	5.80±0.75	430.9±3.15a	82.32±1.45abc	60.27±0.08	55.40±0.56
E	0.593±0.01	1.40±0.02	42.28±0.46	92.30±2.85	99.29±0.17	4.7±2.1c	5.70±0.36	435.0±5.63a	83.74±0.13a	60.98±1.09	56.43±0.31
Concerto Mix	0.587±0.02	1.33±0.04	44.12±1.01	92.47±0.91	99.33±0.29	6.7±2.5c	5.67±0.83	425.8±1.69a	82.48±0.24abc	60.97±1.05	56.57±0.42
Sienna Mix	0.587±0.01	1.32±0.07	44.63±2.30	94.65±0.79	99.35±0.35	5.3±2.1c	5.80±0.87	433.4±0.80a	83.38±0.76a	60.58±0.18	55.85±0.22

[a] Mean values (n=3) are expressed as mean ± standard deviation.

[b] Fractions which do not share a letter for each of the measured parameters are significantly different from one another.

All measures of malt N content which included soluble N, total N and the soluble N ratio showed no significant differences between fractions. Friability was lowest in the smallest fraction, fraction A with 89.17% and highest in fraction D with 94.89%. Homogeneity was lowest in fraction B2 with 98.43% and highest in Sienna Mix with 99.35%. The number of whole corns ranged from 4.7 in fraction E, the largest grain size fraction, to 18.7 in fraction A, the smallest grain size fraction (**Figure 5-1B**). Fraction A was significantly higher than all D fractions, fraction E and the two remaining mixed fractions. Hot water extract was lowest in fraction B1 with 80.57% and highest in fraction E with 83.74% (**Figure 5-1C**). No significant differences were observed between malt moisture contents. Predicted spirit yield showed interesting differences across the fractions created in this study (**Figure 5-1D**), fraction A had the lowest PSY with 411 litres of alcohol per tonne (LA tonne^{-1}) which was significantly different from all other fractions apart from B1 and B2. Fraction E had the highest PSY with 435 LA tonne^{-1}. The rheological properties of starch in the ten fractions were investigated through RVA. Fraction A had the highest peak gelatinisation temperature with 61.17°C and fraction D2 the lowest with 60.27°C. The temperature for the onset of gelatinisation varied from 54.77°C with fraction A, to 57.38°C with fraction C.

5.3.3 Correlations Between Grain and Malt Quality Parameters

Table 5-4 summarises the correlations between both grain and malt quality parameters which are displayed in a matrix of the Pearson correlation coefficients (r).

The friability of the malted samples negatively correlated with malt nitrogen (r = -0.65, *P* < 0.05) and positively with both predicted extract (r = 0.65, *P* < 0.05) and soluble nitrogen ratio (r = 0.64, *P* < 0.05). Friability also correlated with the key malt quality parameters PSY (r = 0.79, *P* < 0.01) and HWE (r = 0.64, *P* < 0.05). Malt homogeneity exhibited a strong positive

correlation with predicted extract (r = 0.89, *P* < 0.001) but not HWE. Homogeneity did however show a strong positive correlation with PSY (r = 0.77, *P* < 0.01). Furthermore, the homogeneity of the fractions also correlated with the packing efficiency of the grain (r = 0.66, *P* < 0.05). The PSY of fractions strongly correlated with the SW of the sample (r = 0.84, *P* < 0.01) and also one of the components of SW, GD (r = 0.65, *P* < 0.05). However PSY did not correlate with the other component of SW, PE (r = 0.5, *P* > 0.05). Hot water extract showed much the same relationship as PSY with grain parameters positively correlating with SW (r = 0.82, *P* < 0.01) and GD (r = 0.67, *P* < 0.05). Starch rheological properties showed correlations with both malt quality parameters and grain parameters. Peak gelatinisation temperature negatively correlates with PSY (r = -0.65, *P* < 0.05), SW (r = -0.69, *P* < 0.05) and GD (r = -0.65, *P* < 0.05). Whereas, the temperature for the onset of gelatinisation shows a positive correlation with HWE (r = 0.76, *P* < 0.05).

Table 5-4 Correlation matrix of Pearson's correlation coefficients (r) for grain and malt parameters.

		Malt											Starch		Grain			
		Total Nitrogen (%)	Moisture (%)	Predicted Extract (NIR)	Soluble Nitrogen (%)	Soluble Nitrogen Ratio (%)	Friability (%)	Homogeneity (%)	Whole corn number	Whole corn weight (g)	Predicted Spirit Yield (LA/tonne)	Hot Water Extract (%)	Peak Gelatinisation Temperature (°C)	Onset of Gelatinisation Temperature (°C)	Nitrogen (%)	Specific Weight (kg hl^{-1})	Density (g cm^{-3})	Packing Efficiency (%)
Malt	Total Nitrogen (%)	1	-0.37	-0.25	0.04	-0.89***	-0.65*	-0.5	0.38	0.51	-0.18	-0.06	0.12	-0.2	0.71*	-0.01	-0.12	-0.71*
	Moisture (%)		1	0.05	0.28	0.47	0.07	0.11	0.09	-0.06	-0.26	-0.47	0.12	-0.28	0.04	-0.56	-0.47	-0.17
	Predicted Extract (NIR)			1	0.02	0.24	0.65*	0.89***	-0.9***	-0.81***	0.79***	0.57	-0.53	0.43	-0.06	0.53	0.44	0.51
	Soluble Nitrogen (%)				1	0.42	0.11	-0.12	-0.02	0.1	0.14	0.03	-0.35	0.08	0.19	0.1	0.08	-0.33
	Soluble Nitrogen Ratio (%)					1	0.64*	0.41	-0.36	-0.42	0.23	0.07	-0.28	0.2	-0.56	0.05	0.15	0.49
	Friability (%)						1	0.77***	-0.8**	-0.74***	0.79**	0.64*	-0.53	0.59	-0.57	0.53	0.34	0.59
	Homogeneity (%)							1	-0.94***	-0.97***	0.77**	0.59	-0.46	0.42	-0.31	0.42	0.39	0.66*
	Whole corn number								1	0.93***	-0.92***	-0.78**	0.57	-0.52	0.37	-0.68*	-0.59	-0.68*
	Whole corn weight (g)									1	-0.75**	-0.58	0.43	-0.34	0.4	-0.42	-0.38	-0.69*
	Predicted Spirit Yield (LA/tonne)										1	0.91***	-0.65*	0.62	-0.34	0.84**	0.65*	0.5
	Hot Water Extract (%)											1	-0.41	0.76*	-0.37	0.82**	0.67*	0.45
Starch	Peak Gelatinisation Temperature (°C)												1	-0.01	0.1	-0.69*	-0.65*	-0.24
	Onset of Gelatinisation Temperature (°C)													1	-0.2	0.42	0.29	0.29
Grain	Nitrogen (%)														1	-0.39	-0.37	-0.77**
	Specific Weight (kg hl^{-1})															1	0.87**	0.5
	Density (g cm^{-3})																1	0.58
	Packing Efficiency (%)																	1

"***", "**", "*" were significant at P < 0.001, P < 0.01 and P < 0.05 respectively.

In order to explore the relationships between parameters further, PCA was used to examine trends in multiple parameters together. Principal component (PC) 1 contributed 94.6% of the total variance, fractions with a high score in PC1 have an increased PSY and reduced Wc. PC2 contributed 4% of the total variance, fractions with a high score in PC2 have a high Wc, high SW, high HWE and low homogeneity. A PC biplot of PC1 and PC2 (**Figure 5-2**) displays how grain fractions differ according to the aforementioned parameters. **Figure 5-2** separates the grain fractions of poorer malting quality from the clustered higher quality fractions. Fraction B2 is separated as a result of its high Wc resulting in a higher score in PC2. Fraction A and B1 are separated due to both a low SW and PSY resulting in negative scores for both PCs. Concerto mix is closest to the group of good malting quality fractions which is representative of its quality status, but is separated along PC2 as a result of a combination of lower SW and PSY.
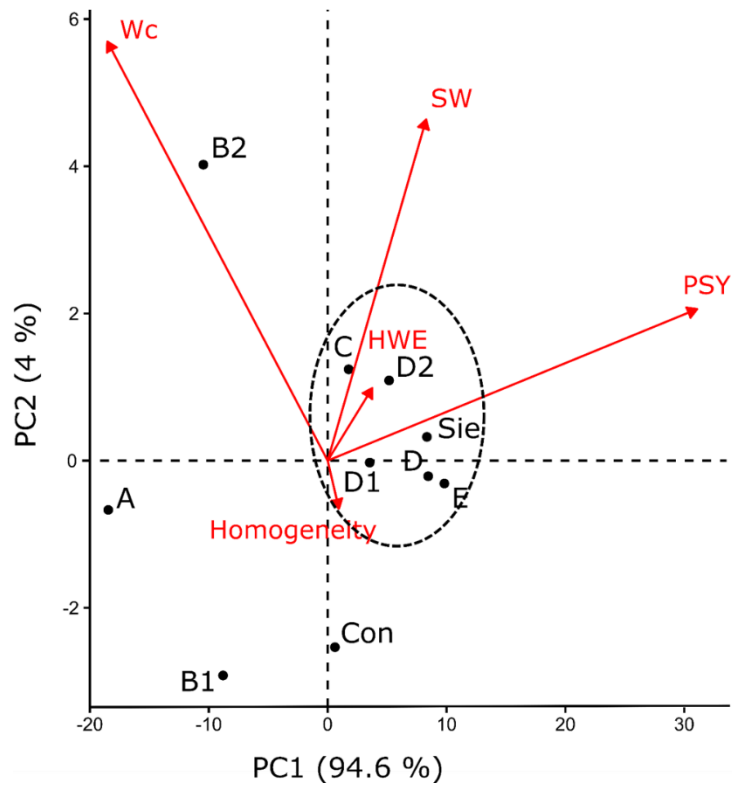
Trait	PC1	PC2
Homogeneity		
Number of whole corns (Wc)	-0.496	-0.738
Predicted spirit yield (PSY)	0.833	-0.267
Hot water extract (HWE)	0.101	-0.128

Figure 5-2 Biplot of the principal component analysis of specific weight and malt quality parameters of the ten grain fractions used in this study. Arrows starting at the centre of the plot represent the loadings of specific weight and malt quality parameters, with the length of the arrows representing the relative importance of each trait. Loadings for PC1 and PC2 are shown in the table beneath the figure.

5.4 Discussion

Specific weight of barley is an established measure of grain quality, used by maltsters to determine if the grain is of high enough quality for acceptance to malting and consequently have a premium paid for it. The effect of changing SW, GD or PE of spring barley on either the malting process or outputs has not been investigated before now. Specific weight or either of its components could influence this process, or the malt product across numerous stages. Grain composition has been shown to determine GD, therefore different SWs can arise as a result of varying composition. Composition is directly related malt quality (Fox, 2010). Starch complexes determine gelatinisation temperature and also the amount of sugars available for conversion into alcohol (Evers et al., 1999). High protein content has also been associated with a limited modification of the barley endosperm (Agu, 2003). Furthermore, the endosperm structure has the potential to contribute to GD, which in turn influences water distribution within the endosperm and consequently the spatial distribution of enzyme activity (Chandra et al., 1999). Packing efficiency will influence the flow of water between grains during steeping which may affect the rate at which water is imbibed into the grain and the rate or uniformity of germination.

In this study, a bulk of grain of the cultivar Concerto was manipulated into different fractions through sequential sieving and additional sorting by grain weight. An additional Sienna bulk was also used, without sorting (i.e. with its natural variation in grain size). Therefore ten fractions were investigated in total. This resulted in significant differences in both grain characteristics and malt quality parameters among fractions. Of these grain characteristics, grain volume and weight increased in fractions with increasing grain size as expected. Specific weight generally in fractions with increasing grain size, which was expected since larger and plumper grains traditionally are associated with a higher SW. Packing efficiency was highest in the two mixed fractions, indicating that too much homogeneity of grain size may be

detrimental for SW despite being a favoured trait by maltsters to ensure uniform modification (Wade and Froment, 2003). Fraction had little effect on grain composition, with N content differing between Sienna and all Concerto fractions apart from Fraction B1, highlighting that cultivar rather than sieve fraction had a greater influence on composition. This is in accordance with other studies which demonstrated that when creating fractions by sieving, weighing alone or pneumatic classification, cultivar had a greater effect on protein content than the effect of the parameters by which the fractions were sorted (Elfverson et al., 1999).

All ten fractions were micromalted using the same malting regime. Fractions had no effect on the levels of soluble N, total N or the soluble nitrogen ratio. Friability is effectively a measure of how crumbly a material is, and for malt this is one indication that the grains have malted successfully and undergone sufficient modification. Friability did not vary with fractions suggesting all fractions malted well and achieved similar levels of modification. However, the high Wc in the smallest fractions, particularly fraction A indicated that these fractions did not malt as effectively as the larger fractions. In terms of malt output, both HWE and PSY were significantly affected by fraction. In general, smaller grain size fractions with lower SWs had both reduced PSY and HWE. This was particularly evident in fraction A which had the lowest SW, PSY and HWE. Despite fraction A only contributing 5.5% to the overall mix by weight, its significantly lower malt quality will be detrimental to the total mix fraction. On the other hand fraction B2 achieved a relatively high SW with lower levels of malt output, in comparison to fractions which had a similar SW, such as fractions D and E. Apart from this exception, the general pattern agrees with the concept of a higher SW being beneficial for malt output. Attempts to link GD and PE to malting output have not been reported before. However a previous analysis of DP from different grain fractions has shown that larger grains have an increased DP, which is beneficial for converting complex starch into fermentable sugars during

mashing (Agu et al., 2007). Therefore this enhanced malt output could be contributed to by the increased enzymatic activity in larger grains, which also had an increased GD.

In order to understand how grain attributes that are associated with an increased SW influence malt quality, relationships between and among grain and malt parameters were investigated. Both SW and GD correlated with the two main measures of malt output used in this study, PSY and HWE. However PE did not, which implies that it is the GD aspect of SW which influences malting output rather than PE. Samples with a higher SW also have a reduced Wc implying a greater of level of modification in comparison to low SW samples. Interestingly a high SW and GD results in a reduced peak gelatinisation temperature which could contribute to the explanation of why higher SW fractions have an increased malt output. A lower peak gelatinisation temperature means that during mashing at 65°C there is an increased chance of full conversion of starch to fermentable sugars. Therefore as well as malt output, SW could be related to an increased malt efficiency with an increased conversion of starch, resulting from the same energy input to reach mashing temperature. Again PE does not share this correlation, further highlighting the importance of GD over this component.

This study has shown that through the manipulation of grain size and grain weight in a bulk, SW can be altered. In general, samples formed from the lower size fractions have a lower SW. When SW is altered in this way it is a good indicator of malt quality in the majority of cases. Higher SW fractions on the whole had increased malt output demonstrated by an increased HWE and PSY. Furthermore, these data suggests that efficiency in downstream processes, where the malt undergoes a mash, could be improved with a higher SW, as it was associated with a reduced peak gelatinisation temperature. However, it is important to note that in this study the changes in SW were due to GD rather than PE. Therefore it is the GD component

of SW that is responsible for changes in malt output, rather than PE. If SW had been altered through a changing the PE, I cannot yet tell if it is likely to have the same effect on malt output.

5.5 Acknowledgements

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Visualization of a Correlation Matrix.

6 Chapter Six

Discussion

Chapter 6. *Discussion*

6.1 Overview

The grain quality trait at the centre of this thesis is SW. The primary reason for investigating SW is because correlations between it and the malting process have not yet been established. There was also no quantitative information about how grain level or bulk level parameters determine SW. The main aims of this thesis have been addressed through i) the identification of grain attributes of spring barley which contribute to SW, ii) uncovering which grain compositional characteristics contribute to the density of barley grains, iii) enhancement of the current understanding of how environmental conditions influence plant development and consequently SW and iv) examination of the effects of SW and its components on the malting process. The results of experimental chapters (Chapters 2-5) show how SW is determined by GD and PE, describe how grain composition (N content and starch B-type granules) is associated with GD, demonstrate how under a moderate but prolonged water stress SW can be maintained, and finally established the impact of changing SW and its components on the malting process. In this final discussion chapter, the findings and limitations of this work are collated and discussed as a whole. Finally, suggestions for future work on SW and related topics will be discussed, to build on this current progress in understanding SW as a grain quality measure.

Specific weight had previously been thought of as a singular grain quality trait, however initial work demonstrated that this is not the case (Hoyle et al., 2018). Specific weight is in fact a product of two components, the mean GD of a sample and the PE of this sample. Each of these components are in turn determined by many additional grain characteristics. **Figure 6-1** is not an exhaustive list of the grain traits which have the potential to influence SW, but summaries the main traits to help portray the complexity of this measure. All of the measures outlined are a result of the interaction of barley genotype and environmental conditions, in this case environmental conditions also include post-harvest grain handling. This is what

differentiates SW from many other grain quality traits, which are solely a measure of one characteristic, for example N content. In terms of SW's relevance to the malting industry, it is unknown whether these two components of SW are beneficial for malting, deleterious, or if their effects change according to the factors which contribute to them (e.g. grain composition).

Specific weight is quick to measure, however GD at the grain level and PE at the bulk level are not, which may explain why little research has been done on these components (Walker et al., 2013). Both of these components involve measuring the volume of irregularly shaped barley grains, which is in itself a science with many years of research dedicated to it (Walker and Panozzo, 2012). However, the difficulties in measuring these components does not undermine the important role they could have upon malting. Initial work also established that both GD and PE contribute significantly to the variation in SW i.e. it is not only one component which causes changes in SW. Therefore both need to be addressed in this research and all future work on SW.
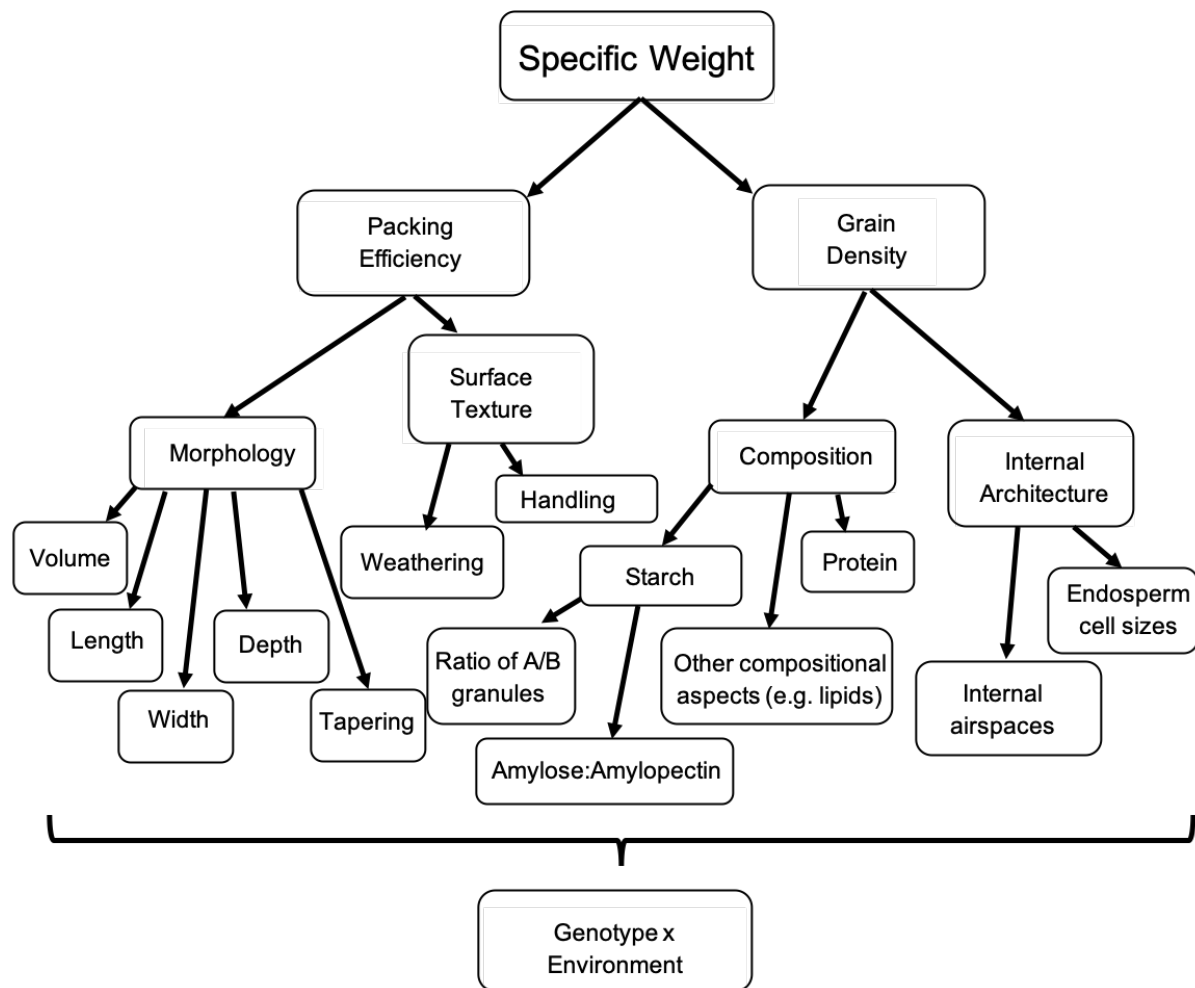
Figure 6-1 Specific weight and its components packing efficiency and grain density. Potential grain characteristics which contribute to SW are shown.

6.2 Packing Efficiency

6.2.1 Specific weight is determined by packing efficiency at the grain bulk scale

As previously mentioned there is a shortage of research on the PE of barley grains, however some research exists on the PE of oat and wheat grains. I hypothesise that principles of PE are similar across the majority of cereal species. For example if smaller wheat grains have greater PEs I assume that smaller barley grains also would. However this may not be the case when comparing barley and millet due to their divergent morphology. This was not tested in this thesis, but could be extremely useful in terms of sharing and comparing research in PE across different cereal species. The PE of oats has been shown to be influenced by genotype, but appears not to be influenced by environment (Doehlert and McMullen, 2008). In addition to this, an increased PE in oats was associated with smaller grains (Doehlert and McMullen, 2008). Recent work with wheat has shown that long and narrow grains result in an increased PE, in comparison with more spherical grains (Yabwalo et al., 2018). Small, needle-like grains are considered detrimental for malting, since they have lower proportions of starch and higher proportions of protein in comparison with larger grains. Therefore if smaller, or more needle-like barley grains increase PE, SW may not be the best indicator for an efficient malting process or indicative of a high quality malt.

The PE of grains within a volume can be dissected further into: the number of grains in the given volume, and the mean volume of these (Hoyle et al., 2018). Therefore, variation in either of these can result in a change in PE. Work from chapter 2 demonstrated that individual grain dimensions and other measures of grain size negatively correlated with PE, but none of them significantly so. However, when investigating the effect of these on the number of grains in a given volume, grain length exhibited a significant negative correlation. In addition to this, when grain dimensions were combined the sum of grain length and depth significantly and negatively correlated with both: the number of grains in a given volume and the PE of these.

These observations are akin to those from previous studies on different cereal grains, which also show that smaller grains can increase PE. This highlights a concern for the way in which PE and SW are increased.

Later work in chapter 5 suggests that not only mean grain dimensions, but the variation in grain sizes within a sample may influence PE. This hypothesis was formulated since the two mixed fractions, containing grain from all size fractions had the highest PEs. Therefore, future studies could investigate not only the effect of grain dimensions on PE, but also the manipulation of the variation of these dimensions. A similar study has been performed on oats, oat grain size distribution is different from other cereal species. Oats do not exhibit a normal distribution because of the presence of secondary and tertiary grains (Doehlert et al., 2006). The presence of triple-grain spikelets, effectively produces three grain sizes, and consequently a trimodal distribution can be observed. Therefore mixed distributions of different size fractions in oats can be produced with relative ease, and it has been hypothesised that increasing the ratio of smaller grains will increase PE in oats by filling in those gaps left between larger grains. This was tested in a different study, however the data did not support this hypothesis (Doehlert et al., 2004).

6.2.2 Packing efficiency: genotype and the environment

Specific weight is a complex grain trait, influenced by many different grain characteristics, each of which can be influenced by environmental change. In this thesis, a prolonged but moderate water stress treatment did not alter SW or PE. However water stress had significant effects on plant development, which consequently influenced other aspects of grain quality. It was also demonstrated that SW is a product of GD and PE, even under water stressed conditions.

Despite water stress significantly reducing ear number, grain number, plant biomass, grain yield, harvest index and the length of grain filling, grain weight and morphology was maintained. Despite the multitude of traits being changed by the water stress, PE was in fact similar across all cultivars and growing conditions. If a study compared the PE of water stressed and control plants in the field, the effect on PE may be very different in comparison to my glasshouse study. These differences could be a result of physical weathering of the grain. Rainfall has been shown to result in the loosening and swelling of the seed coat in wheat (Gan et al., 2000). Which when it is dried is shrivelled, which reduces SW.

6.2.3 Packing efficiency and malting

The water stress study in this thesis examined only the physiological effect of a water stress due to a reduction in soil moisture and did not include potential physical effects on the grain due to above ground conditions. The effect of misting on the grain quality trait, skinning has previously been studied, however a similar experiment to investigate the effects of this on PE and malting quality would be of interest (Brennan et al., 2017). This would come with logistical problems of ensuring the misting treatment does not increase the moisture content of the misted pots. Also enough grain would need to be produced to be used in a micromalting study, so the effect of this physical damage on malting can be investigated.

In future work, it would be interesting to investigate how PE influences steeping. This could determine if PE influences malting efficiency through another mechanism, rather than just the quantity of grain which can be included in a steeping vessel. Theoretically if PE was increased to the extreme, difficulties would arise during steeping because grains would be in contact with less steep water.

6.3 Grain Density

6.3.1 Specific weight is determined by grain density at the grain level

The GD of barley grains was investigated thoroughly in chapter 3. The primary finding was that N content and the number of starch B-type granules, explained roughly half of the observed variation in GD (Hoyle et al., 2019). Both of these positively contribute to GD. Endosperm texture was not examined in this study, but these findings seem consistent with the aforementioned research. Endosperm texture is not to be confused with surface texture. Surface texture refers to the roughness of the barley husks whereas, endosperm texture describes the hardness or susceptibility to crumbling. Barley grains are either classified as mealy or steely, mealy gains crumble easily, but steely grains tend to fracture cleanly.

Nitrogen content is a proxy for protein content in cereal grains, with a range of conversion factors recommended, depending on the cereal species (Mariotti et al., 2008). Therefore since denser grains have a higher N content, it is presumed that these are steely, with a more compacted endosperm, as a result of an increased protein content. However, this is just speculation, because in this study endosperm texture was not assessed alongside composition. This additional information would be useful to hypothesise further about the effect of altered GD on the malting process through links with endosperm texture. Additional questions to be asked in future work include: Are higher density grains always steely? Do lower density grains have an increased volume of airspaces in the endosperm? Can higher density grains be created without increased protein content and steeliness? These are key research questions which were not addressed in the current thesis, but would contribute greatly to the debate about the value of SW as a malt quality indicator.

Other studies, have shown that lower density grains have a mealy endosperm texture, containing loosely packed starch granules in a patchy protein matrix (Walker et al., 2013). Whereas steely grains have higher levels of C hordeins, a glycoprotein commonly referred to as gluten (Ferrari et al., 2010). Mealiness and steeliness are measures of endosperm texture and have been shown to be intercorrelated with GD. However, whether composition is linked to GD, remained unknown. In turn, grains with a mealy endosperm texture in comparison to steely textured grains are associated with increased modification rates in malting (Ferrari et al., 2010). Therefore it may be a lower GD and increased mealiness which is beneficial for malting through achieving a more uniform modification. This could result in samples with a low SW, malting efficiently due to having a mealy grain texture.

6.3.2 Grain density: genotype and the environment

Grain density was not significantly affected by the water stress, but was affected by cultivar, suggesting a strong genotypic effect on GD. Grain density had the same rank order as SW in this study for all cultivars, providing more evidence for the importance of GD in contributing to SW. In oats it has also been demonstrated that across different genotypes GD is more important than PE at accounting for observed variation in SW (Doehlert et al., 2009). In both chapter 3 and chapter 4, Sienna had the highest GD of the cultivars examined. Sienna is marketed as a high SW cultivar and this thesis indicates that this is a result of its high GD rather than a high PE, so in effect it is a high GD cultivar. Despite GD not being affected by water stress, composition was affected by this stress. Nitrogen content increased in all cultivars as a result of the stress. This was not statistically significant, but the reduction in the C:N ratio was. This is an important finding, demonstrating that this change in composition and consequently grain quality is masked by a stable SW. Starch content and the composition of this starch in terms of amylose/amylopectin ratios were the same under water stressed and well watered conditions. One aspect of starch composition which was not analysed in this

study was the ratio of A-type and B-type starch granules. This could have implications for downstream processing, so would be a good trait to measure in future in relation to this, and other environmental stresses.

6.3.3   Grain density and malting

The final experimental chapter advanced on previous experimental chapters, and examined the effect of different SWs on the malting process. In terms of its application to industry, this chapter is the most important, aiming to provide stakeholders with information about how SW affects the malting process. This is of particular importance due to the lack of studies which have attempted to link SW with malting.

Specific weight was manipulated within one cultivar through changing both grain size and grain weight. This primarily resulted in variation in the GD component of SW, rather than PE. Although as previously mentioned in this chapter, PE was highest in the two mixed fractions used, which may be of interest to future work investigating if a mix of grain sizes is beneficial for PE. In general, SW was a good predictor of malting output, correlating strongly with both HWE and PSY. Of the components of SW, GD also correlated with HWE and PSY, but PE did not.

In each micromalting run, samples consisted of 500 g of grain, and when malt analyses were performed in the laboratory 50 g of grist were used. This is standard procedure for assessing malt quality. However, 500 g of grain occupies a different volume depending upon its SW. Therefore in assessing the output of each malting batch it may be worth changing micromalting protocols to require a volume of grain rather than weight of grain. This would be a more accurate reflection of industry processes, because it is the volume of tanks which dictates how

much can be malted, as opposed to how much weight can be held by them. This may be a reason why PE appears to show no correlation with malt output, whereas in maltings where tanks are filled by volume, I predict it could result in a higher throughput of malt.

This study manipulated SW within one genotype, Concerto. Therefore this needs to be taken into consideration when relationships are observed between SW and malt quality parameters in this study. I believe this was the best way to manipulate SW, without changing many other traits that may have impacted upon malt quality. For example, if cultivars were used to create variation in SW additional parameters may have been altered which may have impacted malt quality. This is demonstrated by the significantly reduced N content in the Sienna sample included in the micromalting in comparison to all Concerto fractions apart from Concerto B1.

6.3.4 Future work

The work done throughout this thesis has increased the current understanding of SW, but has also opened up opportunities for more research. Although the contributing factors to PE have been elaborated on in this study, there is scope for more investigation on this. When investigating PE it is important to highlight whether changes in PE are between genotypes or within genotypes. Data from chapter 5 suggested that the variation in grain sizes may influence PE. This could be tested by the sequential sieving of more cultivars and measuring the PE of each fraction and the PE of the natural mix. This would confirm whether the pattern of increased PE in samples with a higher variation in grain size is consistent across cultivars.

The stratification and grouping of grains by GD was a useful method to examine differences between groups of grains of varying GD. This work could be developed further by including more measures. Since grain texture in terms of mealiness and steeliness is known to affect

the modification of grains, this would be an interesting trait to investigate. This is particularly so as the initial work demonstrated that higher density grains had increased levels of protein, which is typically associated with a steely grain texture. Additionally, the proportion of internal airspaces within the grain was not measured in this thesis, which would negatively contribute to density.

Exploring the relationships between environmental conditions and SW is an almost infinite task, with the multitude of different conditions barley can be exposed to and the differing magnitudes of these. This work highlighted that it is not only SW and its components which need to be measured when investigating the potential effects of environmental conditions on malt quality, but also other quality traits shown to be masked by stable SW. A useful further study would also include the physical effects of rainfall on SW. In recent years, increased rainfall at the harvest time for spring barley has become more common, causing numerous harvesting issues. The effect of this on SW has not been investigated and neither has the downstream effects of this on malting. Therefore a controlled environment experiment could examine how wetting of mature grains close to harvest affects SW and malt quality.

As a result of recent progress on barley genetics, particularly the sequencing of the barley genome, possibilities in barley genetics have expanded greatly (Mayer et al., 2012). Quantitative trait loci (QTLs) have been identified for many malt quality parameters such as: malt extract, diastatic power, free amino acid content, protein content and soluble protein content (Fang et al., 2019). Numerous QTLs have been identified for GD on chromosomes 2H and 6H (Walker et al., 2013). However no research has uncovered QTLs for PE, although it would be expected that these would be similar to those previously identified QTLs for grain dimensions. Identification of these, and potential SW QTLs would allow marker assisted

selection for SW, or either of its components. If SW could be manipulated in this way it would allow for further malting studies, to investigate how extremes of SW affect malting.

6.3.5 Conclusions

Specific weight is the product of two components: GD and PE; these are in turn influenced by numerous other grain characteristics. Specific weight can be influenced simultaneously, both positively and negatively by many of these characteristics (**Figure 6-1**). Despite SW being contributed to by many important grain traits which are indicative of grain quality for malting uses, it does not capture details of them all at once. For example a high SW is presumed to always be beneficial for malting conferring higher levels of starch and, large and plump uniform grains within the sample. However if GD has been increased through a high protein content within the grain, the increase in SW may not necessarily result in higher quality grain for malting. Similarly if PE has been increased through an altered grain morphology to more needle-like grains, the higher SW from this may not convey higher quality. Nevertheless on the whole SW is a useful and rapidly measurable indicator, which is generally indicative of barley grain quality. However due to the complexity of this measure and its multifarious nature, important grain or malt quality traits can be masked. Therefore it is important to measure this trait in tandem with other well established and understood grain quality parameters, in order to gain a reliable understanding of how a sample of grain will perform in maltings and downstream uses.

6.4 References

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Appendix A. Supplementary Information for Chapter 2
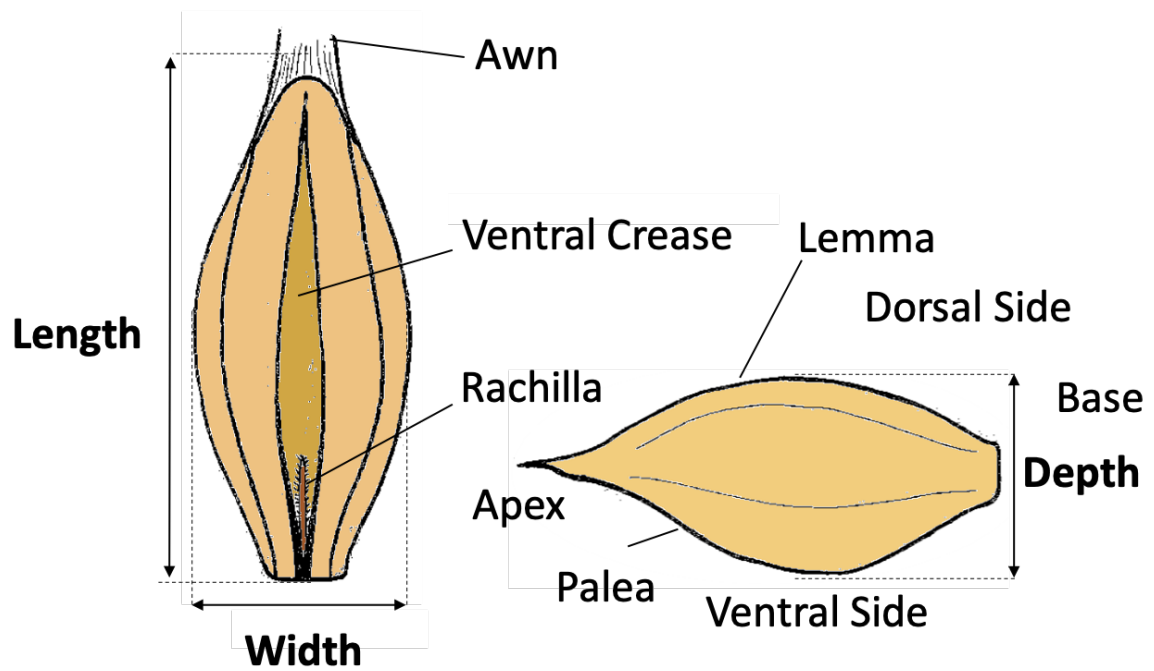
Figure A-1 Anatomical diagram of a barley grain, indicating the orientation of dimensions measured in this study.
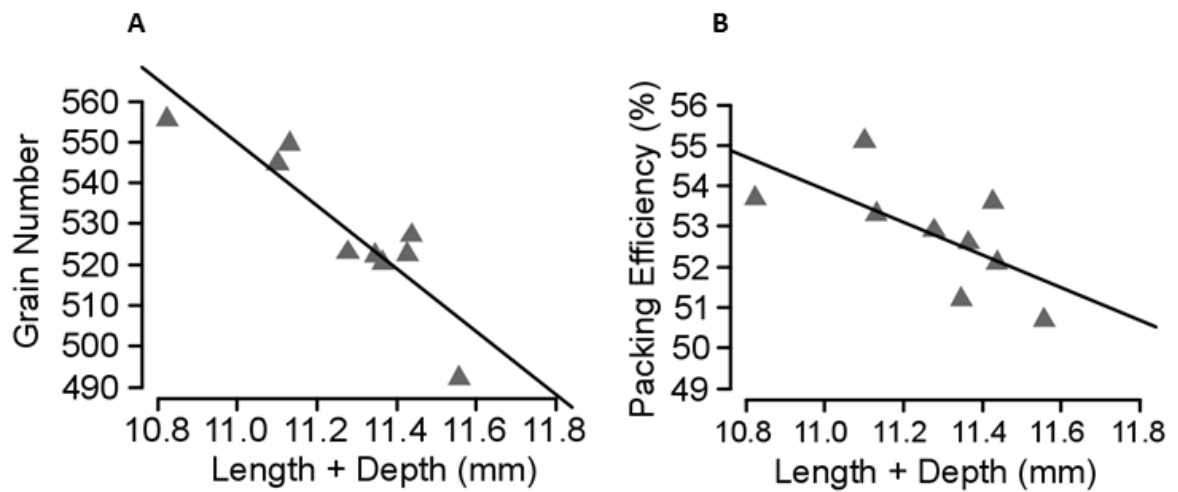
Figure A-2 Linear regression plots of the sum of grain length and depth correlated with (A)

grain number (r^2 = 0.81, P < 0.01) and (B) packing efficiency (r^2 = 0.44, P = 0.05), for the nine

cultivars.

Chapter 6. *Discussion*

Table A-1 Difference between the mean weight of 100 representatively sampled grains, and

the mean weight of the measuring cylinder full of grains.

Cultivar	100-Grain Sample Mean (mg)	Grain Mean Weight in Cylinder (mg)	Difference (mg)
Sienna	51.20	49.90	1.30
Propino	50.97	50.96	0.01
Olympus	48.32	47.71	0.61
Concerto	47.49	47.12	0.37
Origin	49.36	49.42	-0.06
Laureate	52.45	52.77	-0.32
Odyssey	50.01	49.42	0.59
Octavia	48.61	49.13	-0.52
KWS Irina	49.67	48.56	1.11

Chapter 6. *Discussion*

Table A-2 Loadings and proportion of variance explained in the principal component analysis

of grain dimensions among the nine cultivars.

	PC1	PC2	PC3
Loadings			
Depth	0.130	0.854	0.503
Length	-0.981	0.185	0.000
Width	-0.146	-0.485	0.862
Variance explained			
Standard Deviation	0.236	0.057	0.041
Proportion of Variance	0.918	0.053	0.028
Cumulative Proportion	0.918	0.972	1.000

Appendix B.   Supplementary Information for Chapter 3

Figure B-1 Scatter plot of measured grain density using Archimedes' Principle

and predicted grain density using the equation built in 3.3.4 for 25 samples

from five cultivars.

Table B-1 Grain density and aspects of grain composition from five spring malting barley cultivars.

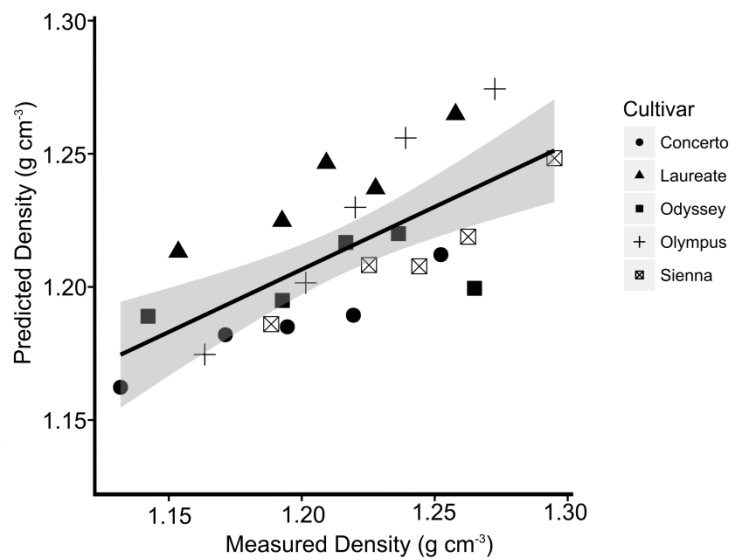| Cultivar	Density class	Grain density (g cm^{-3})	Nitrogen (%)	Carbon (g/100g)	Total Starch (g/100g)	Amylose (%)	Amylopectin (%)	B granule number (%)	B granule volume (%)	B granule surface area (%)
Concerto	1	1.13	1.37	40.23	58.05	21.92	78.08	96.47	15.37	47.40
Laureate	1	1.15	1.43	40.07	58.99	21.43	78.57	97.73	22.83	59.13
Odyssey	1	1.14	1.36	39.76	57.73	18.86	81.14	97.20	21.00	55.67
Olympus	1	1.16	1.27	39.71	59.00	20.61	79.39	97.40	22.10	57.20
Sienna	1	1.19	1.38	40.40	59.45	21.00	79.00	97.27	19.70	54.57
Concerto	2	1.17	1.42	40.37	58.94	21.73	78.27	96.40	16.97	49.33
Laureate	2	1.19	1.42	39.99	58.70	18.18	81.82	97.90	25.57	62.10
Odyssey	2	1.19	1.38	40.18	58.50	16.72	83.28	97.13	21.30	55.57
Olympus	2	1.20	1.36	40.21	58.61	16.15	83.85	97.43	23.53	58.63
Sienna	2	1.23	1.41	40.38	58.69	20.05	79.95	97.60	22.73	58.77
Concerto	3	1.19	1.40	39.49	58.37	20.35	79.65	96.97	18.37	52.47
Laureate	3	1.21	1.50	39.33	59.32	15.85	84.15	97.90	26.33	62.73
Odyssey	3	1.22	1.48	40.17	57.95	16.91	83.09	97.27	21.13	56.13
Olympus	3	1.22	1.51	40.41	59.18	17.82	82.18	97.40	22.70	57.90
Sienna	3	1.24	1.39	39.83	59.08	20.75	79.25	97.67	23.47	59.57
Concerto	4	1.22	1.43	40.43	58.27	18.36	81.64	96.67	17.90	50.97
Laureate	4	1.23	1.55	40.04	57.91	17.16	82.84	97.60	22.20	58.90
Odyssey	4	1.24	1.45	39.68	57.57	16.01	83.99	97.40	23.20	58.37
Olympus	4	1.24	1.54	40.36	58.93	15.96	84.04	97.77	26.57	62.43
Sienna	4	1.26	1.39	40.20	61.07	17.39	82.61	97.90	25.60	62.23
Concerto	5	1.25	1.47	39.93	57.89	20.88	79.12	97.23	20.70	55.57
Laureate	5	1.26	1.62	39.96	58.74	19.83	80.17	97.60	24.43	59.70
Odyssey	5	1.26	1.39	39.46	57.95	20.22	79.78	97.40	21.83	57.20
Olympus	5	1.27	1.63	39.96	60.15	16.20	83.80	97.63	25.90	61.03
Sienna	5	1.30	1.54	40.31	58.38	19.89	80.11	97.93	24.90	61.73

Table B-2 Probability values for the correlation matrix for grain density, elemental analysis and starch analyses.

	Grain density (g cm^{-3})	Nitrogen content (%)	Carbon content (%)	Starch content (%)	Amylose content (%)	B granule volume (%)	B granule number (%)	B granule surface area (%)
Grain density (g cm^{-3})	0	0.001231	0.9482	0.3475	0.1309	0.004003	0.008991	0.004075
Nitrogen content (%)		0	0.6507	0.6595	0.07201	0.02726	0.09488	0.04403
Carbon content (%)			0	0.3744	0.804	0.4114	0.3556	0.3826
Starch content (%)				0	0.5714	0.03253	0.06479	0.0509
Amylose content (%)					0	0.002659	0.0545	0.008071
B granule volume (%)						0	0	0
B granule number (%)							0	0
B granule surface area (%)								0

Appendix C. Supplementary Information for Chapter 4

Table C-1 Statistical analyses of the impact of drought and cultivar on grain characteristics

using mixed models with rep as a random effect.

Response Variable	Treatment*Cultivar	Cultivar	Treatment
Ear and grain traits			
Grain weight (mg)	ns	ns	ns
Ear Number	ns	ns	8.20E-10
Ear Length (mm)	ns	6.67E-05	0.00699
Grains per pot	ns	ns	1.66E-08
Grains per ear	0.01653	*	*
Biomass partitioning			
Plant Dry Biomass (g)	ns	8.89E-09	3.41E-05
Grain Yield (g/pot)	ns	ns	0.000101
Harvest Index	ns	0.04617	4.11E-03
Plant Growth			
Mean theta (mV)	ns	ns	2.20E-16
Fertility	0.03789	*	*
Days Grain Fill	ns	0.00534	1.59E-09

Table C-2 Statistical analyses of the impact of drought and cultivar on grain characteristics using mixed models with rep as a random effect.

Response Variable	Treatment*Cultivar	Cultivar	Treatment
Size Classes			
>3.25 mm	ns	ns	ns
3.00-3.25 mm	ns	ns	ns
2.75-3.00 mm	ns	ns	ns
2.50-2.75 mm	ns	ns	ns
2.25-2.50 mm	ns	ns	ns
Screenings (%)	ns	ns	ns
Dimensions			
Length (mm)	ns	0.04304	ns
Width (mm)	ns	ns	ns
Depth (mm)	ns	ns	ns
2D area (mm^2)	ns	ns	ns
Circularity	ns	0.0087	ns
Specific weight and components			
Specific Weight (kg hl^{-1})	ns	0.003	ns
Packing Efficiency (%)	ns	ns	ns
Density (g cm^{-3})	ns	0.00857	ns
Composition			
Total starch	ns	0.02794	ns
Amylose (%)	0.03979		
Carbon (%)	ns	ns	ns
Nitrogen (%)	ns	ns	0.04456
C:N	ns	ns	0.02958

Table C-3 p-values associated with the correlation matrix in **Table 4-2**.

	Grain weight (mg)	Length (mm)	Width (mm)	Depth (mm)	Volume (mm³)	Area (mm²)	Perimeter (mm)	Circularity	Density (g cm⁻³)	Packing Efficiency (%)	Specific Weight (kg hl⁻¹)	Nitrogen Content (%)	Carbon Content (%)	Starch Content (%)
Grain weight (mg)	0	0.177	0.0053	0.00916	0.0125	0.197	0.328	0.00404	0.0447	0.00615	0.00885	0.0509	0.465	0.00199
Length (mm)		0	0.0321	0.0222	0.903	0.00116	0.00141	0.0482	0.0152	0.76	0.00369	0.159	0.886	0.234
Width (mm)			0	0.000185	0.0592	0.756	0.0538	0.00013	0.14	0.0144	0.00024	0.019	0.123	0.00573
Depth (mm)				0	0.063	0.937	0.0232	0.0468	0.28	0.00644	0.000237	0.0368	0.162	0.00989
Volume (mm³)					0	0.0425	0.942	0.0689	0.729	0.0258	0.0225	0.0758	0.215	0.00472
Area (mm²)						0	0.0518	0.125	0.4068	0.135	0.722	0.609	0.933	0.855
Perimeter (mm)							0	0.0127	0.0765	0.908	0.0169	0.0853	0.68	0.219
Circularity								0	0.0473	0.172	0.000281	0.025	0.579	0.0342
Density (g cm⁻³)									0	0.512	0.002445	0.4654	0.601	0.361
Packing Efficiency (%)										0	0.0224	0.341	0.429	0.0445
Specific Weight (kg hl⁻¹)											0	0.0931	0.887	0.00419
Nitrogen Content (%)												0	0.00197	0.00025
Carbon Content (%)													0	0.26
Starch Content (%)														0