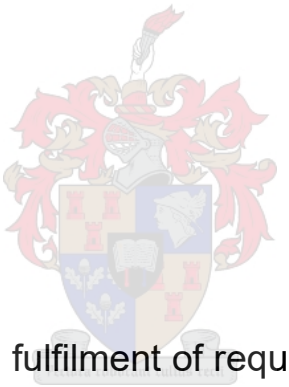
Factors that have an influence on splitting of malting spring barley (*Hordeum vulgare* L.)

by

Louis Wilhelm Carstens



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DECLARATION

I hereby declare that the work contained for the degree MSc (Agric) Agronomy at the University of Stellenbosch is my own original work and that I have not previously in its entirety or in part submitted it for any other University for a degree.

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ABSTRACT

In South Africa barley (*Hordeum vulgare* L.) is the second most produced small grain crop, where most of the barley is used by the beer-brewery industry for malting purposes. The Southern Cape (dryland) and Northern Cape (irrigation) is South Africa's two main barley production areas.

The term "splitting" refers to when the grain is cracked open through the pericarp, aleurone or testa, exposing the starchy endosperm. Split grain can result in non-uniform malt, the formation of foam in steep tanks or possible fungal growth, which is not suitable for the brewing process. This was recently detected in barley produced in the Northern Cape and consequently threatens the beer-brewing industry of South Africa.

Some studies suggest that splitting is caused by abnormal climatic conditions or high nitrogen (N) application during the later stages of crop development. However, there is a lack of information regarding the causes of the splitting of barley under South African conditions. Therefore, the aim of this study was to identify such factors by investigating the influence of temperature, water stress, different light intensity conditions, and the rate of nitrogen application on a low-risk (*Cristalia*) and a high-risk (*Overture*) cultivar for splitting during different growth stages. Pot trials were conducted in a glasshouse at the Welgevallen Experimental Farm in Stellenbosch. For the temperature experiment, temperatures were lowered by 10°C at grain filling; maintaining 15°C/5°C for a week and then transferred back to normal growing conditions. The nitrogen experiment involved a total of 100 kg N ha^{-1} (low N), 150 kg N ha^{-1} (control) and 200 kg N ha^{-1} (high N) divided into increments and applied at planting, six weeks after planting and grain filling respectively. The shade experiment entailed no shading (control), 40% and 60% shade introduced at the end of tillering and removed at early milk development. For the water experiment, normal daily irrigation was applied until stem elongation after which low, medium and high water stress treatments were introduced. The effect of the treatments on vegetative and reproductive growth parameters and grain quality was determined. Low temperature during grain filling and high rate of nitrogen fertiliser resulted in an increase in splits. Different shade levels and water stress had minor effects on splitting. Therefore, a

follow-up trial was conducted the next year where the combined influence of temperature and nitrogen on splitting was tested. It involved low-temperature conditions for three hours during grain filling. Nitrogen rates only differed at grain filling (0, 30, 40, 50 and 60 kg N ha^{-1}) with a standard nitrogen rate applied at planting and six weeks after planting respectively. It was found that only nitrogen had a significant ($p < 0.05$) effect on splitting, as rates above 40 kg N ha^{-1} during grain filling resulted in an increase in split grain. The results of this study will aid in finding methods to prevent splitting of barley in the future.

UITTREKSEL

Gars (*Hordeum Vulgare* L.) is die tweede belangrikste kleingraangewas wat in Suid-Afrika geproduseer word, waar die meeste gars gebruik word vir die vermoutingsproses deur die bierbrou industrie. Die twee hoof gars produksie areas in Suid-Afrika is die Suid-Kaap (droëland) en die Noord-Kaap (besproeiing).

Splitsing is die term wat gebruik word om die toestand waar graan deur die perikarp, aleuroon en testa oopkraak en die styselagtige endosperm blootgestel word, te beskryf. Dit affekteer die vermoutingsproses deur oneweredige ontkieming, die vorming van skuim in fermentasietenke of moontlike groei van swamme. Dit was onlangs in gars wat in die Noord-Kaap geproduseer word waargeneem en gevolglik hou dit ‘n bedreiging vir die bierbrou industrie van Suid-Afrika in.

Sommige studies stel voor dat splitsing deur abnormale klimaatsomstandighede of hoë stikstoftoedienings gedurende die latere stadiums van gewasontwikkeling veroorsaak word. Daar is wel ‘n tekort aan inligting rakende die oorsake van splitsing van gars onder Suid Afrikaanse toestande. Daarom is die doel van die studie om sulke faktore te identifiseer deur die impak van temperature, waterstremming, verskillende vlakke van lig-intensiteit en die vlak van stikstoftoedienings op ‘n lae risiko (*Cristalia*) en ‘n hoë risiko (*Overture*) kultivar vir splitsing gedurende verskillende groeistadiums te toets. Potproewe is in ‘n glashuis by die Welgevallen-proefplaas in Stellenbosch uitgevoer. Vir die temperatuureksperiment is temperatuur verlaag met 10°C gedurende graanvul om 15°C/5°C vir ‘n week te handhaaf waarna dit na normale groei-omstandighede teruggeplaas is. Die stikstofeksperiment het behels dat ‘n totaal van 100 kg N ha^{-1} (lae N), 150 kg N ha^{-1} (kontrole) en 200 kg N ha^{-1} (hoë N) opgedeel en onderskeidelik tydens plant, ses weke na plant en tydens graanvul toegedien is. Die skaduwee-eksperiment het bestaan uit geen skaduwee (kontrole), 40% en 60% skaduwee wat aan die einde van halmvorming toegevoeg is en weer met vroeë melk-ontwikkeling verwyder is. Tydens die water-eksperiment is ‘n normale daaglikse besproeiing tot en met stamverlenging toegedien waarna dit deur lae, medium en hoë waterstres behandelinge opgevolg is. Die impak van die behandelinge op die vegetatiewe en reproduktiewe groei-parameters en graankwaliteit is bepaal. Die lae temperatuur gedurende graanvul en hoë stikstoftoedienings het ‘n verhoogde impak op splitsing gehad. Die impak van

verskillende skaduwee-vlakke en waterstres op splitsing was minimaal. Daarom is ‘n opvolgproef die volgende jaar uitgevoer waar die gekombineerde impak van temperature en stikstof getoets is. Dit het behels dat lae temperatuurtoestande vir drie ure tydens graanvul toegepas is. Die stikstofvlakke het slegs verskil gedurende graanvul (0, 30, 40, 50 en 60 kg N ha^{-1}), terwyl ‘n standaard stikstofvlak toegedien was met plant en ses weke na plant onderskeidelik. Daar is gevind dat slegs stikstof ‘n betekenisvolle invloed op splitsing gehad het aangesien vlakke bo 40 kg N ha^{-1} gedurende graanvul ‘n verhoging in splitsing tot gevolg gehad het. Die resultate van hierdie studie sal dus help om metodes te vind om splitsing van gars in die toekoms te voorkom.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of variance
DM	Dry mass
EC	Electrical conductivity
e.g.	exempli gratia (for example)
GS	Growth stage
h	Hour
ha	Hectare
N	Nitrogen
ns	not significant
SAB	South African Breweries
SAC	Special Area of Conservation
SWC	Soil water content
TGM	Thousand grain mass

CHAPTER 1

1. Introduction

1.1 Background

Barley (*Hordeum vulgare* L.) is next to wheat the most valuable small grain crop produced in South Africa and ranks about fourth in the world along with wheat, rice and maize (Aynewa et al. 2013). The two main barley production areas within South Africa, the southern Cape and Northern Cape, produce approximately 315 and 20 thousand tons per annum under dryland and irrigation respectively (DAFF 2017). Most barley is used for malting purposes by the beer-brewery industry (Maenetje and Dutton 2007), while only a small portion is used for animal feed. The feed market for barley is very small due to large quantities of maize produced in South Africa that is used as the main ingredient in animal feed production (DAFF 2017).

Apartheid had a huge influence on the South African economy by restricting direct investment and preventing capital from leaving the country as it was cut-off from world markets (Mager 2008). South African Breweries (SAB) was ranked as the number one industrial company on the Johannesburg Stock Exchange during that time (Tregurtha and Vink 1999), due to the significant income derived from tax on its brewing operations and the lack of competitors to gain important market shares (Mager 2008). In the late 1990s, SAB developed a strategic objective to retain market power (keeping prices low) and building on South Africa's economy (Mager 2008). SAB is still today the only major buyer of malting barley and contributes on average between 46 and 121 thousand tons to South Africa's gross value of agricultural production per annum (DAFF 2017). The requirement for malting barley is more than the amount produced and the deficit is imported from Canada and the European Union (Tregurtha and Vink 1999).

Therefore, it is within South Africa's financial interest to produce enough high-quality malting barley. On the other hand, the import of malting barley supports the size of the local crop by smoothing the annual fluctuations (Tregurtha and Vink 1999).

1.2 Problem statement

Recently, splitting was detected in barley grain produced in the Northern Cape, which has a major impact on its quality for malting. “Splitting” is the term used to describe a condition where the grain is cracked open through its pericarp, aleurone or testa, exposing the starchy endosperm (Hoad et al. 2003). This affects the malting process by leading to uneven germination, the formation of foam in steep tanks or possible fungal growth (ARC 2018). Kai et al. (2003) found that split grains are caused by low-temperature conditions, not enough sunlight, and moisture stress during hull development. Hoad et al. found in a study in 2003 that the application of nitrogen fertiliser at high rates during late crop development also lead to split occurring. This data was unfortunately not published.

An unexpected decrease in temperature of 10°C during the grain filling period, at the soft dough stage, accompanied by rain causes the grain to rapidly harden. The rainwater absorbed by the plant roots and moved to the filling grain caused the grain to swell whilst the dry, non-expanding husk split at the side, crease or the back of the grain. Under warm and dry conditions barley will start to 'turn', which causes the outside of the grain to form waxes and the husk to harden. The endosperm will consequently change from a milky to a doughy texture (DPIRD 2017).

By using split grains in the malting process, water enters through the open area and saturate the starchy endosperm. This will result in uneven germination and the production of non-uniform malt which makes the grain unsuitable for the brewing process.

However, there is a lack of research in South Africa on the causes of split in barley. Therefore, a glasshouse study was conducted on the Welgevallen Experimental Farm in Stellenbosch, where certain climatic conditions were simulated to determine its effect on barley grain. The results of this study will not only aid in identifying the causes of splitting grain, but it will also help to improve barley management practices and increase the production of high-quality malting barley.

1.3 Aim and objectives

The aim of this study is to identify factors that might lead to splitting of malting spring barley by investigating the influence of temperature, water deficiency, different levels of light intensity, and the rate of nitrogen application on a low-risk (*Cristalia*) and a high-risk (*Overture*) cultivar for splitting during different growth stages. The following objectives were established to achieve the goal for this study:

Objective 1: Evaluate the effect of lower temperatures during the period of grain filling on splitting of barley grain.

Objective 2: Evaluate the effect of different rates of nitrogen applications on splitting of barley grain.

Objective 3: Evaluate the effect of reduced light intensity on splitting of barley grain.

Objective 4: Evaluate the effect of water stress during different growth stages on splitting of barley grain.

Objective 5: Evaluate the effect of the combined influence of temperature and nitrogen during grain filling on splitting of barley grain.

CHAPTER 2

2. Literature review

2.1 Production of barley

Barley (*Hordeum vulgare L.*) originated in the Middle East and was the first crop to be domesticated (Rajasekaran et al. 2004). Ranking fourth after wheat, rice and maize, barley is still today one of the world's most important small grains (Aynewa et al. 2013). In numerous parts of the world, barley is the main staple food and is normally found in regions that are recognised for having low rainfall, soil salinity and high altitudes or areas where other small grains do not perform well. Barley is extremely adaptable and can be produced in various climates that range from subtropical to sub-Arctic (Gupta et al. 2010). "Barley is arguably the most widely adopted cereal grain species with production at higher altitudes and latitudes and farther into deserts than any other cereal crop" (Baik and Ullrich 2008). Dry areas with rainfall less than 300 mm remain the most suitable condition for barley production (Mobtaker et al. 2010). Due to the rise in importance of wheat and rice, barley developed from food primarily into feed, malting and brewing grain (Baik and Ullrich 2008). Throughout the history of barley, it has been and still is a very important source of food in countries all around the world, like Asia, northern and eastern Europe, North Africa and the Middle East (Newman and Newman 2006; Baik and Ullrich 2008). In the early years of the 21st century, barley has been the fifth most produced crop in the world on a dry mass basis (Tavakoli et al. 2009). In South Africa, the cultivation area for malting barley under dryland conditions is at present restricted to the Western and southern Cape, which stretches from Piketberg in the west to Heidelberg in the east. Barley is produced under irrigation in the Northern Cape in places such as Vaalharts, Douglas, Barkley West and Rietrivier/Modderrivier irrigation schemes (SABBI 2012).

After domestication, throughout successive cycles of breeding, the crop was established to produce grain suitable for malting (Swanston and Ellis 2002). These days most of the barley produced is used for malting purposes and it is only used as animal feed and about 2% for human consumption when quality is a problem (DAFF 2017).

2.2 Morphology of barley

Barley grain consist of about 65 to 68% starch, 10 to 17% protein, 4 to 9% β-glucan, 2 to 3% free lipids and 1.5 to 2.5% minerals (Czuchajowska et al. 1998; Izydorczyk et al. 2000; Quinde et al. 2004). The compound structure of the barley grain consists of tissues of gametophytic and sporophytic origin (Rajasekaran et al. 2004). The four main tissues of mature barley grain, the pericarp, aleurone, testa and husk (Figure 2.1), play an important part in the malting and brewing processes (Wallwork et al. 1998). The husk which consists of two glumes, the palea and lemma, protects the embryo from being damaged during the handling of the grain and also prevent germination losses (Brennan et al. 2017). The palea covers the ventral side which is characterised by a central crease, while the lemma covers the dorsal side of the grain (Hoad et al. 2016). The husk differs from other tissues in the way it is formed; it is not formed from or within the ovary (Rajasekaran et al. 2004).

The caryopsis, in other words, the kernel, consists of mainly the endosperm and the embryo (Hoad et al. 2003). More or less 80% of the fully developed grain is comprised of the endosperm, which is separated from the husk by several layers. The ovary wall or pericarp is the layers directly beneath the husk, which also supports and protects the embryo and the developing endosperm (Hoad et al. 2003). As the grain matures, the husk (palea and lemma) becomes attached to the pericarp due to the release of a sticky substance from the pericarp (Gaines et al. 1985). Approximately two weeks after anthesis, it is very hard to remove the husk from the caryopsis (Hoad et al. 2003). The process in malting where the grain is evenly modified or completely degraded of its protein matrix is promoted by distal abrasion (Rajasekaran et al. 2004). Damages to the pericarp enable the exogenous gibberellic acid to enter through the opening and stimulate the production of hydrolytic enzymes from the aleurone into the endosperm (Freeman and Palmer 1984). The barley endosperm cell walls are formed by approximately 75% mixed linked (1-3), (1-4)-β-D-glucans together with 20% arabinoxylans and protein (Gupta et al. 2010). Both β-glucans and arabinoxylans form a barrier for hydrolytic enzymes to attack the starch and protein within the endosperm cell walls causing a few potential health benefits (Freeman and Palmer 1984). These benefits include the prevention of constipation, reducing the risk of colorectal cancer, lowering blood cholesterol, and controlling diabetes management (Gupta et al. 2010).

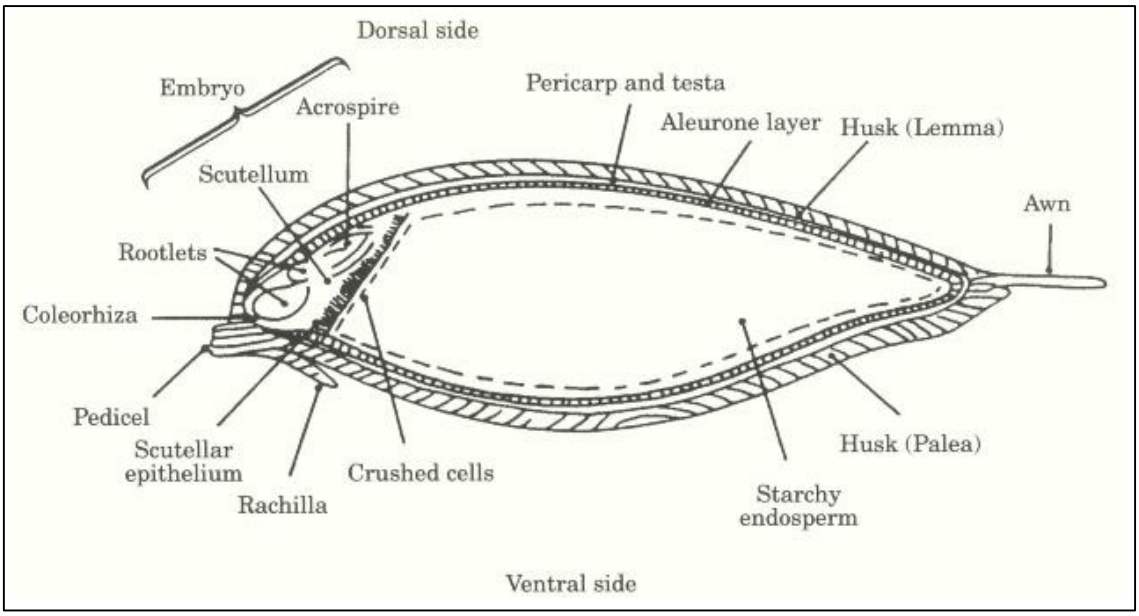


Figure 2.1: Longitudinal section of barley grain (Wallwork et al. 1998).

2.3 Malting of barley grain

Barley is the world's most important cereal crop used in malt production (Gupta et al. 2010; Jamar et al. 2011). The malting industry is challenged by the demand of sufficient volume and quality of barley that they have to meet (O'Donovan et al. 2011). The characteristics of a malting barley grain are clean, bright, yellow-white, plump, thin-husk, medium-hard and uniformly sized grain (Pomeranz and Shands 1974). Six- and two-row barley are the two types that are frequently used for the malting process. Six-row barley produces malt with a small extract, darker colour and more enzyme content than the two-row type (Broderick 1977). The quality requirements for two-row barley includes relatively low protein concentration of between 100 to 125 mg g^{-1}, ≥80% plump kernels and ≤3% thin kernels. Six-row barely must have a protein concentration of 105 to 130 mg g^{-1}, ≥70% plump kernels and ≤4% thin kernels (McKenzie et al. 2005). These grains must also be free from disease, weathering, damage such as splitting and contamination. Only two-row barley is used as malting barley in South Africa. Fast hydration and germination are the necessary traits for good quality malting barley (Ulonska and Baumer 1976; Briggs 1998). During the process of malting, barley goes through an incomplete, natural germination process that involves a series of enzyme degradations of the endosperm (Gupta et al. 2010). The results of enzyme degradation cause the

removal of the internal cell wall barriers that leads to the production of enzymes, which stimulate and promote colour and flavour development (Lafond et al. 2011). In malting and brewing, barley husk contributes to the beer's flavour and serves as a filtering aid. That is why hulled barley is preferred above hull-less barley (Burger and La-Berge 1985).

Gupta et al. (2010) defined malting "as the controlled germination of cereals, to ensure a given physical and biochemical change within the grain, which is then stabilised by grain drying". To ensure that these changes take place, three steps are necessary: steeping, germination and kilning. Steeping is the soaking of a solid in a liquid, usually to extract flavours or to soften it. Steeping is important for good absorption of water (at least 40% of moisture) by the grain. Germination will maintain the growth of the embryo and enzyme synthesis as well as limit the breakdown of the endosperm. Lastly, kilning is the heating of germinated barley to dry it and develop malty, biscuit-like flavours. Kilning will ensure the stability of the product (Gupta et al. 2010).

2.4 Splitting of malting barley

In breweries, malting barley containing a high amount of biologically damaged grains can cause significant problems throughout the malting process (Olkku et al. 2003). Baumer et al. (1998) stated that if this type of damaged grain is less than 2% in a pile, it is acceptable. Studies show that grain split through the palea and endosperm occurs more regularly than grain split through the lemma and endosperm (Psota et al. 2011). During steeping in the malting process, grain with splits (regardless of the type of split) absorb water more rapidly than normal grain (Psota et al. 2011). This will result in early germination and therefore in a batch of malt that is over-modified, the potential malt extract will reduce (Bryce et al. 2010).

Malting barley has a strict quality requirement. Thus, grain with minimum physical defects, relatively low protein (<125 g kg^{-1}), and large plump grains (>800 g kg^{-1}) of uniform size (BMBRI, 2010) are required to produce a standard quality malt. The quality and premium of malting barley are affected by three main physical defects: splitting, gape and skinning (as described below) (Hoad et al. 2003). The occurrence of these types of defects differs depending on weather conditions during the grain-

filling period, cultivar susceptibility and, to a lesser extent, agronomic practices (Hoad et al. 2003).

Splitting (Figure 2.2 A and B), also referred to as cleaving, is when the grain is cracked open through the pericarp, aleurone or testa, exposing the starchy endosperm to other elements (Hoad et al. 2003). The uncovering or exposing of the endosperm is the result of mechanical weaknesses or excessive grain filling. Splitting has lately become a serious problem for the barley industry due to the decrease in the quality of the grain (Kai et al. 2003). Damage to the barley grain can either be inconsequential with minor damage to the appearance of the grain, or it can be more serious with damage caused by mycotoxins (Psota et al. 2011). Mycotoxins are secondary metabolites of moulds which can endanger the health (toxic effect) of both people and livestock (Lucic et al. 1999). The toxic effect, referred to as mycotoxicosis, occurs more frequently in hot and humid climate areas suitable for the growth of moulds (Lucic et al. 1999).

Splitting can appear in three forms on the barley grain, each of them causing the same amount of damage (Hoad et al. 2003). The first form is lateral (side) splitting that occurs on the side of the grain, revealing an opening or crack in the pericarp, aleurone or testa which surrounds the endosperm, similar to gape. (Hoad et al. 2003). The last two forms are dorsal (back) and ventral (front) splitting. They are similar to each other due to the fact that the husk is attached to the pericarp and damage to both the husk and pericarp or testa uncovers the endosperm (Hoad et al. 2003).

Gape occurs due to poor development of the husk or excessive expansion that forms a gap between the two husk tissues (palea and lemma) and does not cover the caryopsis (Hoad et al. 2016). Gape can be defined as a gap of 0.5 mm or more separating the palea and lemma (Hoad et al. 2003). Throughout the development of gape, the pericarp remains undamaged and the endosperm covered (Hoad et al. 2003).

Grain skinning (Figure 2.2 C and D), also referred to as ‘hull peeling’, is the result of poor adhesion causing the husk to separate from the caryopsis (Brennan et al. 2017). In other words, the grain has skinned when the tissues covering the husk and caryopsis lose their grip and the husk becomes partially or completely stripped from

the caryopsis (Hoad et al. 2016). Based on scientific literature, there is very little information regarding the causes of skinning. However, some studies suggest certain weather patterns (odd wet and dry conditions as well as humidity) may lead to skinning (Hoad et al. 2016). There are also other causes such as mechanical damage during harvesting or rough handling during post-harvest (Hoad et al. 2003). Over-threshing during harvesting with combines can be the cause of split-grain, damaged embryo or skinning (Rajasekaran et al. 2004). Transporting wet grain to malt houses can also result in skinning (Psota et al. 2011).

Skinned grain (without husks) will germinate much quicker and absorb more water than grain with undamaged husks (Brennan et al. 2017). The occurrence of these type of grain in a batch of malt will cause uneven malting due to under- or over-modification of starch (Roumeliotis et al. 1999; Agu et al. 2002, 2008; Bryce et al. 2010). The skinned grain will leave the embryo unprotected with greater chances of being damaged and altogether increasing germination rates (Roumeliotis et al. 2001; Agu et al. 2002; Olkku et al. 2005).

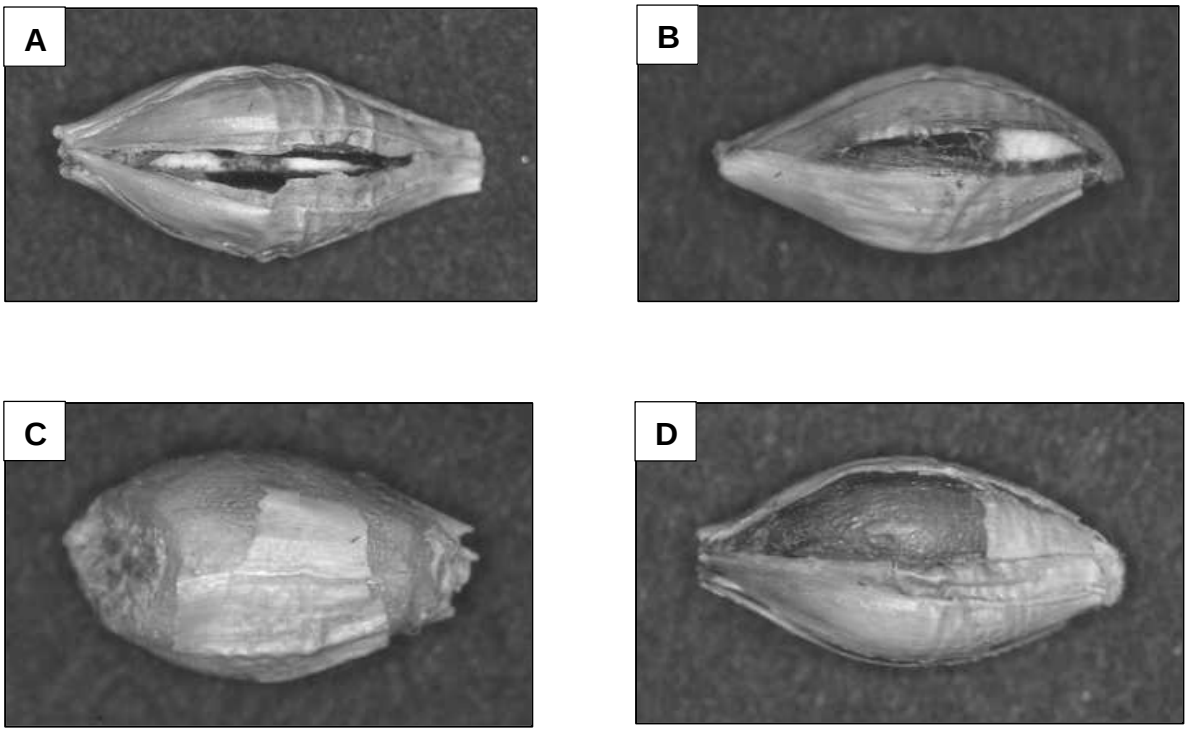


Figure 2.2: Ventral split of barley grain (A), lateral split of barley grain (B), skinned barley grain (80% loss of husk) (C) and partially skinned barley grain (20% loss of the husk) (D) (Hoad et al. 2003).

Skinning and splitting affect starch modification and germination, which reduces malting production efficiency and is the reason for batches being rejected for malting purposes (Hoad et al. 2003). Micro-organisms will attack these damaged grains and have an effect on the brewery filtration. In fields where there is split-grain, starch will be converted to sugars before harvest, resulting in lower yields and low potential malt extract levels (Hoad et al. 2003).

The industry has a low tolerance for splitting, whereas the tolerance for skinned and gape with no signs of mould found in underlying tissues are higher (Hoad et al. 2003).

2.5 High-risk factors for causing splitting

2.5.1 Environment

The main environmental and crop warning signs of an increased risk for splitting are: high soil nitrogen, a dry spring, low spring sunshine intensity, stress during stem extension, a wet summer, high summer sunshine, long canopy duration (days from leaf appearance to complete leaf fall), a very long grain filling period, repeated wetting and drying and delayed harvest (Hoad et al. 2003). These signs are more associated with spring barley than winter barley. Winter barley is more commonly used for feed, because of the high protein content, and as a cover crop. Therefore, winter barley is rarely used for malt production (Hoad et al. 2003). The risk for grain to split may differ between areas, but the weather is most possibly the reason for it (Hoad et al. 2003). Certain weather patterns play an important role in the splitting of grain. The effect of severe weather conditions during the ripening phase of barley grain causes physiological problems such as microbial contamination, morphological and anatomical changes (Psota et al. 2011). Baumer et al. (1998) stated that the existence of split-grain had increased in recent years due to severe weather conditions occurring at critical times of grain development and maturation. The change in global climate and increased variability of rainfall and temperature is happening so fast that the selection programmes (cultivar selection etc.) cannot keep up (Psota et al. 2011). The impact of climate change on spring barley production is expected to be mostly negative (Olesen et al. 2011).

2.5.1.1 Temperature

Kai et al. (2003) found that split-grains are caused by the result of low-temperature conditions during husk development. According to this research, these plants were exposed to lower temperatures during husk development by planting two to three weeks earlier than the normal dates (Kai et al. 2003). Australian research also claimed that a sudden drop around 10°C in temperature, followed by a rainfall event during the period of grain filling, is the main cause of split (DPIRD 2017). The temperature-drop causes the husk to quickly harden, whilst the follow-up rain is subsequently absorbed and causes the grains to swell. The dry husk has no room to expand and will result in split-grain.

2.5.1.2 Sunlight

Kai et al. (2003) found that split-grains can be the result of a lack of sunlight. In this study, the sunlight was reduced by introducing 50% shading on the plants during stem elongation until heading. The result was an increased incidence of grain-splitting (Kai et al. 2003). Hoad et al. (2003) found in a study that splitting occurred more frequently in plants that are shaded before anthesis than in plants that are kept unshaded. There were significantly higher levels of splitting in the shaded plants of Chariot (high-risk for splitting cultivar) than Landlord (low-risk for splitting cultivar). Hamachi et al. (1989) found that shading combined with excess soil moisture resulted in the underdevelopment of the husk, exposing the caryopsis (splitting) through the lemma and palea. They stated that splitting occurred due to an unbalance between the husk and grain size.

2.5.1.3 Rainfall

Studies revealed that in 2001 Scotland had a high incidence of splitting due to abnormal rainfall patterns, which ranged from low rainfall in spring to high rainfall during the months of July and August (Hoad et al. 2003). The low rainfall in spring can be the reason for grain stress during husk development and the high rainfall in summer can create conditions suitable for grain filling but leads to tension in the pericarp, aleurone or testa of the grain. The end result is a grain split. Rajasekaran et al. (2004) indicated that splitting increased in the year 1999 to 2001 when]low rainfall

occurred early in summer followed by higher rainfall in mid to late summer. According to Kai et al. (2003), the effect of water injury (drowning) during husk development slows down the growth of the husk which increase the risk for splitting. Reports from Germany (Zimmerman 1998; Muller and Schildbach 1998) suggest that the high levels of splitting during that year's barley harvest were due to repeated exposure to heavy rain followed directly by hot dry conditions (warmer temperatures combined with windy conditions).

2.5.2 Agronomy practices

According to a study in the United Kingdom, which revolved around agronomy treatments, the main influence on the quality of malting barley is the way barley was managed in the field. These agronomy treatments included the rate of nitrogen application, cultivar selection, seeding rate and other management practices (Lafond et al. 2011). Any agronomy treatments that cause excessive grain-filling or prolonged canopy greenness may increase the risk of grain splitting (Hoad et al. 2003). Crop management in terms of agronomy practices must be considered in relation to exposing the crop to grain damage or disease that will result in loss of yield and quality (Hoad et al. 2003).

2.5.2.1 Nitrogen application

During the production of malting barley, the application of nitrogen is certainly one of the most important aspects of agronomy contributing to grain quality and yield (McKenzie et al. 2005). The timing and rate of nitrogen application during the growing season of barley have the highest influence on the risk of splitting. Although the nitrogen rates were not mentioned, it showed that the application of high levels of nitrogen late in the developing stage of the crop was associated with splitting (Hoad et al. 2003). According to trials in Scotland, an increase in the rate of nitrogen application led to an increase in split-grain (Hoad et al. 2003).

2.5.3 Mechanical damage during harvest or post-harvest

These types of damage occur when the grain is harvested with combines, transported from the fields after harvest, during post-harvest handling of the grain

and grain storage. No evidence was found in studies to assume that certain combine settings can result in splitting and gape (Hoad et al. 2003). Although this is not the case, abrasive combining can cause other types of damages to the grain such as removing the embryo and chipping of the grain (Hoad et al. 2003).

Harvest and post-harvest processes have a strong influence on skinning and can worsen the effect of physical damages (Hoad et al. 2003). Cultivars that are vulnerable to skinning should not be exposed to any abrasive harvesting, grain handling and transport (Hoad et al. 2003). Fornal et al. (2000) stated that barley grain's mechanical properties and genotype are related to each other. The mechanical resistance of barley grain genotypes with floury endosperm is lower than those with glassy endosperm (Psota et al. 2011). The amount of damaged grain is associated with the fracture resistance and corresponds with the grain's water content and direction of compression force (Psota et al. 2011).

2.5.4 Genetic factors

2.5.4.1 Cultivars

Cultivars differ in their susceptibility to splitting, skinning, and gape due to the significant genetic relations (grain dimension) of these defects (Hoad et al. 2003). There is evidence found that grain dimension is an important factor determining the risk for split (Hoad et al. 2003). Rajasekaran et al. (2004) found in studies between 1999 and 2000 that the occurrence of split-grain was relatively higher for the cultivar Tankard than Livet - this could have been due to the fact that these two cultivars differed in grain dimensions with Tankard producing on average shorter and broader grains than those of Livet, which resulted in a higher width/length ratio. The influence of agronomy practices differs in cultivars of high and low risk for splitting, for example in high-risk cultivars any treatment that causes excessive grain-filling or extends the canopy greenness will possibly increase splitting (Hoad et al. 2003). Thousand grain mass (TGM) differs between cultivars and is therefore not a good indicator of splitting. However, within a cultivar, changes in TGM seems to be important. These studies showed that the risk for splitting of barley grown in Scotland, where it is on average cooler and wetter for longer than in England, was higher compared to situations where TGM tends to be higher (Hoad et al. 2003). The risk of certain

cultivars to split was identified in the recent Special Area of Conservation (SAC) surveys. Chariot was categorised as a high-risk cultivar, while cultivars such as Decanter, Optic, and Prisma had a medium risk and Charlice and Cellar a low risk (Hoad et al. 2003). In South Africa the cultivar Overture was recorded as the high risk for split cultivar, while Cristalia had a low risk for split. The Scottish cultivar, Tankard, was removed from the United Kingdom's list of recommended cereals of 1999 as a result of its high splitting incidence in that year and the next year (Psota et al. 2011).

2.6 Grain-filling

The period of grain-filling is a sensitive time for the barley grain and the tendency to split are more likely. Excessive expansion during this period could cause the grain to split (Hoad et al. 2003). By combining a high-risk cultivar with any treatment that will increase excessive grain-filling may increase the risk of splitting (Hoad et al. 2003). When the structure of the grain is stressed by grain filling, it can reduce the mechanical strength of the grain which will cause the grain to be more likely to split (Hoad et al. 2003). A very long grain-filling period is an important factor or warning sign indicating the risk of splitting (Hoad et al. 2003). Long grain filling can result in split-grain because they had filled in excess (Hoad et al. 2003). The duration of grain filling period is determined by time of planting, weather conditions and cultivar genotypes (Pržulj and Momcilovic 2012). Lower temperatures combined with earlier flowering causes a decrease in rate and an increase in the duration of grain filling (Pržulj 2001).

CHAPTER 3

3. Material and methods

3.1 Plant material

Glasshouse/Controlled environment experiments were conducted in the Western Cape at Welgevallen, the experimental farm of Stellenbosch University (33°56'33"S 18°51'56"E). Spring barley (*Hordeum vulgare* L.) seeds of cultivar Overture (a high-risk for split) and Cristalia (a low-risk for split) were used in this study. Overture is a medium maturing cultivar with low kernel nitrogen, high kernel plumpness and low screenings. Cristalia is an early maturing cultivar with medium kernel nitrogen, kernel plumpness and medium screenings (Burie Erasmus, AB InBev 2017). Barley seeds obtained from AB InBev (Caledon) were used. Ten seeds from each cultivar, Overture and Cristalia, were sown into 3 L pots filled with a sand medium on 25 May 2018. At the three-leaf growth stage (GS13), plants were thinned from ten to five per pot.

3.2 Irrigation and fertilising

The barley was irrigated daily by means of drip irrigation to the soil's field capacity until it reached maturity (except for the water experiment). The field capacity for the water experiment was determined by using three 3 L pots filled with a completely dry sand medium. At first, the soil was weighed to determine the dry mass. Then each of the pots was placed in a 5 L ice cream bucket filled with 1 L of water, allowing for the pots to take up the water by means of capillary action. When the sand was completely saturated, the sand medium was again weighed to determine the wet mass and the remaining water in the 5L buckets was measured. Afterwards, the dry mass was subtracted from the wet mass and the remaining water from the 1 L. An average reading was calculated and it was found that the amount of water needed to irrigated the soil to field capacity was approximately 600 ml.

To determine the amount of water to be applied daily, these pots were placed in the glasshouse for a week and was weighed every 24 hours to determine the water loss over time. A water loss of approximately 20 ml of water per day was measured. Therefore, after watering the pots to field capacity with planting, the plants were

irrigated with 25 ml of water every day from plant emergence onwards. The amount of water was increased according to visual inspection of the soil surface as the plant increased in size and used more water for growth.

Figure 3.1: Pots were placed in 5 L ice cream buckets filled with 1 L of water. Capillary action was used to determine the field capacity of the sand medium.

A standard Steiner nutrient solution (Steiner, 1984) at an electrical conductivity (EC) of 2 ms cm^{-1} (Table 3.1) was applied with irrigation for the temperature and shade experiments and a nitrogen-free nutrient solution (Table 3.2) was applied to the nitrogen experiment throughout the crop's growing season.

Table 3.1: Composition of the standard Steiner nutrient solution used for irrigation at an EC of 2 ms cm^{-1}

Micronutrients	Application (g $1000L^{-1}$)	Micronutrients	Application (g $1000L^{-1}$)
K_2SO_4	250	$MnSO_4$	2.23
KNO_3	200	$ZnSO_4$	1.47
KH_2PO_4	136	Boric acid	1.51
$Ca(NO_3)_2.2H_2O$	850	$CuSO_4$	0.20
$MgSO_4.7H_2O$	440	NH_4 molybdate	0.09
		Fe-EDTA	6.54

Table 3.2: Composition of the nitrogen-free nutrient solution for the nitrogen experiment

Macronutrients	Application (g 1000L^{-1})
K_2S0_4	287,1
KH_2PO_4	272
$CaSO_4.2H_2O$	476
Mg $SO_4.7H_2O$	369

Micronutrients	Application (g 1000L^{-1})
Fe	1.738
Mn	0.36
Zn	0.214
Cu	0.024
B	0.44
Mo	0.038

NPK 2:3:2 (22) and urea were used for the water-stress and nitrogen experiments as prescribed by AB InBev (Burie Erasmus, AB InBev 2017). Nitrogen was applied equivalent to 100 kg N ha^{-1} at planting, 20 and 30 kg N ha^{-1} six weeks after planting and at grain filling (soft dough stage) respectively.

3.3 Growing conditions

Plants were grown in a glasshouse in which day/night temperatures were maintained at 20 °C/10 °C for a 12 h/day. Plants were either shaded (reduced light by 40 or 60%) from the end of tillering (GS25) until early milk development (GS71) or kept unshaded. The study was divided into four main experiments: temperature, water-stress, nitrogen, and shade experiments. The crops in all four experiments were irrigated every day to the soil's field capacity (normal irrigation regime) until it reached the maturity stage, except for the crops in the water stress treatments.

3.4 Experiments and treatments

3.4.1 Experiment 1

The effect of cooler temperature during grain filling on splitting of malting spring barley (*Hordeum vulgare* L.) grain

Plants were grown in a glasshouse with day/night temperatures maintained at 20 °C/10 °C for a 12 h/day. Temperatures were increased to 25 °C/20 °C after ± six weeks. After anthesis, 50% of the pots were transferred to a cool room every night for

a week where a temperature of 5 °C was maintained. During the day the pots were returned to the glasshouse running at 15 °C. After a week the pots were transferred back to normal growing conditions that were 25 °C/20 °C until maturity. The remaining 50% of the pots were kept under normal growing conditions throughout the growing season.

3.4.2 Experiment 2

The effect of different nitrogen rates at planting, six weeks after planting and grain filling on splitting of malting spring barley (*Hordeum vulgare* L.) grain

This experiment consisted of three treatments; each with different nitrogen rates. The control treatment was a total of 150 kg N ha^{-1} which was prescribed by AB InBev (Table 3.3). All of the nitrogen-treatments received a nitrogen-free nutrient solution (Table 3.2) with irrigation throughout the duration of the study. Treatment 1 received 33.3% less N and Treatment 2 received 33.3% more N than the control treatment (Table 3.3).

Table 3.3: Amount of nitrogen in kg N ha^{-1} applied at planting, six weeks after planting and at dough stage for each of the treatments

	Amount of nitrogen (kg N ha^{-1})		
	Control	Treatment 1	Treatment 2
Total N	**150**	**100**	**200**
Planting	100	66,7	133,3
6 Weeks after	20	13,3	26,7
Soft dough	30	20	40

Nitrogen had been applied by hand to each treatment according to the ratios in Table 3.4. It was applied in the form of granules NPK 2:3:2 (22) at planting (Table 3.5). Urea was applied in granule form six weeks after planting and at dough stage.

The amount of nitrogen needed per pot was calculated using the following formula:

$$\{Area\ of\ pot\ (ha)\ x\ Amount\ of\ nitrogen\ (kg\ N\ ha^{-1})\}\ x\ 1000$$

Table 3.4: Amount of nitrogen applied at planting, six weeks after planting and at dough stage for each of the treatments according to the area of the pots

	Amount of nitrogen (g N pot^{-1})		
	Control	Treatment 1	Treatment 2
Total N	**0.181**	**0.121**	**0.242**
Planting	0.121	0.081	0.161
6 Weeks after	0.024	0.016	0.032
Soft dough	0.036	0.024	0.049

Table 3.5: Total fertiliser per pot at planting

	Amount of fertiliser (g pot^{-1})		
	Control	Treatment 1	Treatment 2
Total Fertiliser	1.921 g	1.286 g	2.556 g
Consist of N	**0.121 g**	**0.081 g**	**0.161 g**
Consist of P	0.181 g	0.121 g	0.240 g
Consist of K	0.121 g	0.081 g	0.161 g
Total NPK	0.423 g	0.283 g	0.562 g

3.4.3 Experiment 3

The effect of light intensity during husk development on splitting of malting spring barley (*Hordeum vulgare* L.) grain

Shade netting was used in this experiment to cover the plants to provide different shade levels. The control treatment comprised of a third of the plants left unshaded (0% shade level) for the duration of the experiment. For the second treatment, ambient light was reduced by 40% and for the third treatment, ambient light was reduced by 60%. To facilitate this, square tube steel frames of 1 m high and 0.5 m wide were constructed and covered with shade netting. Each structure covered eight pots and was placed 0.5 m apart to prevent additional shading from nearby structures. Shading was initiated on the two treatments at the end of tillering (GS25) and was removed at early milk development (GS71).

All the shade-treatments had received a nutrient solution (Standard solution used at Welgevallen experimental farm, Table 3.1) with irrigation throughout the crop's growing period.

3.4.4 Experiment 4

The effect of water stress troughout the growing season on splitting of malting spring barley (*Hordeum vulgare* L.) grain

All the treatments were normally irrigated until the crops have reached stem elongation. The crops in the control treatment were not subjected to water stress and continued to receive normal irrigation until maturity (Figure 3.2).

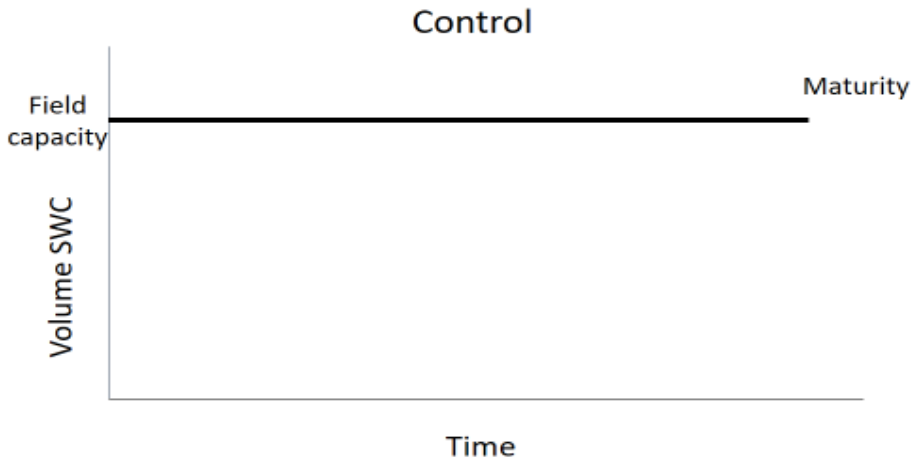


Figure 3.2: Application of water at field capacity throughout the growing season until maturity. Water was applied continuously throughout the duration of the experiment.

The crops in Treatment 1, that were subjected to low water stress, were irrigated daily at field capacity until stem elongation when stress was applied (Figure 3.3). From that point forward, plants were irrigated every fourth day until it reached anthesis, where water was rapidly increased to the normal irrigation regime.

The crops in Treatment 2, which were intermediately (medium) water-stressed, received normal daily irrigation until plants reached stem elongation (Figure 3.4). At this point, stress was applied and plants were irrigated every fourth day until anthesis. From anthesis, water application was slightly increased to every second day until the hard dough stage, whereafter it was increased to normal irrigation

regime until maturity. Figure 3.4 shows that water is gradually increased after stress until maturity.

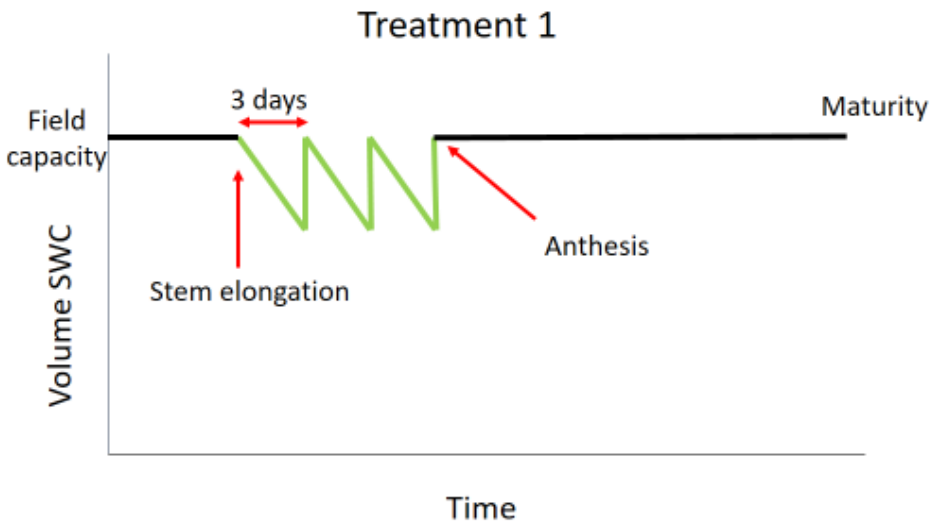


Figure 3.3: Application of water at field capacity daily until stem extension whereafter plants were stressed by irrigating only every fourth day until anthesis. After anthesis it received the normal irrigation regime.

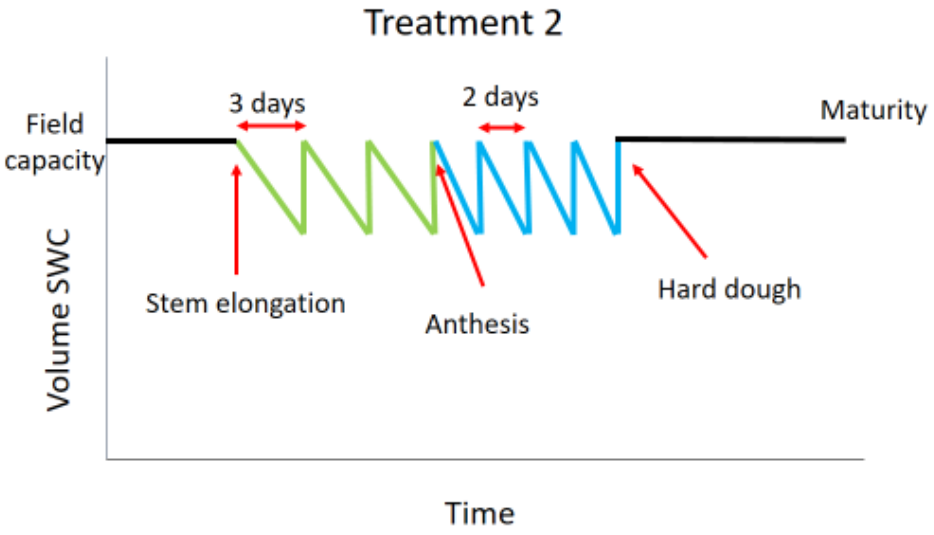


Figure 3.4: Application of water at field capacity until plants were stressed at stem elongation by only irrigating every fourth day until anthesis. After anthesis, plants received water every second day until the hard dough stage. Thereafter, the normal irrigation regime was followed until maturity.

The crops in Treatment 3, which were highly water-stressed, were irrigated daily to field capacity up to stem elongation (Figure 3.5). At this stage, the plants were stressed and plants were only irrigated every fourth day until they reached maturity.

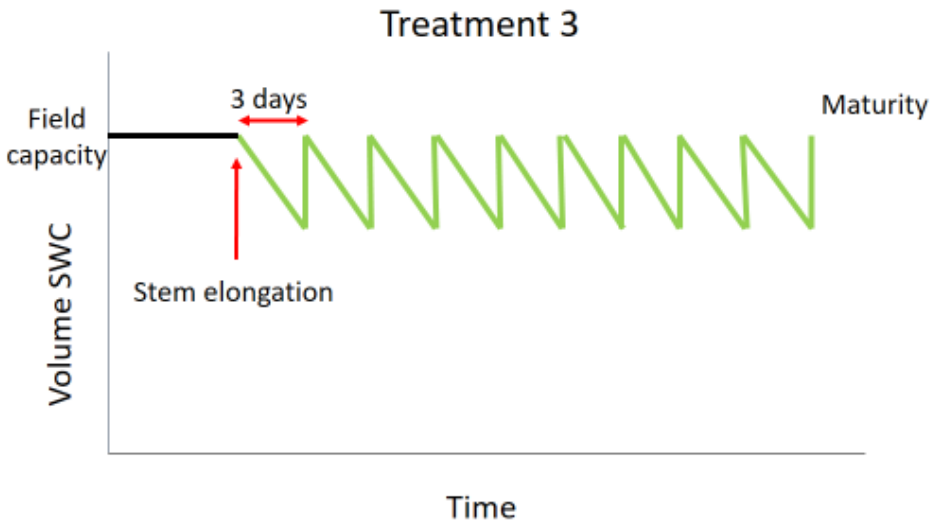


Figure 3.5: Application of water at field capacity daily until plants were stressed from stem elongation by irrigating only every fourth day until maturity.

NPK 2:3:2 (22) was applied to all the water treatments in the form of granules at planting (Control in Table 3.5). At six weeks after planting and at grain filling (dough stage) urea were applied in the form of granules (Control in Table 3.4).

3.5 Data collected

3.5.1 Measurements

The following data were collected for each experiment. One plant per pot was cut at soil level at tillering, flag leaf and anthesis. The total tiller and number of active leaves were determined per plant before cutting. Plant height was measured as the vertical length between the point of cut at ground level and the highest point of the plant. Afterwards, the leaf area was calculated by means of the Li-Cor leaf area index machine. The leaves and the rest of the plant material were oven-dried at 60°C for 72 hours before determining the dry matter.

After the three stages of cutting, there were two plants remaining in each of the pots to harvest at maturity. Before harvest, the total number of spike bearing tillers and spikes per plant were determined. The plants were cut at ground level and each

spike's grains were counted separately and collected. The total number of grains for each plant was calculated and weighed.

Thousand grain mass was calculated using the following formula:

$$\frac{Grain\ dry\ mass\ (g)}{Number\ of\ grain}\ x\ 1000$$

Harvest index was calculated using the following formula:

$$\frac{Grain\ dry\ mass\ (g)}{Above\ ground\ mass\ (g)}$$

Grain samples (1 g) were collected and percentage N determined by the Department of Agriculture of the Western Cape. Protein content was calculated as N x 6.25. The percentage of nitrogen of the harvested grain was multiplied by 6.25 to get the calculated protein content of each sample.

After completing all the measurements and collecting the samples, split grains were identified by grain inspection (visual) and EC (electrical conductivity) tests.

3.5.2 Visual test

Due to unavailability of published visial test results, a criterion was developed to identify splits. Seeds that were used for planting were classified as normal grain (Figure 3.6, A) and were compared with the harvested grain. When comparing these grains with each other, anything deviating from the normal grain (planting seeds) were classified as splits.

A hundred seeds from each treatment, cultivar and replicates were obtained after harvest for the visual test (inspection) to determine the percentage of split-grain. Three degrees of splitting were noted using the visual test: ventral (Figure 3.6, B), dorsal (Figure 3.6, C) and lateral splitting (Figure 3.6, D). There were also some grains that formed a thin soft layer in the middle with black content visible underneath (Figure 3.7, A). Applying pressure to this area, cracked open the thin soft layer and exposed the black content within (Figure 3.7, B). There were also grains with a deep groove in the middle which looked as if the black content had already leached (Figure 3.7, C).

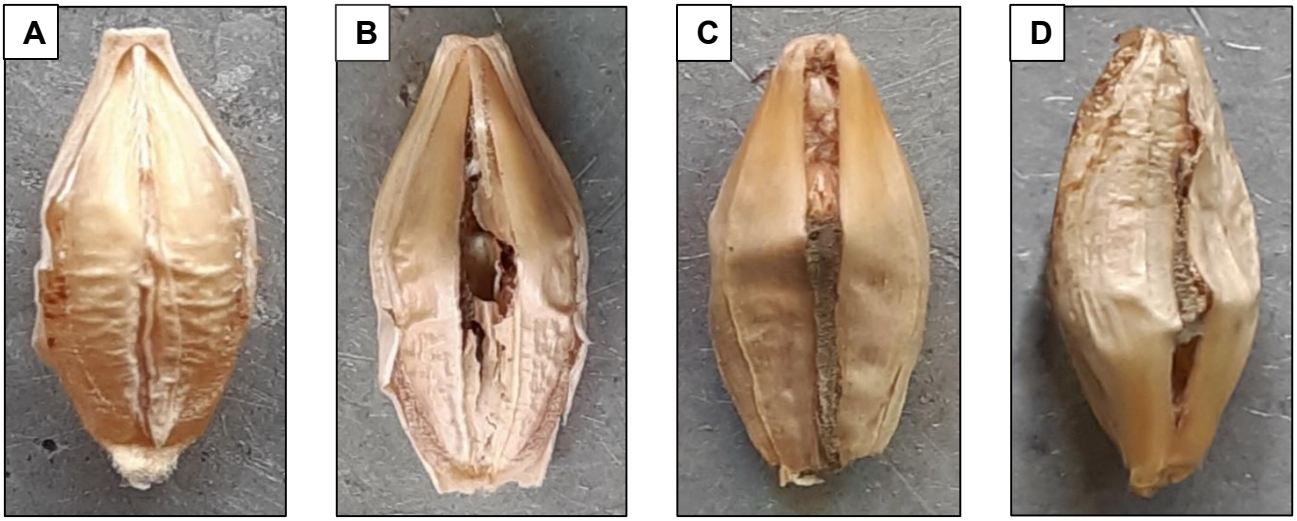


Figure 3.6: (A) Normal barley grain, (B) Ventral splitting, (C) dorsal splitting and (D) lateral spitting.

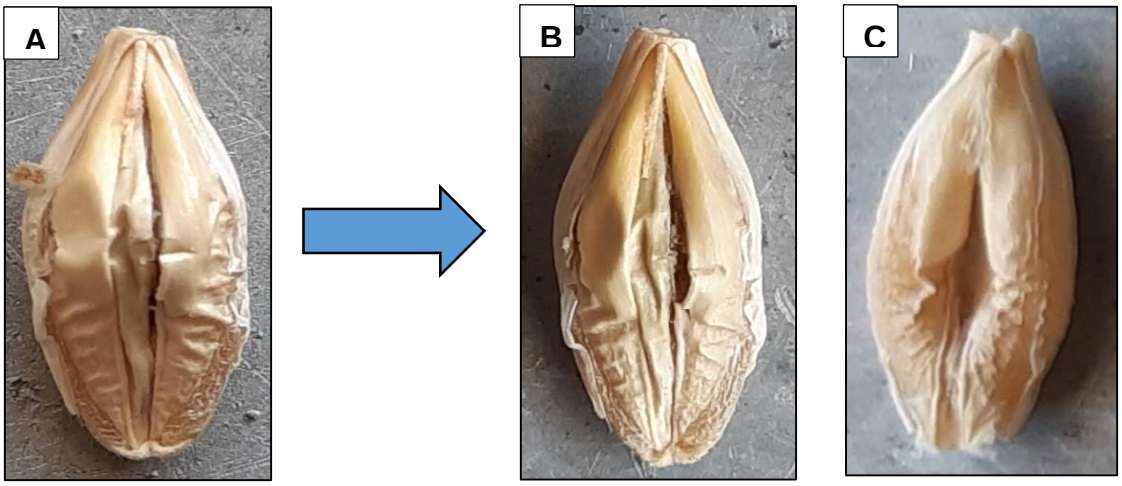


Figure 3.7: (A) Grain with a thin soft layer, (B) cracked grain and (C) grain with a deep groove.

3.5.3 EC test

An electrical conductivity (EC) test is said to identify splits not visible to the eye. The test was conducted to determine if any content had leached out of the grain (Samarah and Al-kofahi, 2008) and would indicate if the grain had split or not.

Firstly, the EC of the seeds that were left over from the previous season's planting was determined to identify the value of normal grain for each cultivar that did not split. Fifty seeds of each treatment, cultivar and replicates were obtained after the harvest for the EC test. The seeds were soaked in a flask with 75 ml of distilled water. The

flask was covered with cling wrap to reduce evaporation and placed in a growth chamber (Figure 3.8) at 25°C for 24 hours. The electrical conductivity of seed leachates was measured using an electrical conductivity meter (Figure 3.9, Oakton PCTestr® 35). After each measurement, the EC meter was rinsed with distilled water and dried to avoid any contamination that may result in inaccurate readings.

Figure 3.8: Growth chamber

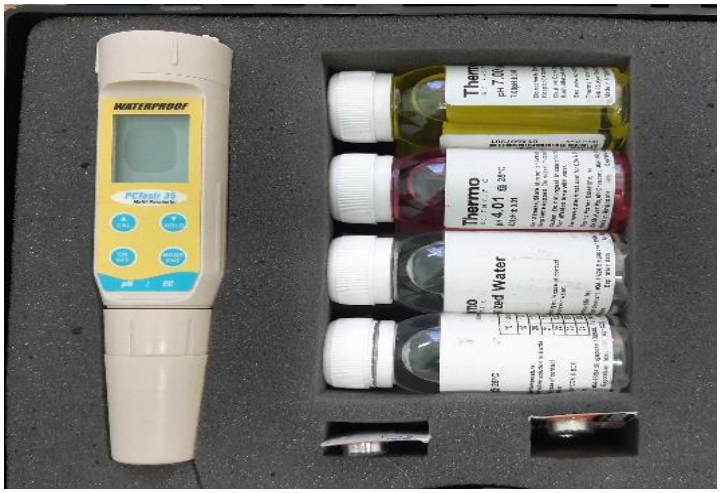


Figure 3.9: Oakton PCTestr® 35 pH and conductivity meter

3.6 Experimental design and statistical analyses

The trial was conducted using a completely randomised factorial design. This project consisted of four separate experiments that investigated the following four-factor combinations:

3.6.1 Temperature

Two cultivars and two temperature-levels formed the factors in a 2 x 2 factorial experiment with eight repetitions, a total of thirty-two pots for experiment 1.

3.6.2 Nitrogen

Two cultivars and three rates of nitrogen applications formed the factors in a 2 x 3 factorial experiment with five repetitions. This gave a total of thirty pots for experiment 2.

3.6.3 Shade

Two cultivars and three shading-levels formed the factors in a 2 x 3 factorial experiment with four repetitions. This gave a total of twenty-four pots for experiment 3.

3.6.4 Water

Two cultivars and four water levels formed the factors in a 2 x 4 factorial experiment with four repetitions, a total of thirty-two pots for experiment 4.

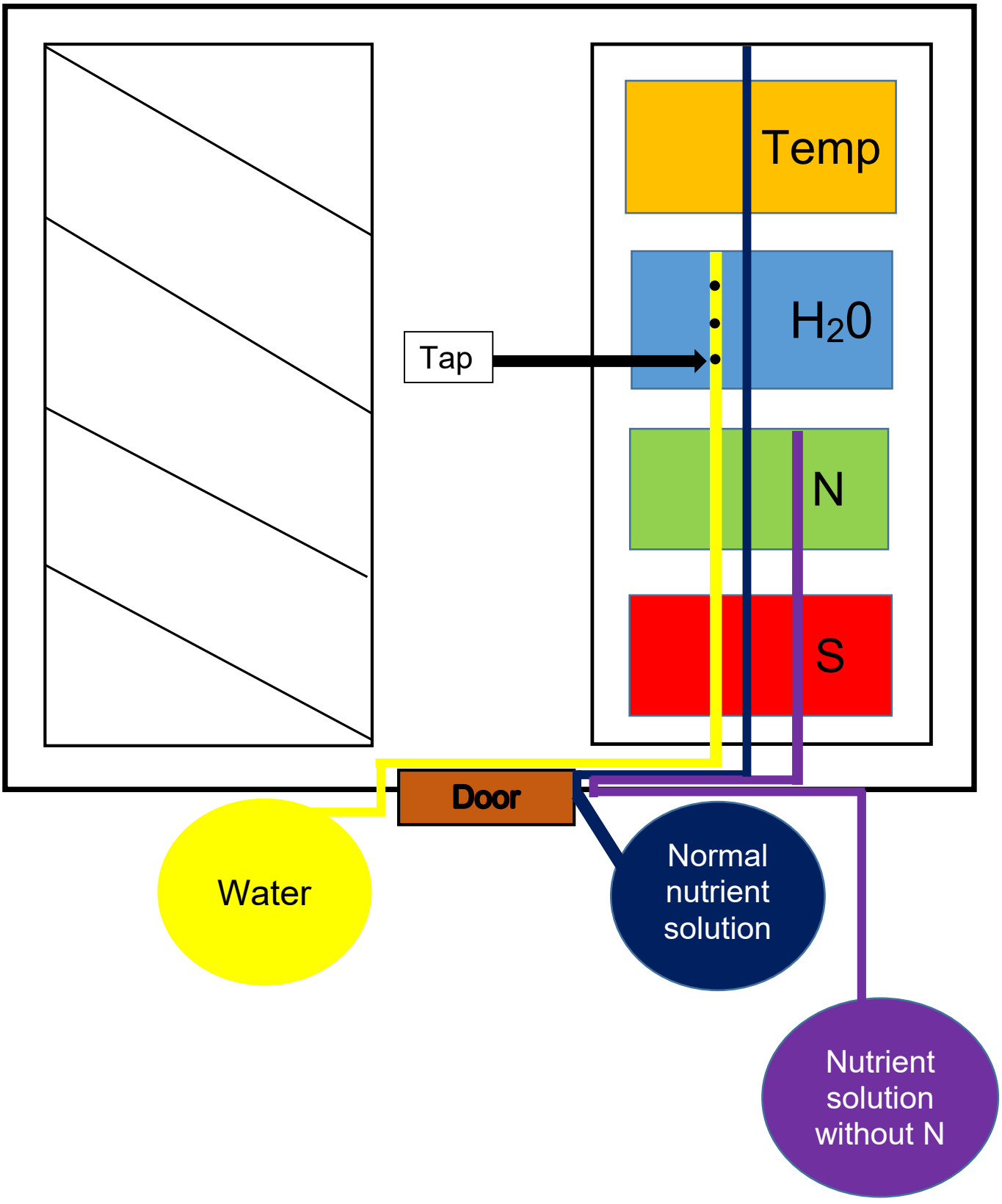


Figure 3.10: The experimental layout in the glasshouse

The data were analysed by means of ANOVA analyses by making use of Statistica (a statistical program) to test for significant interactions between treatments or significant differences within treatments. Significance level (Pr > F) of the main effects were calculated at the 5% probability level.

3.7 Follow-up trial

A follow-up trial was conducted in the Western Cape at Welgevallen, the experimental farm of Stellenbosch University in 2019, where the combined effect of temperature and nitrogen on the possible splitting of malting spring barley (*Hordeum vulgare* L.) was tested. Barley seeds of only the high-risk for split cultivar, Overture were used in this study. Overture is a medium maturing cultivar with low kernel nitrogen, high kernel plumpness and low screenings. Barley seeds obtained from AB InBev (Caledon) were used. Two seeds from the cultivar Overture were sown into a 3 L pot filled with a field soil medium on 5 June 2019. The field soil medium was collected at Welgevallen and sent for soil analyses. The soil was supplemented with calcitic agricultural lime to raise the pH and calcium levels to the preferred standard according to AB InBev (Burie Erasmus, AB InBev 2017).

3.7.1 Growing conditions

Plants were grown in a glasshouse in which day/night temperatures were maintained at 20 °C/15 °C day/night for 12 h/day. After approximately two months (in August), temperatures were increased to 25 °C/20 °C day/night and at ear emergence, it was further increased to 30 °C/20 °C day/night. The crops in this experiment were irrigated daily by means of drip irrigation to the soil's field capacity (normal irrigation regime) until they reached maturity. The crops received a nitrogen-free nutrient solution according to Table 3.2.

3.7.2 Treatments

Temperature

The control for the temperature treatments was to keep the plants at 30 °C/20 °C day/night for the rest of the growing season. At the end of soft dough, a third of the pots were transferred to a glasshouse where the temperature was dropped with 15°C

and 10°C respectively. These treatments were applied for three hours between 12h00 and 15h00. Thereafter, the pots were transferred back to normal growing conditions and were immediately irrigated by means of sprinkler irrigation to simulate approximately 30 mm of rainfall. The length of the simulated rainfall-event was determined by measuring the amount of irrigation water applied in one minute. The average amount of irrigation was calculated from five randomly placed rain gauges. The following formula was used:

$$\frac{30\ mm\ (rainfall)}{Average\ irrigation\ water\ (15\ ml)}\ x\ 60\ seconds$$

Two minutes of irrigation resulted in about 30 mm of simulated rainfall.

Nitrogen

The nitrogen treatment consisted of five different rates of nitrogen application as indicated in Table 3.6. The control treatment was a total of 140 kg N ha^{-1}, which was prescribed by AB InBev (Table 3.6).

Table 3.6: Amount of nitrogen in kg N ha^{-1} applied at planting, six weeks after planting and at soft dough stage for each of the treatments

	Amount of nitrogen (kg N ha^{-1})				
	Control	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Total N	**140**	**170**	**180**	**190**	**200**
Planting	100	100	100	100	100
4-6 weeks	40	40	40	40	40
Soft dough	-	30	40	50	60

3.7.3 Data collected

Measurements

In contrast to the 2018 trial, data were collected only during tillering, flag leaf and anthesis when total tillers, leaf number and plant height were recorded.

The same measurements were done at harvest for each of the pots as with the 2018 trial and the same calculations were used to determine the thousand grain mass and harvest index. After completing all the measurements and collecting the samples, split grains were identified by grain inspection (visual) and EC (Electrical conductivity) tests.

3.7.4 Experimental design and statistical analyses

The trial was conducted using a completely randomised split block design. This trial consisted of a combined factorial experiment that investigated the following two factorial combinations:

3.7.4.1 Temperature and Nitrogen

This experiment consisted of three temperature levels and five nitrogen levels as factors in a 3 x 5 factorial experiment, with six repetitions, a total of ninety pots.

The data were analysed by means of ANOVA analyses by making use of Statistica (a statistical program) to test for significant interactions between treatments or significant differences within treatments. Significance level (Pr > F) of the main effects were calculated at the 5% probability level.

CHAPTER 4

4. Results and discussion - Temperature

The effect of cooler temperature during grain filling on splitting of malting spring barley (*Hordeum vulgare* L.) grain

4.1 Vegetative growth parameters

Table 4.1 summarises the results of an Analysis of Variance (ANOVA) for the temperature experiment. Treatments were introduced after anthesis which means there were no significant differences and interactions with regards to treatments and therefore was not included in the ANOVA table.

As the temperature treatment was done after anthesis no significant ($p > 0.05$) differences for the vegetative growth parameters were found. There was however significant ($p < 0.05$) differences within cultivar and growth stage treatments respectively (Table 4.1).

Table 4.1: ANOVA table of main effects (cultivar and growth stage) and their interactions in terms of the number of tillers and leaves, leaf area, plant height and dry matter production

	Tillers	Leaves	Leaf area	Plant height	Dry matter
Cultivar	*	*	*	ns	ns
Stage	*	*	*	*	*
C*S	*	*	ns	ns	ns

ns = not significant

* = significance at 0.05%

Overture produced more tillers and leaves as well as higher leaf area than Cristalia (Table 4.2). However, no significant ($p > 0.05$) differences between Overture and Cristalia for plant height and dry matter production were recorded. Regarding growth stage, there was a significant difference between tillering, flag leaf and anthesis for all of the vegetative growth parameters (Table 4.2). There is an upward trend in all of the vegetative growth parameters from tillering to anthesis, which is normal as plants

increase in size as the growing season progresses. The dynamics of the appearance of leaves and tillers during the early stages of plant development are crucial for the growth of cereal crops. They contribute to the early accumulation of biomass during the initial phases of growth by early expansion of the leaf area index, giving the crop the ability to intercept solar radiation which largely determines growth and grain yield (Prystupa et al. 2003). Under favourable conditions, most cereal crops including barley, produce a great number of tillers in the early stages of crop development, however, not all of them progress to produce a fertile spike at maturity (Garcia del Moral et al. 2003).

Table 4.2: Means from the analysis of variances for the effect of growth stage and cultivar on the number of tillers and leaves, leaf area, plant height and dry matter production

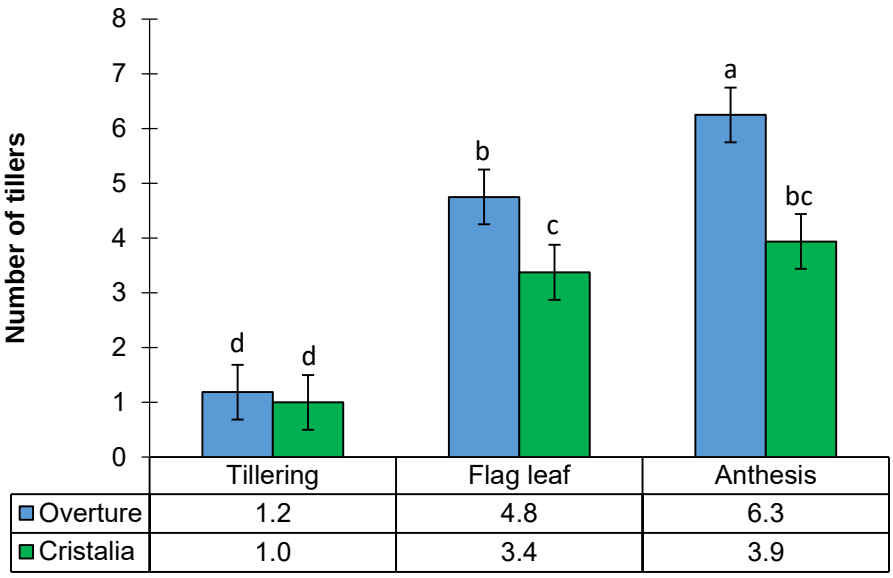
	Tillers	Leaves	Leaf area (cm^2)	Height (cm)	Dry matter (g)
Stages					
Tillering	1.1c	2.5c	20.6c	26.1c	0.1c
Flag leaf	4.1b	24.7b	338.4b	52.2b	2.5b
Anthesis	5.1a	38.8a	505.2a	68.9a	5.1a
Cultivars					
Overture	4.1a	25.2a	323.9a	49.7a	2.8a
Cristalia	2.8b	18.8b	252.3b	49.3a	2.4a

Means followed by the same letters within the same column are not significantly different at the 5% probability level.

Tillers

The process where tillers are formed can be divided into four phases: (i) the early phase in which tillers appears, (ii) a second phase where the maximum number of tillers is formed, (iii) the third phase where smaller tillers dies off due to competition with bigger tillers for nutrients and water until (iv) the final amount of tillers is established at the last phase (Alzueta et al. 2012). According to Table 4.1, cultivar and growth stage, as well as the interaction between them, had a significant ($p < 0.05$) effect on tiller production. The cultivar Cristalia, had a significant increase in the number of tillers from the tillering stage to the flag leaf stage whereafter no further

significant increases occurred (Figure 4.1). Overture had a significant increase in the number of tillers from tillering to anthesis (Figure 4.1). This reaction showed that Overture is quicker to form tillers than Cristalia. This is contrary to what was found by Berry et al. (2003), who found that higher tiller initiation is associated with lower tiller survival, which results in a counterbalance between the rates of tiller initiation and tiller mortality.



	Tillering	Flag leaf	Anthesis
Overture	1.2	4.8	6.3
Cristalia	1.0	3.4	3.9

Figure 4.1: The average number of tillers of cultivars Overture and Cristalia at tillering, flag leaf and anthesis stage. Vertical bars indicate 95% confidence intervals. Bars indicated with different letters differed significantly at p = 0.05.

Leaves

The process of tiller and leaf appearance is synchronised with each other (Prystupa et al. 2003), which means with an increase in the number of tillers throughout the development of the plant, the number of leaves would also increase. According to Table 4.1, cultivars and growth stages, as well as the interaction between them, had a significant ($p < 0.05$) effect on the number of leaves formed. Both cultivars had a significant increase in the number of leaves from the tillering stage to the anthesis stage (Figure 4.2). Figure 4.2 shows that there were only during anthesis a significant ($p < 0.05$) difference between the two cultivars because Overture produced more leaves than Cristalia. Several studies found that the rate of leaf appearance is constant throughout the growing phase of the plant (Baker et al. 1980; Kirby et al.

1985; Cao and Moss 1991; Slafer et al. 1994), which supports the results shown in Figure 4.2.

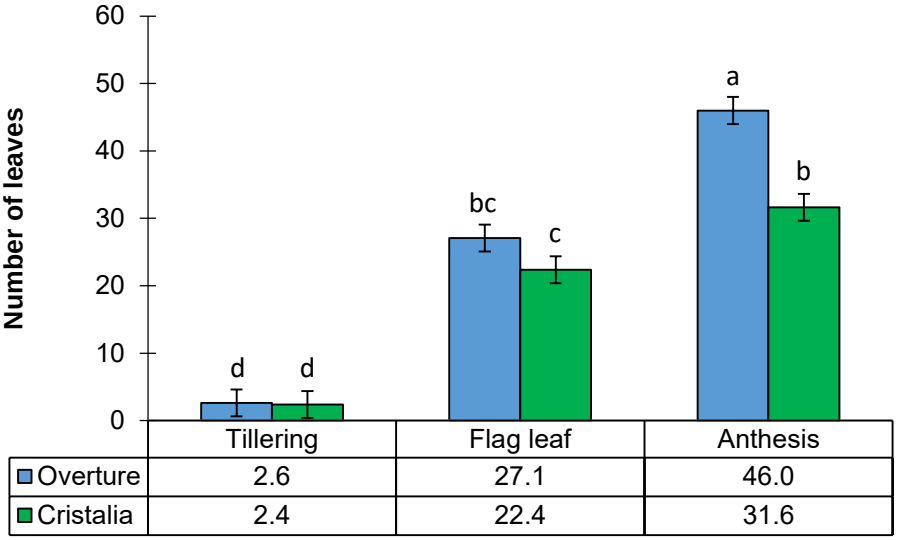


Figure 4.2: The average number of leaves of cultivars Overture and Cristalia at tillering, flag leaf and anthesis stage. Vertical bars indicate 95% confidence intervals. Bars indicated with different letters differed significantly at p = 0.05.

4.2 Reproductive and quality parameters

Table 4.3 summarises the results of an Analysis of Variance (ANOVA) for the reproductive and quality parameters of the temperature study.

Table 4.3: ANOVA of main effects (cultivar and treatment) and their interactions in terms of the number of tillers (T) and spikes (S), total number of grains (TNG), grain per spike (G/S), grain mass (GM), biomass (B), harvest index (HI), thousand grain mass (TGM), visual test (VT) and electrical conductivity (EC) test

	T	S	TNG	G/S	GM	B	HI	TGM	VT	EC
Cultivar	ns	*	*	ns	*	ns	*	*	*	*
Treatment	ns	ns	ns	ns	ns	ns	ns	ns	*	ns
C*T	ns	ns	ns	ns	*	*	ns	*	*	ns

ns = not significant

* = significant at 0.05%

Tillers

There were no significant ($p > 0.05$) differences within treatments and cultivars as well as no interaction between treatments and cultivars (Table 4.3).

Spikes

Post-anthesis temperature treatments had no effect on the number of spikes formed (Andersson and Holm 2011), which supports the results of this experiment. Although low temperatures had no effect on the number of spikes formed, the emergence of the spikes was delayed (Sakata et al. 2000). Results from Table 4.3 show that only cultivars had a significant ($p < 0.05$) effect on the number of spikes. The cultivar Overture produced on average 32 spikes compared to Cristalia's 26 spikes (Table 4.4).

Table 4.4: Influence of cultivars (Overture and Cristalia) on number of spikes, total number of grains (TNG), harvest index and electrical conductivity (EC) test under controlled conditions at Welgevallen. Different letters after the values in a column indicate significant differences at $p = 0.05$

	Spikes	**TNG**	**Harvest index**	**EC (µS/cm)**
Overture	31.6a	500.4a	0.38a	247.1a
Cristalia	26.0b	410.9b	0.32b	139.1b

Total number of grains

Low temperature (cold stress) is one of the main factors affecting crop production and reducing the yield potential (Zhang et al. 2011). The exposure of plants to low temperatures during the reproductive phase causes a reduction in metabolic rates, which results in lower yields (Thakur et al. 2010). Pržulj and Momčilović (2012) found that unfavourable climatic conditions such as high or low temperatures during the period of grain filling reduce grain yield and quality of spring barley. According to Table 4.3, only cultivars showed significant ($p < 0.05$) differences with Overture producing on average a total of 500 number of grains in comparison to Cristalia's 411 (Table 4.4). This result was expected due to more spikes produced by the cultivar Overture. Temperature treatments had no effect on the number of grains produced (yield) for this experiment, which does not support the findings mentioned above. The

reason for that is probably because the exposure to lower temperatures for this experiment was only for a week at grain filling and not for most of the reproductive phase as in the case of the previously mentioned studies.

Grains per spike

There were no significant ($p > 0.05$) differences within treatments and cultivars as well as no interaction between them (Table 4.3). The average number of grains per spike was sixteen (Data is not shown).

Grain mass

Grain mass is influenced by both the duration and rate of grain filling, with the duration being rather less important than the rate (Thakur et al. 2010). Low temperatures (<20 °C) may affect the development of the grain by changing cell division and differentiation as well as increasing the duration and reducing the rate of grain filling (Thakur et al. 2010) which results in increased grain mass. Andersson and Holm (2011) found that low temperatures after anthesis resulted in higher grain mass in contrast to high temperatures. Results from Table 4.3 showed that only cultivars, as well as the interaction between cultivars and treatments, had a significant ($p < 0.05$) effect on grain mass.

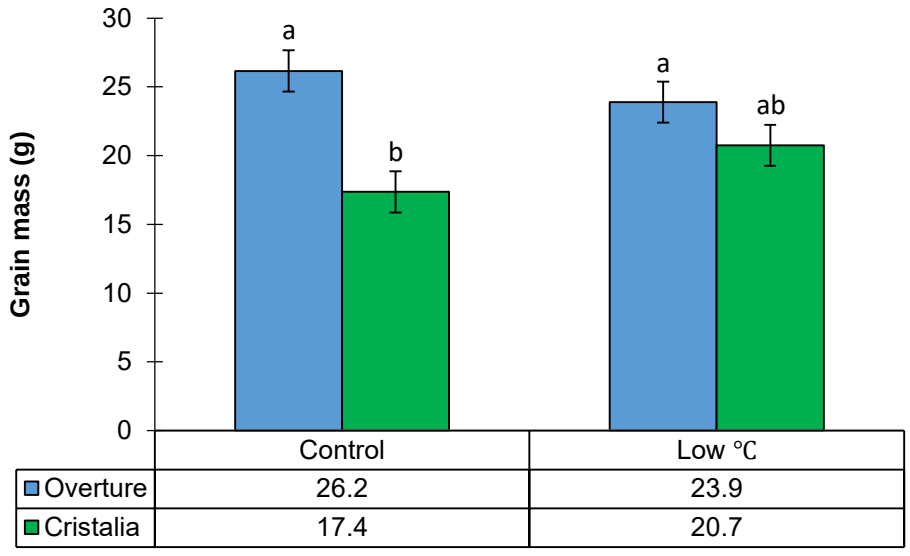


Figure 4.3: The average grain mass of cultivars Overture and Cristalia as affected by day/night temperatures of 25°C/20°C (control) and 15°C/5°C (low temperature) after

anthesis. Vertical bars indicate 95% confidence intervals. Bars indicated with different letters differed significantly at p = 0.05.

There were no differences between the treatments within each cultivar (Figure 4.3). However, grain produced by Cristalia under the control treatment weighed significantly less than both Overture's control and low temperature treatments (Figure 4.3). This result does not agree with the findings of Andersson and Holm (2011) and may be due to the short duration of exposure to lower temperature after anthesis.

Biomass

Grace (1988) found that both high and low temperatures have a negative effect on dry matter production and can also prevent biomass production under extreme conditions. According to Table 4.3, only the interaction between cultivars and treatments had a significant ($p < 0.05$) effect on biomass. There was only a significant difference between the treatments of Cristalia (Figure 4.4). The low-temperature treatment resulted in significantly higher biomass than the control treatment.

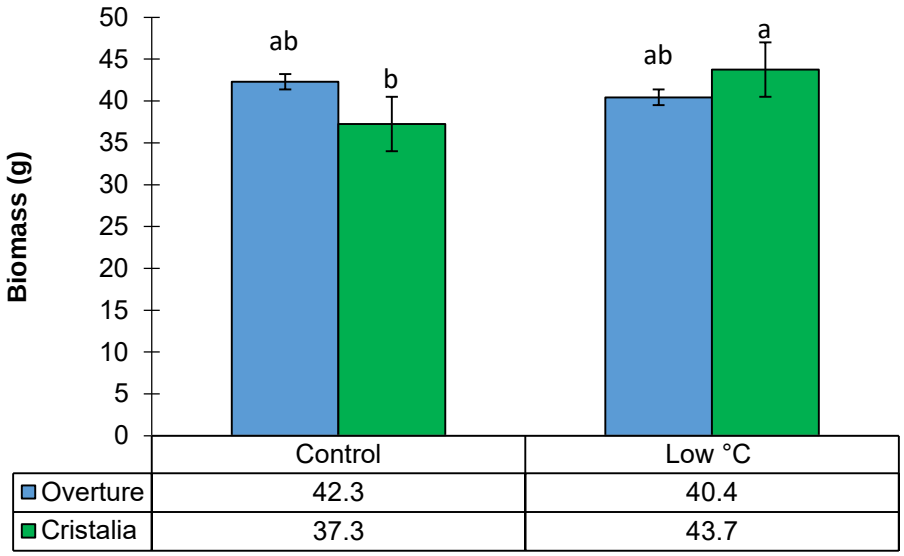


Figure 4.4: The average biomass of cultivars Overture and Cristalia as affected by day/night temperatures of 25 °C/20 °C (control) and 15 °C/5 °C (low temperature) after anthesis. Vertical bars indicate 95% confidence intervals. Bars indicated with different letters differed significantly at p = 0.05.

Harvest index

Andersson and Holm (2011) found that a low-temperature regime after anthesis increased grain mass and therefore produced a higher harvest index than a high-temperature regime after anthesis. Results from Table 4.3 showed that only cultivars showed significant ($p < 0.05$) differences with the cultivar Overture producing a harvest index of 0.38 compared to Cristalia's 0.32 (Table 4.4). Therefore, the results of the harvest index do not support the findings of Andersson and Holm (2011).

Thousand grain mass (TGM)

Results from Table 4.3 shows that cultivars, as well as the interaction between cultivars and treatments, had a significant ($p < 0.05$) effect on TGM. There were no differences in the TGM for the Cristalia treatments and also no difference between the two cultivars at low temperatures (Figure 4.5). However, Overture's control treatment had significantly ($p < 0.05$) higher TGM then all of the rest. There was also a significant ($p < 0.05$) difference between Cristalia's control treatment and Overture's low-temperature treatment (Figure 4.5).

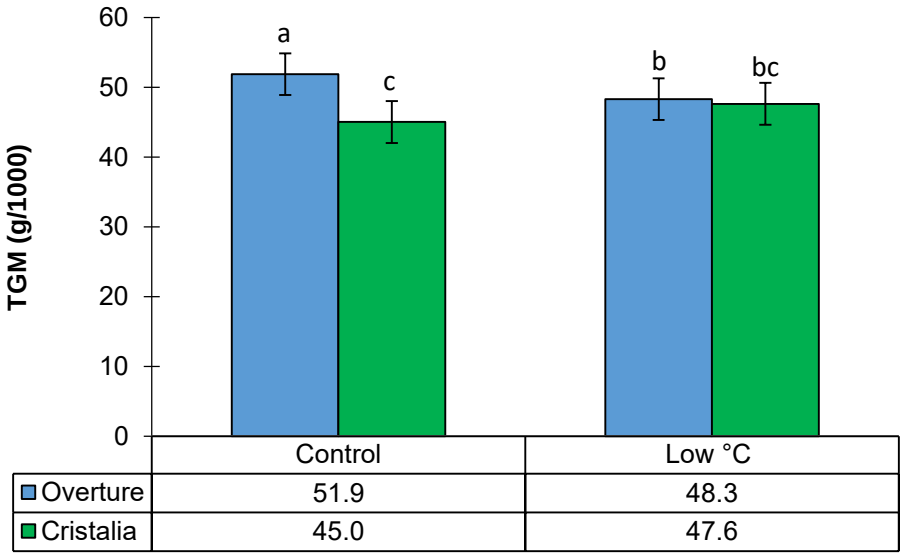


Figure 4.5: The average thousand grain mass (TGM) of cultivars Overture and Cristalia as affected by day/night temperatures of 25 °C/20 °C (control) and 15 °C/5 °C (low temperature) after anthesis. Vertical bars indicate 95% confidence intervals. Bars indicated with different letters differed significantly at $p = 0.05$.

Visual test (% split)

The visual test was used to identify split-grain according to the criteria mentioned in Chapter 3. Results from Table 4.3 shows that cultivars and treatments, as well as the interaction between them, had a significant ($p < 0.05$) effect on the visual splitting test. There were no differences in the percentage split between the two cultivars of the control treatment (Figure 4.6). There was, however, a higher percentage of splits in the low-temperature treatments and significant differences between the two cultivars. Overture had more splits than Cristalia. Low temperatures (below 10-15°C) are a major threat for several crops and can cause severe deformation of cell membranes (Mahajan and Tuteja 2005) and dehydration of intercellular spaces (Mutlu et al. 2013). Kai et al. (2003) found that a drop in temperature during husk development caused the grain to split due to under developing of the husks exposing the caryopsis through the lemma and palea. Australian research also claimed that a decrease in temperature of ±10°C, followed by a rainfall event during the period of grain filling, is the main cause of split (DPIRD 2017). Figure 4.6 shows a clear difference in percentage split between the different treatments for both cultivars Overture and Cristalia, which indicates that a decrease in temperature during grain filling had a great effect on splitting.

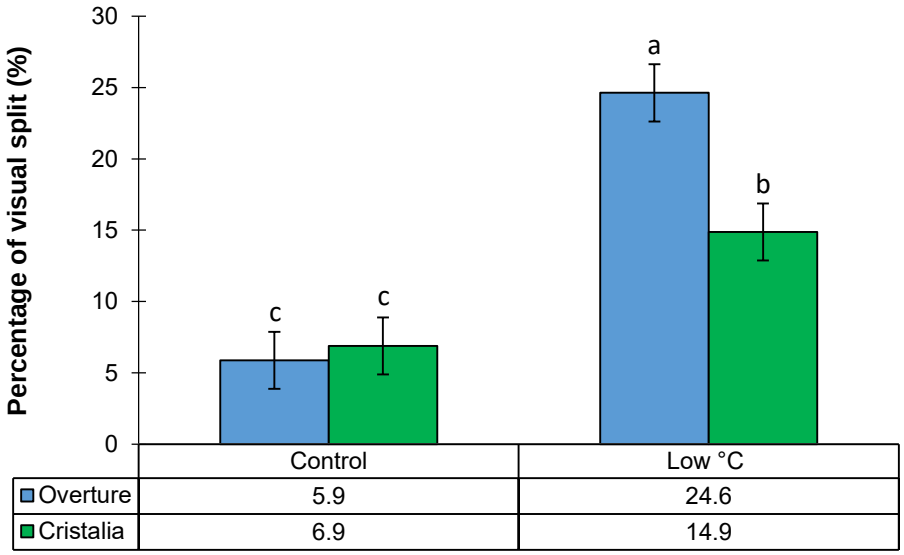


Figure 4.6: The average percentage of visual splits of cultivars Overture and Cristalia as affected by day/night temperatures of 25°C/20°C (control) and 15°C/5°C (low temperature) after anthesis. Vertical bars indicate 95% confidence intervals. Bars indicated with different letters differed significantly at $p = 0.05$.

Electrical conductivity (EC) test

There were no significant ($p > 0.05$) differences between the treatments as well as no interaction between cultivars and treatments for the temperature experiment (Table 4.3). However, there was a significant ($p < 0.05$) difference between the cultivars as Overture (247.1) showed much higher EC-value (higher number of splits) than Cristalia (139.1) (Table 4.4). It was expected from the high-risk cultivar Overture to produce more splits.

Grain nitrogen content

The grain's nitrogen concentration for each of the experiments was not statistically analysed due to the insufficient number of kernels produced. Therefore, one sample was taken by combining the replicates from each treatment and cultivar. The grain's protein concentration is an essential quality parameter when producing malting barley (Schelling et al. 2003). Post-anthesis temperatures have a direct effect on protein concentration in the grain, as nitrogen assimilation is more sensitive to temperatures than carbon assimilation. (Smith and Gooding 1999). Results from Table 4.5 shows a high percentage of protein content in the grain, ranging from 17 to 18.5%. This may be due to excessive nitrogen received by the plant by means of the standard nutrient solution (Table 3.1) that was applied daily. High N rates can increase splittting (Hoad et al. 2003).

Table 4.5: The grain nitrogen content of cultivars Overture and Cristalia as affected by day/night temperatures of 25ºC/20ºC (control) and 15ºC/5ºC (low temperature) after anthesis

Cultivars	Grain nitrogen content (%)	
	Control	Low ºC
Overture	2.9	2.7
Cristalia	2.9	3.0

4.3 Conclusion

The results indicate that cooler and warmer temperatures during post-anthesis (grain filling) have no effect on the development of the reproductive parameters of the barley plant. The influence of a drop in temperature during the period of grain filling (post-anthesis treatment) had a significant effect on the splitting of the barley grain. As expected Overture, the cultivar that has high risk for split, produced much more split-grain than Cristalia, the cultivar that has low risk for split.

CHAPTER 5

5. Results and discussion - Nitrogen

The effect of different nitrogen rates at planting, six weeks after planting and grain filling on splitting of malting spring barley (*Hordeum vulgare* L.) grain

5.1 Vegetative growth parameters

Table 5.1 summarises the results of an Analysis of Variance (ANOVA) for the nitrogen study.

Table 5.1: ANOVA of main effects (cultivar, treatment and growth stage) and their interactions in terms of the number of tillers and leaves, leaf area, plant height and dry matter production

	Tillers	Leaves	Leaf area	Plant height	Dry matter
Cultivar	*	*	ns	ns	ns
Treatment	*	*	*	*	*
Stage	ns	*	*	*	*
C*T	ns	ns	ns	ns	ns
C*S	ns	ns	ns	ns	*
T*S	ns	*	*	*	*
C*T*S	ns	*	*	ns	*

ns = not significant

* = significance at 0.05%

Tillers

The potential number of tillers is initiated very early in the season but the development and survival depends on environmental conditions (Dabbert et al. 2010). Nitrogen was applied at planting, six weeks after planting and at grain filling (soft dough) for each of the different treatments.

Results from Table 5.1 show that only cultivar and treatment had a significant ($p < 0.05$) effect on tiller production. The cultivar Overture (3) had a significantly higher

number of tillers than Cristalia (2) regardless of the different N treatments. According to Hoad et al. (2003), N can have different effects on the production and survival of tillers in different cultivars.

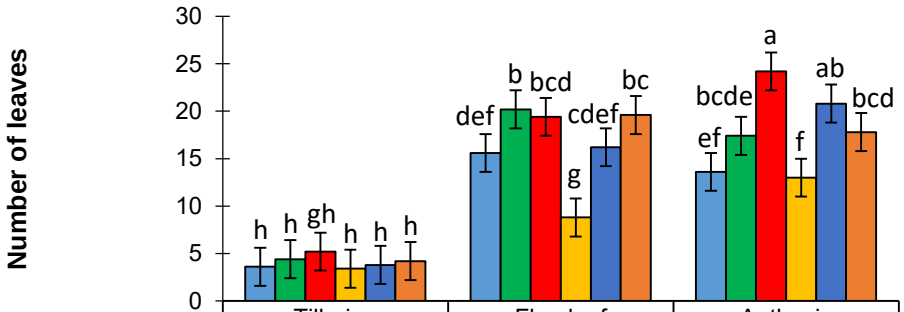
Regarding N treatments, there was a significant ($p < 0.05$) difference between the low N (2.0), control (2.7) and high N (3.2) treatments. The results of an increase in tillers at higher N rates can be due to excessive development of secondary or higher-order tillers (Flectcher and Dale, 1974). The increase in the number of tillers at higher N rates can also be mediated due to the positive effect of N on available carbohydrates at an early stage of crop development (Pinthus and Millet 1987; Van Keulen and Seligman 1987).

Leaves

The dynamics of leaf appearances are critical processes in the earliest stages of crop development (Prystupa et al. 2003). Generally, when a shortage or lack of N affects the rate of leaf appearance, it leads to a decrease in the total number of leaves formed (Dale and Wilson 1978; Longnecker et al. 1993).

Results from Table 5.1 shows that cultivars, treatments and growth stages, as well as the interaction between the factors, had a significant ($p < 0.05$) effect on the number of leaves. There were no significant ($p > 0.05$) differences between the two cultivars Overture and Cristalia for each of the different treatments at the tillering stage (Figure 5.1).

During the flag leaf stage, there was a significant ($p < 0.05$) difference in the number of leaves between cultivars under the low N treatment. This reaction shows that lower rates of N have an effect on the number of leaves formed for both cultivars in their earlier growth stages and supports the findings of Dale and Wilson (1978) and Longnecker et al. (1993). The increase in the number of leaves from flag leaf to anthesis was significant for Overture's high N treatment, as well as for Cristalia's low N and control treatments. The availability of N at a later stage of crop development kept increasing the number of leaves formed as seen with the control and high N treatments of Cristalia and Overture respectively in Figure 5.1.



	Tillering	Flag leaf	Anthesis
Overture Low N	3.6	15.6	13.6
Overture Control	4.4	20.2	17.4
Overture High N	5.2	19.4	24.2
Cristalia Low N	3.4	8.8	13.0
Cristalia Control	3.8	16.2	20.8
Cristalia High N	4.2	19.6	17.8

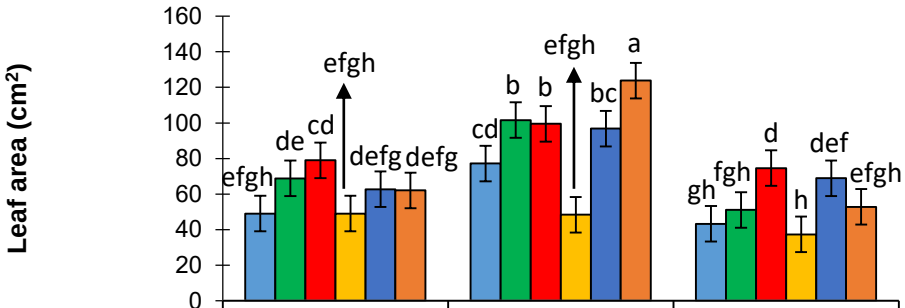
Figure 5.1: The average number of leaves of cultivars Overture and Cristalia as a result of low (100 kg N ha^{-1}), control (150 kg N ha^{-1}) and high N (200 kg N ha^{-1}) rates. Vertical bars indicate 95% confidence intervals. Bars indicated with different letters differed significantly at p = 0.05.

Leaf area

Leaf area index (LAI) is the ratio of leaf surface area per ground surface for a given unit area that characterises plant canopies (Breda 2003). An increase in leaf area is due to the development of broader and longer leaves as well as the formation of new tillers and leaves during its vegetative stage under favourable conditions.

Table 5.1 shows that the interaction between cultivars, treatments and growth stages had a significant ($p < 0.05$) effect on leaf area. It was found by Grashoff and d'Antuono (1997) that higher rates of N application increased the leaf area index of barley. Figure 5.2 shows that only Overture's high N treatment produced a significantly ($p < 0.05$) higher leaf area than the low N treatment at the tillering stage, but that there was no difference in leaf area with higher N rates for Cristalia. By flag leaf stage, there was a significant increase in leaf area with an increase in N rates for the Cristalia cultivar as well. From tillering to flag leaf stage, there was a significant increase in leaf area for Overture's control, low and high N treatment, as well as for Cristalia's control and high N treatments. However, during anthesis, there was a significant ($p < 0.05$) decrease in leaf area for both cultivars and their treatments, except for Cristalia's low N treatment.

Hoad et al. (2003) found that the loss of green leaf area is delayed when there is an increase in N rate in cultivars, Landlord (low risk for split) and Chariot (high risk for split). Therefore, the decrease in leaf area is contradictory to Hoad et al.'s (2003) finding. This reaction can either be due to the loss of N through leaching and not being available for the plant to use at a crucial stage of crop development, and/or that loss of green leaf area of Overture and Cristalia cannot be delayed with an increase in N rate.



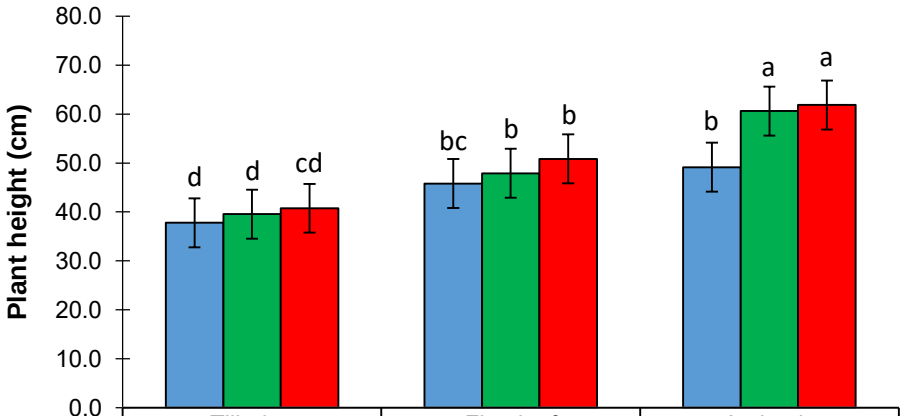
	Tillering	Flag leaf	Anthesis
Overture Low N	49.1	77.2	43.3
Overture Control	68.8	101.6	51.1
Overture High N	79.0	99.5	74.6
Cristalia Low N	49.0	48.4	37.3
Cristalia Control	62.7	96.8	68.9
Cristalia High N	62.1	123.8	52.8

Figure 5.2: The average leaf area of cultivars Overture and Cristalia as a result of low (100 kg N ha^{-1}), control (150 kg N ha^{-1}) and high N (200 kg N ha^{-1}) rates. Vertical bars indicate 95% confidence intervals. Bars indicated with different letters differed significantly at p = 0.05.

Plant height

Plant height increases as cereals develop, but N is the most important component for supporting plant growth and will further increase the size of cereal when an optimal amount of N is applied. Rashid et al. (2007) noted that with an increase in the rate of N application, the plant height tended to increase linearly. Results from Table 5.1 shows that treatments and growth stages, as well as the interaction between them, had a significant ($p < 0.05$) effect on plant height. There were no significant differences in plant height at tillering and flag leaf stage for the different treatments (Figure 5.3). This means that different N rates had no effect on the plant height in the earlier stages of crop development, contradicting Rashid et al. (2007). However,

there was a significant difference at the anthesis stage where both the control and high N treatments produced taller plants than the low N treatment (Figure 5.3).



	Tillering	Flag leaf	Anthesis
Low N	37.8	45.8	49.2
Control	39.6	47.9	60.6
High N	40.8	50.9	61.9

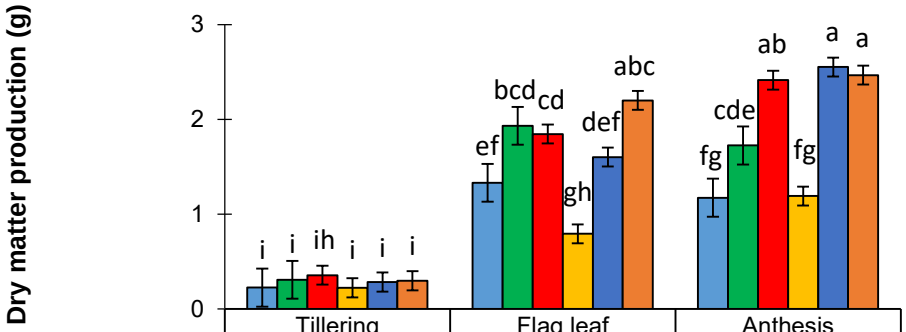
Figure 5.3: The average plant height of treatments low N (100 kg N ha^{-1}), control (150 kg N ha^{-1}) and high N (200 kg N ha^{-1}) at tillering, flag leaf and anthesis stage. Vertical bars indicate 95% confidence intervals. Bars indicated with different letters differed significantly at p = 0.05.

Dry matter production

Dry matter production (DM) is the measurement of the plant mass when completely dried. Higher rates of N application resulted in an increase in DM of barley plants, with an increase in the number of leaves and total leaf area index of the plant (Cantero-Martinez et al. 2003). According to Table 5.1, treatments and growth stages had an effect on dry matter production respectively, but there was also a significant ($p < 0.05$) interaction between cultivars, treatments and growth stages. There were no significant ($p > 0.05$) differences in dry matter production at the tillering stage (Figure 5.4).

During the flag leaf stage, DM production of Cristalia significantly increased as N rate was increased from low to control and control to high There was also a significant increase in DM production of Overture from the low to control N treatments, but no difference between the control and high N treatment (Figure 5.4). However, during anthesis, Overture's high N treatment had a significantly higher DM production than the control treatment, while Cristalia's control and high N treatments were the same.

Dry matter production of Overture was significantly higher than Cristalia under the low N treatment during the flag leaf stage, but during anthesis, these treatments had similar DM production (Figure 5.4). The increase in DM production from flag leaf to anthesis were significant for Overture's high N treatment, as well as for Cristalia's control treatment. These reactions show that N has a greater influence on the DM production of Cristalia earlier in its growing cycle compared to Overture.



	Tillering	Flag leaf	Anthesis
Overture Low N	0.2	1.3	1.2
Overture Control	0.3	1.9	1.7
Overture High N	0.4	1.8	2.4
Cristalia Low N	0.2	0.8	1.2
Cristalia Control	0.3	1.6	2.6
Cristalia High N	0.3	2.2	2.5

Figure 5.4: The average dry matter production of cultivars Overture and Cristalia as a result of low (100 kg N ha^{-1}), control (150 kg N ha^{-1}) and high N (200 kg N ha^{-1}) rates. Vertical bars indicate 95% confidence intervals. Bars indicated with different letters differed significantly at p = 0.05.

5.2 Reproductive and quality parameters

Table 5.2 summarises the results of an Analysis of Variance (ANOVA) of reproductive and quality parameters of the nitrogen study.

Tillers

There was no significant (p > 0.05) interaction between cultivars and treatments (Table 5.2). There was, however, more tillers produced by Overture than Cristalia (Table 5.3). Regardless of the cultivars, the low N treatment had significantly fewer tillers than the control and the high N treatment (Table 5.3). As expected, an increase in N rate stimulated the tiller production (Lafond et al. 2011), increasing the number

of tillers formed which resulted in a positive correlation between N rate and the number of grains produced (Grashoff and d'Antuono 1997).

Table 5.2: ANOVA of main effects (cultivar and treatment) and their interaction in terms of the number of tillers (T) and spikes (S), total number of grains (TNG), grain per spike (G/S), grain mass (GM), biomass (B), harvest index (HI), thousand grain mass (TGM), visual test (VT) and electrical conductivity (EC) test

	T	S	TNG	G/S	GM	B	HI	TGM	VT	EC
Cultivar	*	*	ns	*	ns	ns	ns	ns	*	*
Treatment	*	*	*	ns	*	*	ns	ns	*	ns
C*T	ns	ns	ns	ns	ns	ns	ns	ns	ns	*

ns = not significant

* = significance of 0.05%

Table 5.3: Means from the analysis of variance for the effect of nitrogen rate and cultivar on the number of tillers and spikes and visual test (% split) at harvest

Treatments	Tillers	Spikes	Visual Test (% split)
Nitrogen rates			
Low N	4.5b	3.9b	18.1b
Control	6.5a	5.8a	30.5a
High N	6.7a	6.4a	20.1b
Cultivars			
Overture	6.5a	5.9a	18.9b
Cristalia	5.3b	4.9b	26.8a

Means followed by the same letters within the same column within a treatment are not significantly different at the 5% probability level.

Spikes

There was no significant ($p > 0.05$) interaction between cultivars and treatments (Table 5.2). There was, however, more spikes produced by Overture than Cristalia (Table 5.3). Regardless of the cultivars, the low N treatment had significantly fewer spikes than the control and the high N treatment (Table 5.3). Baethgen et al. (1995) found that applying high rates of N in the early stages of crop development stimulates

tiller production, but many of these tillers do not produce spikes. Spike numbers mainly depend on the tillers produced. When tiller survival is small due to low N availability in the earlier growth stages, the vegetative tillers would compete with spike-bearing tillers for nutrients and water (Wych et al. 1988). This relates to the high N treatment producing the same number of tillers than the control treatment in this study.

Total number of grains

Only treatments resulted in significant ($p < 0.05$) differences (Table 5.2) under the low N treatment producing less grain than both the control and high N treatment (Table 5.4). Higher rates of N fertiliser are commonly associated with an increase in the total amount of grain production. If there is a shortage/lack of N (e.g. low N treatment), both the amount of grain and yield will decrease (Fischer 1993; Baethgen et al. 1995).

Table 5.4: Influence of nitrogen (low N -100 kg N ha^{-1}, control -150 kg N ha^{-1} and high N -200 kg N ha^{-1}) on total number of grains (TNG), grain mass and biomass under controlled conditions at Welgevallen. Different letters after the values in a column indicate significant differences at $p = 0.05$

	TNG	Grain mass (g)	Biomass (g)
Low N	42.6b	2.3b	4.0b
Control	62.7a	3.4a	6.2a
High N	76.4a	4.2a	7.0a

Grain per spike

There was only a significant ($p > 0.05$) difference between the two cultivars Overture and Cristalia (Table 5.2). Cristalia produced an average of thirteen grains per spike compared to Overture's ten grains per spike (Data is not shown). The number of grains per spike is cultivar dependent (Pinthus and Millet 1978), which explain why Cristalia, the cultivar that has low risk for split, produced more grains per spike than Overture (high risk for split). Baethgen et al. (1995) noted where there was an increase in spikes due to N fertilisation, the spikes produced less grain, which corresponds with the cultivar Overture producing more spikes than Cristalia, as

mentioned before. According to Grashoff and d'Antuono (1997), the increase in N rates had no effect on the number of grains per spike. This supports the result observed in this study where no significant differences between treatments occurred.

Grain mass

Only treatments showed significant ($p < 0.05$) differences (Table 5.2). Low N treatment produced less grain mass than both the control and high N treatment (Table 5.4). According to a study done by Baethgen et al. (1995), grain mass was the least affected by N fertiliser of all the yield components. The high rate of N application at an early stage of crop development may increase the number of tillers produced, while tall but weak stems are formed resulting in lodging at a later growth stage (Baethgen et al. 1995; Lafond et al. 2011). Lodging is associated with a reduction in grain mass and can be the result of the high N treatment producing similar grain mass than the control treatment.

Biomass

Only treatments showed significant ($p < 0.05$) differences (Table 5.2) under the low N treatment producing less biomass than both the control and high N treatment (Table 5.4). A higher rate of N increased the total biomass due to the increase in the number of leaves and leaf area shown in Figures 5.1 and 5.2.

Harvest Index

There were no differences within or no interaction between treatments and cultivars (Table 5.2). The average harvest index for the N experiment was 0.36 (Data is not shown).

Thousand grain mass (TGM)

There were no differences within treatments and cultivars as well as no interaction between cultivars and treatments (Table 5.2). The average TGM for the N experiment was 55 g (Data is not shown). The effect of higher rates of N fertiliser application on TGM varied from lower to higher levels across different areas (Hoad et al. 2003). Therefore, no clear effect of an increase in N rate on TGM exists as results obtained in this study showed no significant differences between the different N treatments.

Visual test (% split)

There was no significant ($p > 0.05$) interaction between cultivars and treatments of the N experiment in terms of the visual test to determine if splitting of barley grains occurred. There was, however, more splits in Cristalia than Overture (Table 5.3). Thus, regardless of the amount of N applied to the barley, the Cristalia cultivar resulted in more splits than Overture. Regardless of the cultivars, the control treatment had a significantly higher percentage of splits than the low and the high N treatment (Table 5.3). According to Hoad et al. (2003), trials in Scotland associated splitting to high rates of N application late in the growing season (Unpublished data). However, there were no clear causes identified. They found that the rate of N application had no significant effect on splitting in Landlord, the cultivar that has low risk for split, but for Chariot (high risk for split) an increased N rate led to higher levels of splitting. There was a significant difference in cultivars with Chariot splitting more than Landlord. The results achieved in the current study relate to those of Hoad et al. (2003), except where the visual test showed that the low-risk cultivar had produced the most splits.

Electrical conductivity (EC) test

There was a significant ($p < 0.05$) interaction between cultivars and treatments in terms of EC. Overture displayed significantly ($p < 0.05$) more splits than Cristalia, regardless of the treatment (Figure 5.5). The Overture cultivar's reaction to the N treatments showed only a significant difference between the high and low N treatments, with the high N treatment producing a higher percentage of splits than the low N treatment. On the other hand, Cristalia's reaction to the different N treatments showed only a significant difference between the low N and control treatments, with the low N producing a higher percentage of splits than the control treatment. The results of the visual test were not clear enough and therefore the EC test was conducted to compare the results with each other. The content leaching out of the grain, determined by the EC tests, indicated that the grain had split. Referring to Figure 5.5, the result is contradicting to the visual test where Cristalia split significantly more than Overture. Where there is an increase in the rate of N, splitting was significantly higher for the high risk for split cultivar (Overture); both of those supporting the findings of Hoad et al. (2003).

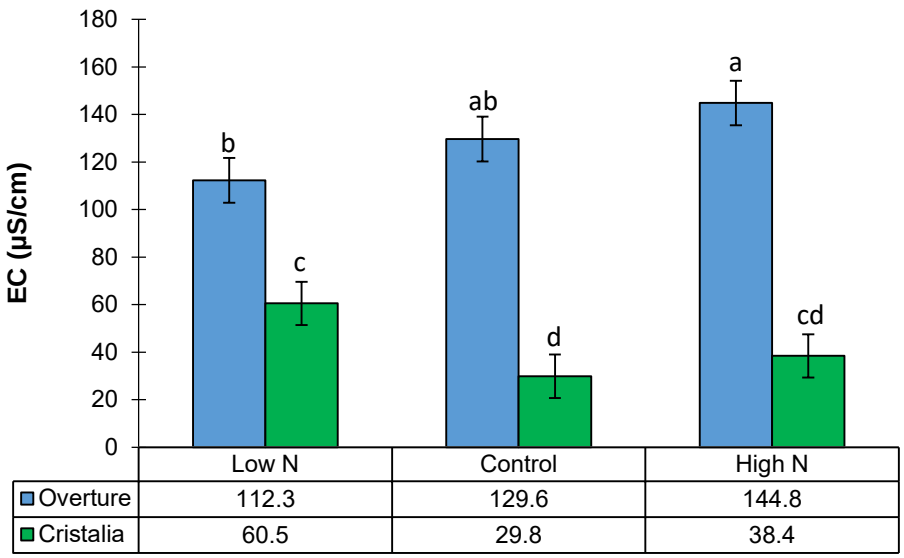


Figure 5.5: The average electrical conductivity (EC) of cultivars Overture and Cristalia as a result of low (100 kg N ha^{-1}), control (150 kg N ha^{-1}) and high N (200 kg N ha^{-1}) rates. Vertical bars indicate 95% confidence intervals. Bars indicated with different letters differed significantly at p = 0.05.

Grain nitrogen content

Mckenzie et al. (2005) found in a study that both the rate of N application and cultivar has an effect on grain protein concentration. High rates of N application can cause an excessive amount of N to be transferred from the vegetative organs to the grains, which increases the protein concentration and decreases the malting quality of the barley grain (Baethgen et al. 1995). Baethgen et al. (1995) also noted that the application of N during stem elongation may result in high grain N content, which is not suitable for the malting process. Grain with a nitrogen content of between 1.5% and 2.0% is accepted as malting barley (ARC 2018). Results from Table 5.5 shows a lower percentage of N for the high N treatment. This may be the result of a loss of N through leaching. Only the Overture cultivar showed a protein content of more than 10% at low N rate and the control treatment.

Table 5.5: The grain's nitrogen content of the cultivars Overture and Cristalia as a result of low N (100 kg N ha^{-1}), control (150 kg N ha^{-1}) and high N (200 kg N ha^{-1}) rates

Cultivar	Grain nitrogen content (%)		
	Low N	Control	High N
Overture	1.8	2.1	1.3
Cristalia	1.4	1.5	1.3

5.3 Conclusion

The different rates of N application during the early stages of crop development had a major impact on the vegetative growth parameters of the plants. Higher rates of N application during grain filling period have an increased effect on the reproductive growth parameters. The results indicate that different N rates and cultivars had a great effect on split grain. However, the visual and EC tests showed different results with regards to which cultivar produced the most splits and the effect of the N levels was not clear. Further investigation into the rate of N application and certain cultivars on the splitting of malting spring barley may explain the variation in results obtained in this study.

CHAPTER 6

6. Results and discussion – Light intensity

The effect of light intensity during husk development on splitting of malting spring barley (*Hordeum vulgare* L.) grain

6.1 Vegetative growth parameters

Table 6.1 summarises the results of an Analysis of Variance (ANOVA) for the light intensity study.

Table 6.1: ANOVA of main effects (cultivar, treatment and growth stage) and their interactions in terms of the number of tillers and leaves, leaf area, plant height and dry matter production

	Tillers	Leaves	Leaf area	Plant height	Dry matter
Cultivar	ns	*	*	*	*
Treatment	ns	ns	*	*	ns
Stage	*	*	*	*	*
C*T	ns	*	ns	ns	ns
C*S	*	ns	ns	ns	ns
T*S	ns	ns	ns	*	ns
C*T*S	ns	*	*	ns	ns

ns = not significant

* = significant at 0.05%

Tillers

Tillers start to form at the beginning of the tillering stage of a cereal crop's growing cycle and will continue to do so until it enters its reproduction stage if conditions are favourable. Shading was introduced at the start of stem elongation when tiller formation is usually greatly completed. Therefore, treatments did not have a significant effect on the number of tillers that formed (Table 6.1). These findings confirm the results of a study by Kőszegi et al. (2005) where the change in light intensity had minor effects on the plant's tillering ability.

There was, however, a significant interaction between cultivars and growth stages (Table 6.1). The cultivar Cristalia had a significant increase in the number of tillers from the tillering stage to the flag leaf stage whereafter no further significant increases occurred (Figure 6.1). Overture had a significant increase in the number of tillers from tillering to anthesis, but not between tillering and flag leaf or flag leaf and the anthesis stage (Figure 6.1). This reaction shows that Cristalia is quicker to form tillers than Overture. It is also important to note that there was no difference in the number of tillers between the two cultivars at each of the growth stages (Figure 6.1).

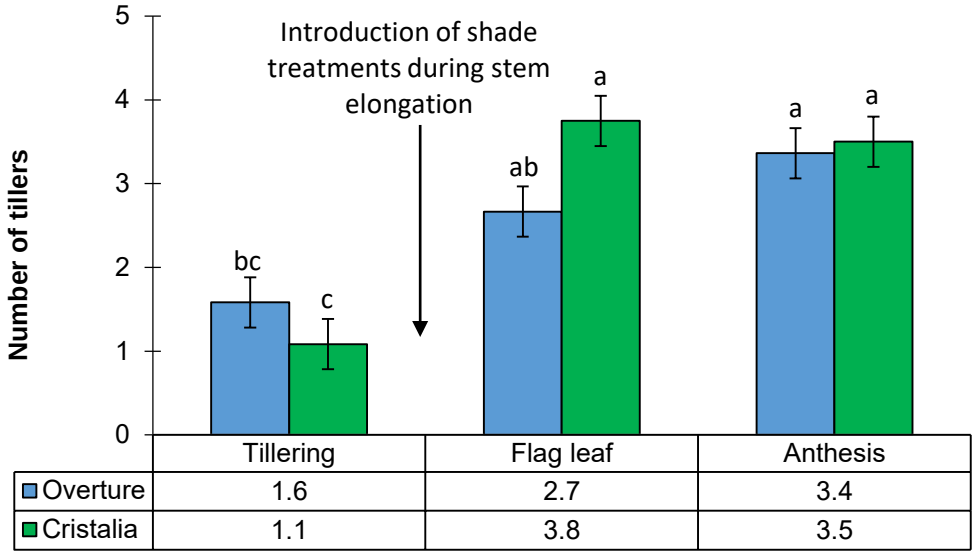


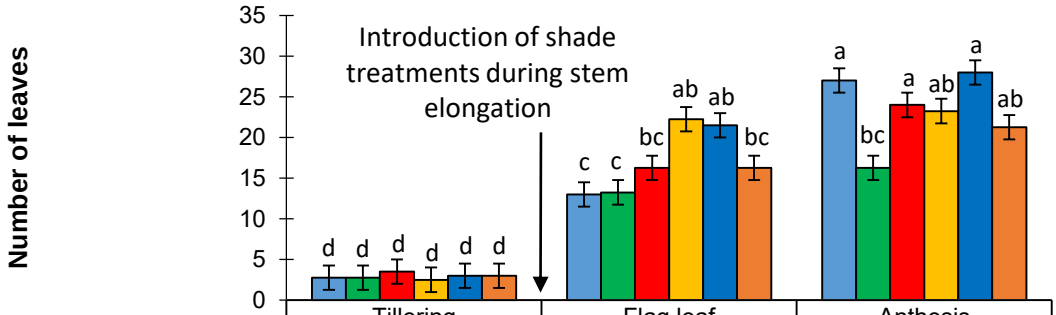
Figure 6.1: The average number of tillers of cultivars Overture and Cristalia at tillering, flag leaf and anthesis stages. Vertical bars indicate 95% confidence intervals. Bars indicated with different letters differed significantly at p = 0.05.

Leaves

The interaction between cultivars, treatments and growth stages had a significant effect on the number of leaves (Table 6.1). Even though there was no significant difference in the number of leaves between the two cultivars Overture and Cristalia, there was an increase in the number of leaves after tillering (when shading was introduced) for the different treatments at the tillering stage (Figure 6.2). Shading has a long-term effect on leaves such as slowing the emergence of later developing leaves (Dale et al. 1972), in other words, reducing the plant growth rate. A delay in later emerging leaves may be due to an increase in sheath length of the shaded leaf,

which means the later developing leaves have to grow longer before emerging (Dale et al. 1972).

During the flag leaf stage, there was no difference between the treatments within the cultivars Overture and Cristalia respectively, which means that shading had no effect on the number of leaves formed at the flag leaf stage. There were however significant differences between Overture and Cristalia under the control and 40% shade treatment. This is the result of gene differences and not the effect of shading (Borràs et al. 2009). The anthesis stage showed a significant increase in the number of leaves from the flag leaf stage for Overture's control and 60% shade treatment. A study by Porter et al. (1950) showed that when the first leaf is shaded, it causes a delay in the appearance of subsequent leaves while shading the later-formed leaves had minor effects. These findings of Porter et al. (1950) relates to the result shown in Figure 6.2, with minor effects on the number of leaves at anthesis.



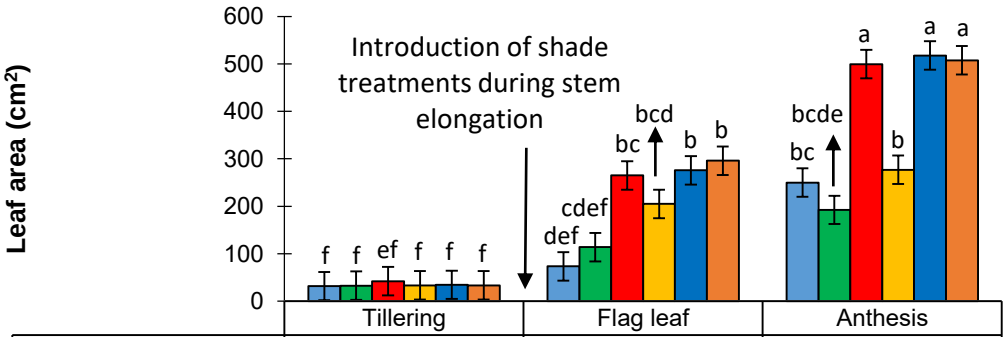
	Tillering	Flag leaf	Anthesis
Overture Control	2.8	13.0	27.0
Overture Shade (40%)	2.8	13.3	16.3
Overture Shade (60%)	3.5	16.3	24.0
Cristalia Control	2.5	22.3	23.3
Cristalia Shade (40%)	3.0	21.5	28.0
Cristalia Shade (60%)	3.0	16.3	21.3

Figure 6.2: The average number of leaves of cultivars Overture and Cristalia as a result of no shading (control), 40% and 60% shading at tillering, flag leaf and anthesis stage. Vertical bars indicate 95% confidence intervals. Bars indicated with different letters differed significantly at p = 0.05.

Leaf area

Under favourable conditions, a cereal crop will keep on producing new tillers and leaves during its vegetative stage and thereby increases its leaf area. A crop will also

increase the length of its lamina and sheath in an attempt to increase its chances of being reached by more sunlight under low-light or shaded conditions (Dale et al. 1972). Results from Table 6.1 shows that cultivars, treatments and growth stages each, as well as the interaction between them, had a significant ($p < 0.05$) effect on the leaf area. From the discussion in the previous paragraph it can also be assumed that any increases in leaf area between flag leaf and the beginning of the anthesis stage are not a result of more tillers and leaves, but an increase in the lamina and sheath of the existing leaves. During the flag leaf stage, there was a difference in leaf area between Overture and Cristalia under the 40% shade treatment, but only the 60% shade treatment had a significant effect on the Overture cultivar (Figure 6.3).



	Tillering	Flag leaf	Anthesis
Overture Control	31.7	73.2	250.0
Overture Shade (40%)	32.5	113.9	192.3
Overture Shade (60%)	42.2	265.0	499.5
Cristalia Control	33.4	204.9	277.0
Cristalia Shade (40%)	34.4	275.9	517.8
Cristalia Shade (60%)	33.3	296.0	507.8

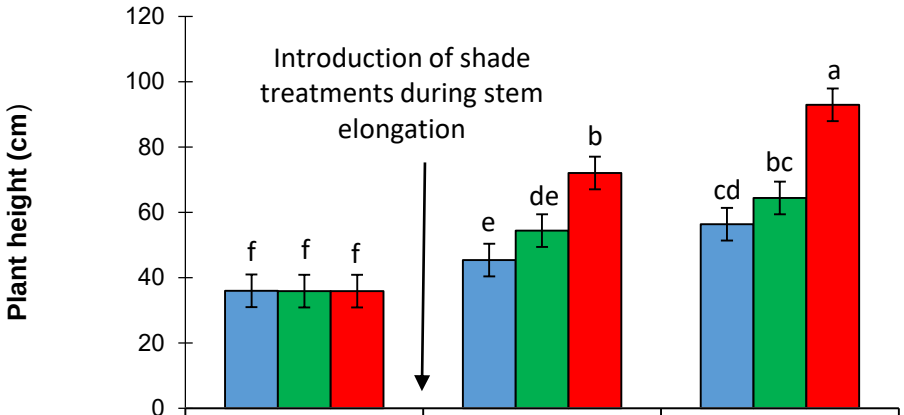
Figure 6.3: The average leaf area of cultivars Overture and Cristalia as a result of no shading (control), 40% and 60% shading, at tillering, flag leaf and anthesis stage. Vertical bars indicate 95% confidence intervals. Bars indicated with different letters differed significantly at $p = 0.05$.

Therefore, this difference is a result of a difference in genes and not a result of less sunlight. By the time the two cultivars reached anthesis, the shade treatments also had an effect on Cristalia. The increase in leaf area from flag leaf to anthesis was significant for Overture's control and 60% shade treatment as well as for Cristalia's 40 and 60% shade treatments. This reaction shows that shade has an influence on the leaf area of Overture earlier in its growing cycle compared to Cristalia, but that this is only the case if the crop receives less than 40% light. On the other hand, the

leaf area of Cristalia will be affected by shade during the last vegetative stages of its growing cycle, but this effect already takes place with 40% shade.

Plant height

The height of a cereal crop will keep on increasing under optimal growing conditions as the growing season progresses. The competition for sunlight between the tillers when shading is introduced at the start of stem elongation may lead to thinner and taller stems, which increases the risk of lodging (Pinthus 1974). Results from Table 6.1 shows that all three main effects (cultivar, treatment and growth stage) had an effect on the plant's height, but only the interaction between treatments and growth stages were significant. There were no significant differences in plant height before shading was introduced (Figure 6.4), however, the effect of shading on plant height can be noticed at the later growth stages.



	Tillering	Flag leaf	Anthesis
Control	36.0	45.4	56.4
Shade (40%)	35.9	54.4	64.4
Shade (60%)	35.9	72.1	92.9

Figure 6.4: The average plant height of treatments control (no shading), 40% shade and 60% shade at tillering, flag leaf and anthesis stage. Vertical bars indicate 95% confidence intervals. Bars indicated with different letters differed significantly at p = 0.05.

During the flag leaf stage, the 60% shade treatment had a greater effect on plant height than both the control and 40% shade treatment, producing much taller plants. The same results were seen at the anthesis stage. There is an upward trend in plant height from tillering to anthesis, which is normal. However, the results show that by

reducing the ambient light by more than 40%, the height of the plants significantly increased as the crops are forced to stretch for more sunlight. Regarding the cultivars, there was a significant ($p > 0.05$) difference with Cristalia (60 cm) producing much higher plants than Overture (50 cm).

Dry matter production

Dale et al. (1972) noted that early shading has a direct effect on dry material mass by reducing photosynthesis levels in shaded leaves which is close to or below the compensation point. The effect of late shading (start of stem elongation) on dry material mass, is much smaller due to the continuing contribution from early developing leaves before shading, keeping newly assimilated carbon available for growth (Dale et al. 1972). Therefore, treatments had no significant effect on DM production due to the late introduction of shading (Table 6.1). Only the cultivars and growth stages had significant effects on DM production (Table 6.1). The cultivar Cristalia (1.5 g) had a significantly higher dry material mass than Overture (0.9 g) regardless of the different treatments (data not shown). This reaction relates to the results achieved at the number of tillers and leaf area, which means Cristalia had more assimilated carbon available for growth due to more tillers and leaves that can contribute towards it. There were significant ($p < 0.05$) differences between the growth stages, tillering (0.2 g), flag leaf (1.1 g) and anthesis (2.4 g) regardless of the cultivar or treatment (data not shown). As previously mentioned, as the growing season progresses the crop grows bigger and taller, which results in higher DM production at the different growth stages.

6.2 Reproductive and quality parameters

Table 6.2 summarises the results of an Analysis of Variance (ANOVA) of reproductive and quality parameters of the light intensity study.

Tillers

Only cultivars showed significant ($p < 0.05$) differences (Table 6.2) with Overture (40) producing more tillers than Cristalia (33) (Data is not shown).

Table 6.2: ANOVA of main effects (cultivar and treatment) and their interaction in terms of the number of tillers (T) and spikes (S), total number of grains (TNG), grain per spike (G/S), grain mass (GM), biomass (B), harvest index (HI), thousand grain mass (TGM), visual test (VT) and electrical conductivity (EC) test

	T	S	TNG	G/S	GM	B	HI	TGM	VT	EC
Cultivar	*	*	ns	ns	ns	ns	ns	*	*	*
Treatment	ns	*	*	*	*	ns	*	*	*	ns
C*T	ns	ns	ns	ns	ns	ns	ns	*	*	*

ns = not significant

* = significant at 0.05%

Spikes

There was no significant interaction ($p > 0.05$) between cultivars and treatment, however, there were significant differences ($p < 0.05$) within cultivars and treatments (Table 6.2) Regardless of the treatment, Overture (26) produced more spikes than Cristalia (23), which corresponds with the number of tillers. Regarding treatments, the control treatment (29) produced significantly more spikes than both the 40% (23) and 60% (20) shade treatments. Arisnabarreta and Miralles (2008) found that by shading plants just before heading reduced the number and size of spikes formed at anthesis by slowing down the rate of crop development during that period.

Total number of grains

Only treatments showed significant ($p < 0.05$) differences (Table 6.2). Regardless of the cultivar, the control treatment produced on average more grains than both the 40% and 60% shade treatment (Table 6.3). Grashoff and d'Antuono (1997) found in a study that grain yield was 5% lower after shading during tillering, 35% lower after shading during stem elongation and 45% lower after shading during grain filling. The spikes of two-row barley can only bear a certain amount of grain and therefore they depend on fertile spikes per unit area (tiller potential) (Arisnabarreta and Miralles, 2008). Slafer et al. (1994) found when conditions are favourable and a high number of tillers is produced, shading can strongly reduce the total amount of grains formed from the tillers. The results of this study are in line with that of Slafer et al. (1994) due

to no significant differences found in the number of tillers being produced compared to the significant reduction in total grain number for the different treatments.

Table 6.3: Influence of shading (control -no shading, 40% and 60% shade) on total number of grains (TNG), grain per spike (G/S), grain mass (GM) and harvest index (HI) under controlled conditions at Welgevallen. Different letters after the values in a column indicate significant differences at p = 0.05

	TNG	G/S	GM (g)	HI
Control	413.1a	14.3a	20.1a	0.28a
40% shade	283.3b	12.3ab	12.2b	0.21b
60% shade	236.6b	11.4b	9.5c	0.15c

Grains per spike

Only treatments showed significant ($p < 0.05$) differences (Table 6.2). Regardless of the cultivar, the control treatment produced more grains per spike than both the 40% and 60% shade treatment (Table 6.3). Shading during stem elongation (the period when grains are formed), significantly reduces the number of grains per spike due to a lack of assimilates which is measured by the water-soluble carbohydrate concentration in the stems and leaves (Grashoff and d'Antuono 1997). Referring to Table 6.3, it is clear that with the reduction of ambient light (level of shading) the grains per spike decreased.

Grain mass

Only treatments showed significant ($p < 0.05$) differences (Table 6.2). Regardless of the cultivar, grain mass was significantly influenced by different degrees of shading tested (Table 6.3). Arisnabarreta and Miralles (2008) noted that pre-anthesis shading decreased the grain mass and this may be due to a lack of stem sugars in a critical time of grain filling, resulting in lighter/smaller grain. Table 6.3 shows a clear decrease in total grain mass as the ambient light is reduced.

Biomass

There were no differences within treatments and cultivars as well as no interaction between cultivars and treatments (Table 6.2).

Harvest index

Only treatments showed significant ($p < 0.05$) differences (Table 6.2). Regardless of cultivars, harvest index was significantly influenced by shading (Table 6.3). It was found that shading during stem elongation (end of tillering) had a greater effect on the total biomass production than shading during tillering. It can decrease the final biomass production by 25-30% (Grashoff and d'Antuono 1997). Therefore, the effect of a decrease in ambient light had a significant effect on harvest index (grain/above-ground biomass) as shown in Table 6.3.

Thousand grain mass (TGM)

Thousand grain mass of Overture was significantly ($p < 0.05$) influenced by degree of shading (Fig 6.5). There was however no differences between the control treatment of both the cultivars Overture and Cristalia. Both the 40 and 60% shade treatments of Cristalia was significantly higher than those of Overture. Regarding Cristalia, the 40% shade treatment was similar to both the control and 60% shade treatment, but the control treatment was significantly higher than the 60% shade treatment.

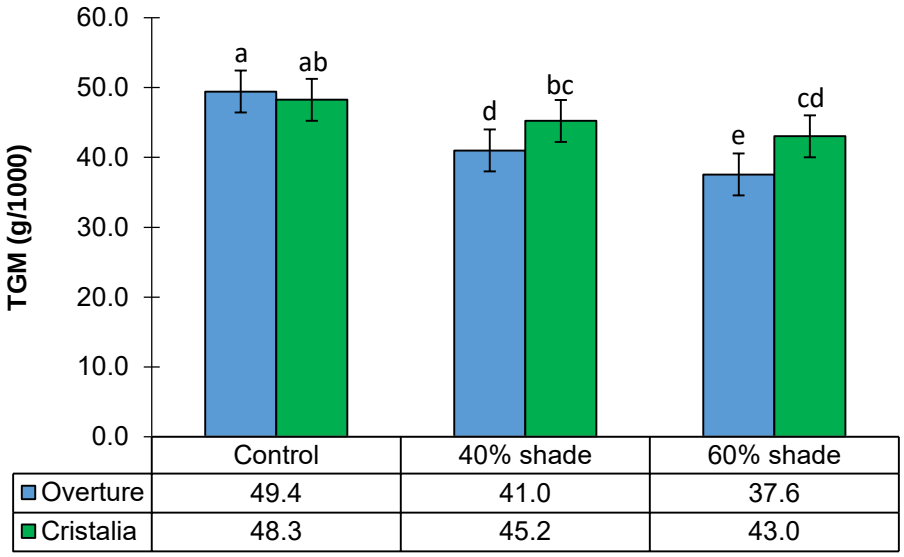
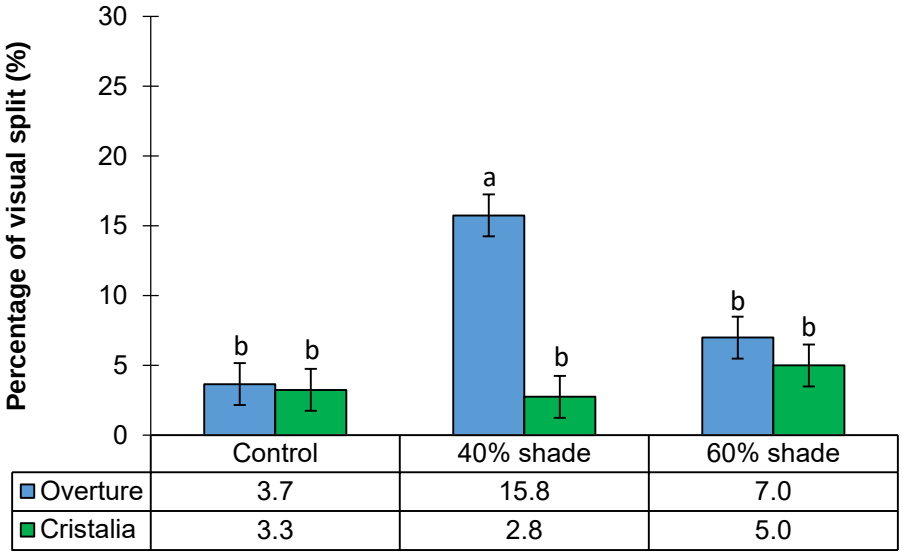


Figure 6.5: The average thousand grain mass (TGM) of cultivars Overture and Cristalia as a result of no shading (control), 40% and 60% shading treatments. Vertical bars indicate 95% confidence intervals. Bars indicated with different letters differed significantly at $p = 0.05$.

Shading before anthesis (end of tillering) has a decreasing effect on TGM. This may be due to a reduction in the amount of carbohydrate stored in the stems that are available at grain filling (Hoad et al. 2003). This supports the results show in Figure 6.5.

Visual test (% split)

There was a significant ($p < 0.05$) interaction between cultivars and treatments in terms of the visual split evaluation. There were no differences between the percentages of splits for the two cultivars in the control treatment as well as 60% shade (Figure 6.6). There was, however, a significantly higher percentage of splits in the Overture cultivar under 40% shade. Kai et al. (2003) found that a lack of sunlight during the development of the husk resulted in split grain. According to Hamachi et al. (1989), the result of grain splitting is due to the unbalance between the husk and grain size where there is a combined influence of shading and excess soil moisture. Hoad et al. (2003) noted that the shading of plants before anthesis had a clear effect on husk dimension. The development of the pericarp cell wall was affected in such a way that the route to grain dehydration has increased resistance, making the grain more vulnerable to split when there is an increase in tension during ripening.



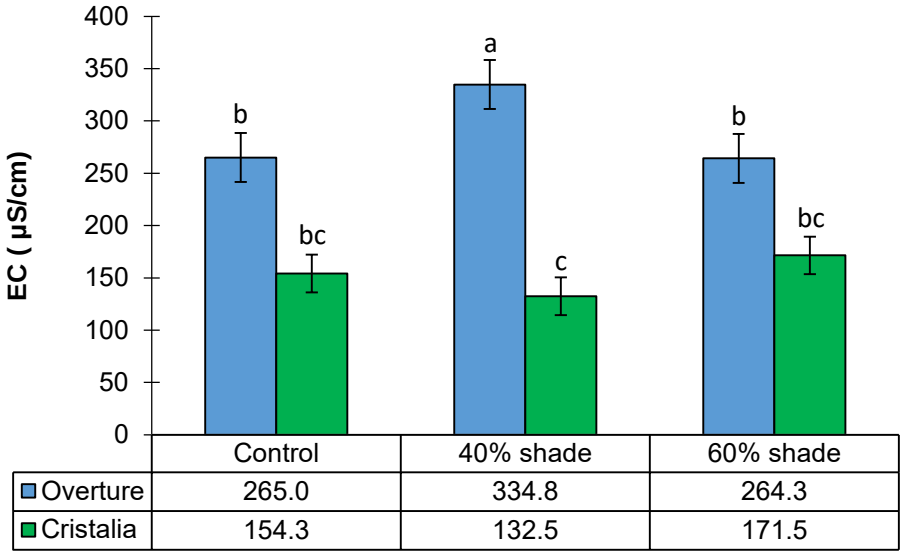
	Control	40% shade	60% shade
Overture	3.7	15.8	7.0
Cristalia	3.3	2.8	5.0

Figure 6.6: The average percentage of visual splits of cultivars Overture and Cristalia as a result of no shading (control), 40% and 60% shade treatment. Vertical bars indicate 95% confidence intervals. Bars indicated with different letters differed significantly at $p = 0.05$.

Figure 6.6 shows that reducing ambient light by 40% had the biggest influence on splitting; possibly as a result of leaving the husk underdeveloped and exposing the caryopsis through the lemma and palea.

Electrical conductivity (EC) test

There was a significant ($p < 0.05$) interaction between cultivars and treatments in terms of electrical conductivity. Figure 6.7 shows that there was a significantly higher percentage of increase in split for Overture under 40% shading. Regarding the Cristalia cultivar, there were no differences between the treatments, but they had a significantly lower percentage of increase in split than those of Overture. The control and 60% shade treatment for Overture was similar. When the results of the EC (Figure 6.7) and visual test (Figure 6.6) are compared to each other, both follow a similar trend, which means that the visual test was accurate and effective.



	Control	40% shade	60% shade
Overture	265.0	334.8	264.3
Cristalia	154.3	132.5	171.5

Figure 6.7: The average electrical conductivity (EC) of cultivars Overture and Cristalia as a result of no shading (control), 40% and 60% shade treatment. Vertical bars indicate 95% confidence intervals. Bars indicated with different letters differed significantly at $p = 0.05$.

Grain nitrogen content

A grain's supply of water-soluble carbohydrates will be reduced when plants are shaded during grain filling and will indirectly increase the N concentration of grain, resulting in a negative impact on the malting quality of barley (Grashoff and

d'Antuono 1997). The extremely high percentage of N concentration shown in Table 6.4 may be a result of the combined influence of shading and an excessive amount of N received from the standard nutrient solution that was applied daily.

Table 6.4: The grain nitrogen content of the cultivars Overture and Cristalia, as a result of no shading (control), 40% and 60% shade treatment

Cultivar	Grain nitrogen content (%)		
	Control	40% shade	60% shade
Overture	2.9	3.4	3.4
Cristalia	2.9	2.8	3.0

6.3 Conclusion

Reducing ambient light had a minor effect on the vegetative growth of the plants. On the other hand, the reduced light intensity had a major impact on cultivars, with Cristalia having the greatest response to light intensity for most of the vegetative parameters. Reducing normal light intensity by 40% had the biggest effect in terms of the quality (splitting) of barley grain on the Overture cultivar. The reduction of ambient light had no significant effect on cultivar Cristalia.

CHAPTER 7

7. Results and discussion - Water

The effect of water stress throughout the growing season on splitting of malting spring barley (*Hordeum vulgare* L.) grain

7.1 Vegetative growth parameters

Table 7.1 summarises the results of an Analysis of Variance (ANOVA) for the water study.

Table 7.1: ANOVA of main effects (cultivar, treatment and growth stage) and their interactions in terms of the number of leaves, leaf area, plant height and dry matter production

	Tillers	Leaves	Leaf area	Plant height	Dry matter
Cultivar	*	*	ns	*	ns
Treatment	*	*	*	*	ns
Stage	*	*	*	*	*
C*T	ns	ns	ns	ns	ns
C*S	ns	ns	ns	ns	ns
T*S	*	*	*	*	*
C*T*S	ns	ns	ns	ns	ns

ns = not significant

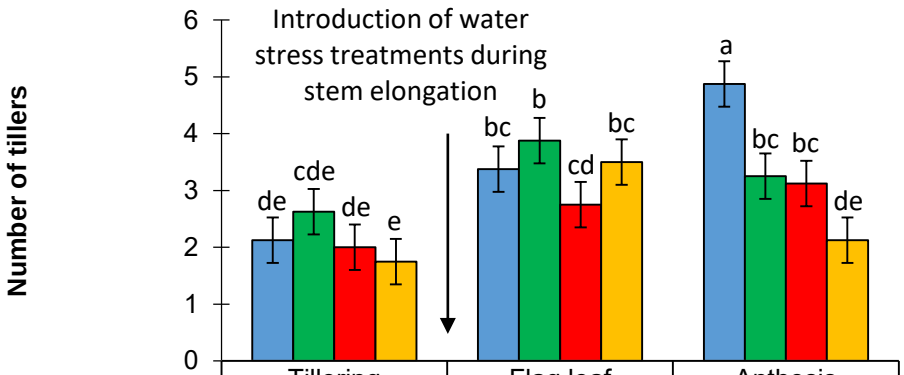
* = significance at 0.05%

Tillers

Primary tillers are additional stems that develop from the main shoot of the plant. When conditions are favourable, secondary tillers may develop from the base of primary tillers (Fletcher and Dale 1974). Water stress was applied at the start of stem elongation when the primary tiller formation is usually greatly completed. Samarah (2005) found in a study that a higher number of tillers were observed under normal watered and medium stressed plants than high stressed plants. Water deficit, or in this case water stress, during different growth stages of the plant reduced the number of tillers formed and survival at maturity (Akram 2011).

Results from Table 7.1 shows that cultivars, treatments and growth stages each, as well as the interaction between treatments and growth stages, had a significant effect on the number of tillers. There was a significant ($p < 0.05$) difference between cultivars with Overture producing on average more tillers (3.4) than Cristalia (2.5), regardless of the different treatments and growth stages. Before water stress was applied, there were no significant differences in the number of tillers (Figure 7.1) which shows that water stress has a great effect on tiller production during the later stages of crop development.

There was a significant increase in the number of tillers from tillering stage to flag leaf except for the medium water stress treatment. The increase in the number of tillers from flag leaf to anthesis was significant only for the control treatment. This shows that with a constant supply of water throughout the growing season, the plant will keep on producing tillers. However, not all of the late tillers will produce fertile spikes (Garcia del Moral et al. 2003). There was a significant decrease in the number of tillers produced from flag leaf to anthesis under the high water stress treatment. This shows that a shortage of water at crucial times of crop development had a major impact on the survival of the number of tillers being produced by the plant.



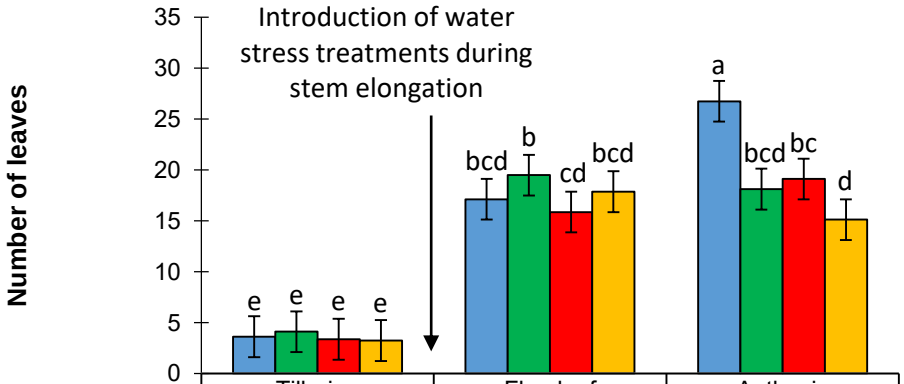
	Tillering	Flag leaf	Anthesis
Control	2.1	3.4	4.9
Low stress	2.6	3.9	3.3
Medium stress	2.0	2.8	3.1
High stress	1.8	3.5	2.1

Figure 7.1: The average number of tillers of treatments control, low, medium and high water stress at tillering, flag leaf and anthesis stage. Vertical bars indicate 95% confidence intervals. Bars indicated with different letters differed significantly at p = 0.05.

Leaves

Leaves are very important for a plant's photosynthesis and gas exchanges. Water deficiency rapidly slows down the expansion of the main-stem leaves and stomatal conductance resulting in disturbance of primal events in the photosynthetic processes (Shah and Paulsen 2003). This reaction decreases the level of water-soluble carbohydrate in the anthers as well as the appearance of the gene responsible for the synthesis of the acidic invertase enzyme (Saini 1997), which affect the growth of the plant. Results from table 7.1 show that cultivars, treatments and growth stages each, as well as the interaction between treatments and growth stages, had a significant ($p < 0.05$) effect on the number of leaves. Cultivars showed significant ($p < 0.05$) differences with Overture producing on average 15 leaves compared to Cristalia's 12.

There was no significant ($p > 0.05$) difference at the tillering stage (Figure 7.2) before the water stress treatments were introduced. However, during the flag leaf stage, after water stress was applied, there was a significant difference between the low and medium water stress treatments (Figure 7.2).



	Tillering	Flag leaf	Anthesis
Control	3.6	17.1	26.8
Low stress	4.1	19.5	18.1
Medium stress	3.4	15.9	19.1
High stress	3.3	17.9	15.1

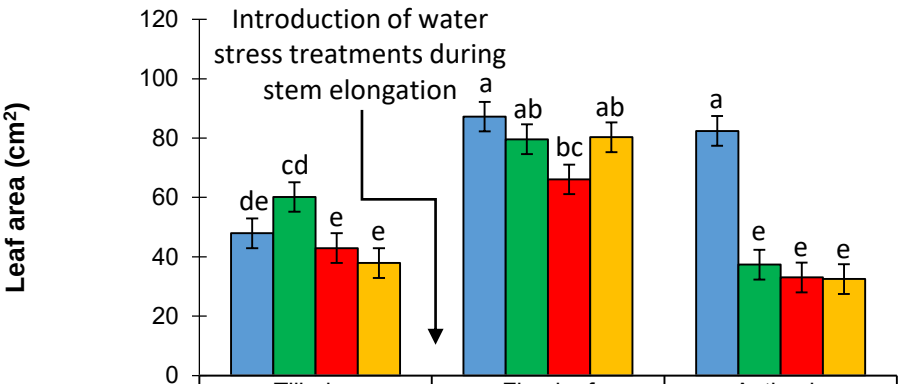
Figure 7.2: The average number of leaves of treatments control, low, medium and high water stress at tillering, flag leaf and anthesis stage. Vertical bars indicate 95% confidence intervals. Bars indicated with different letters differed significantly at $p = 0.05$.

The anthesis stage showed a significant increase and decrease in the number of leaves from the flag leaf stage for the control and high water stress treatments respectively (Figure 7.2). It was expected that the number of leaves will keep on increasing when water was applied daily and that the number of leaves will decrease as the level of water stress increased. The result shown in Figure 7.2 matches the result for the number of tillers formed (Figure 7.1), which is expected to have the same effect with water stress.

Leaf area

The leaf area of a barley crop will increase when grown under optimal conditions due to continuous production of new tillers and leaves during its vegetative stage. Reduced leaf area is commonly associated with water stress (drought) (Reisdorph and Koster 1999). Thameur et al. (2012) found in a study, that leaf area was severely reduced under water deficit stress due to a reduction and abscission in leaf growth. A decrease in leaf area due to fewer tillers produced and smaller leaf size meant that less water was used (Day et al. 1979). Results from Table 7.1 shows that treatments and growth stages, as well as the interaction between them, had a significant ($p < 0.05$) effect on leaf area. There was a significant difference in leaf area between treatments before the water stress treatments were applied (Figure 7.3), with the low water stress treatment producing a higher leaf area than both the medium and high water stress treatment. This result is inexplicable and probably due to experimental error.

After the introduction of the water stress treatments, there were significant differences between treatments at the flag leaf stage. The medium water stress treatment produced a significantly lower leaf area than the control treatment (Figure 7.3). All the water stress treatments had a significant effect on leaf area during the anthesis stage (Figure 7.3). Water stress (drought) decrease the leaf area by preventing the expansion of the leaves (Shah and Paulsen 2003) as well as disturbing the metabolic processes and activities of different enzymes, which have a major effect on the growth of crops (Allen et al. 1998). The leaf area for the water-stressed treatments is expected to be lower due to the low survival of tillers, less leaves produced or smaller leaf size.



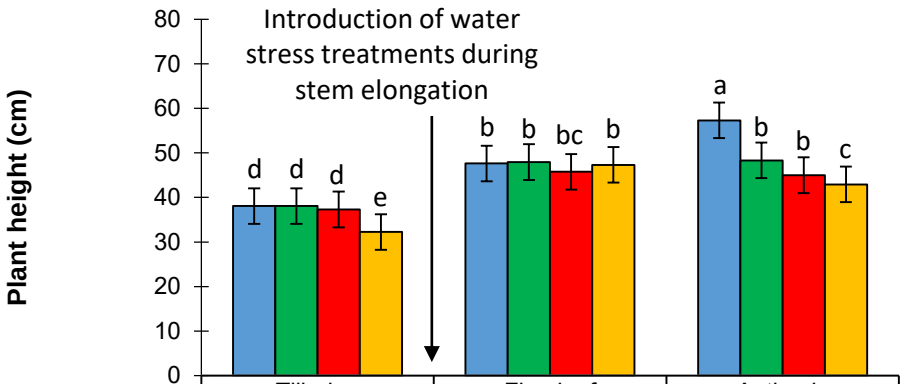
	Tillering	Flag leaf	Anthesis
Control	47.9	87.2	82.4
Low stress	60.2	79.6	37.4
Medium stress	42.9	66.1	33.1
High stress	37.9	80.3	32.5

Figure 7.3: The average leaf area of treatments control, low, medium and high water stress at tillering, flag leaf and anthesis stage. Vertical bars indicate 95% confidence intervals. Bars indicated with different letters differed significantly at p = 0.05.

Plant height

Water is essential at every stage of plant growth and under optimal growing conditions, and the plant will keep increasing in size as the season progresses. Water stress reduces the plant height due to preventing intermodal elongation and also slowing down the tillering capacity of plants, which is caused by a decrease in cell enlargement (Anjum et al. 2003). The decrease in plant height can also be due to lower gross photosynthetic rates and osmotic potential (Taiz and Zeiger 2002).

Results from Table 7.1 show that all three main effects (cultivar, treatment and growth stage) had an effect on the plant's height, but only the interaction between treatments and growth stages were significant. Cultivars showed significant ($p < 0.05$) differences with Cristalia producing on average taller plants (45.8 cm) than Overture (42.9 cm). There was a significant difference in plant height at tillering stage, before water stress was introduced (Figure 7.4), to pots allocated to receive the high water stress treatment later on.



	Tillering	Flag leaf	Anthesis
Control	38.1	47.6	57.3
Low stress	38.1	47.9	48.3
Medium stress	37.3	45.8	45.0
High stress	32.3	47.3	42.9

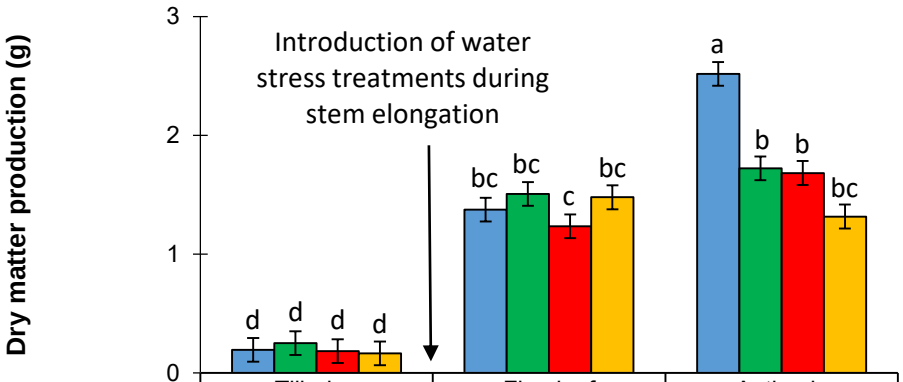
Figure 7.4: The average plant height of treatments control, low, medium and high water stress at tillering, flag leaf and anthesis stage. Vertical bars indicate 95% confidence intervals. Bars indicated with different letters differed significantly at p = 0.05.

After the introduction of the water stress treatments, there was no significant difference during the flag leaf stage (Figure 7.4). There was a significant difference in plant height from the flag leaf to the anthesis stage for the control and high water stress treatments (Figure 7.4). This reaction shows that water is crucial at later stages of crop development, as seen with the control (normal water regime) the height kept on increasing whereas the high water stress treatment decreased in height, which supports the findings of Anjum et al. (2003), as previously mentioned.

Dry matter production

Dry matter (DM) is a more reliable measurement of mass than fresh mass because the former excludes the fluctuating water concentrations in the biological material measured which is present in the latter. DM production is the most affected by differences in leaf area, which includes the total amount of leaves, (Lawlor et al. 1981). If fewer or smaller leaves are formed, DM production will be low. According to Table 7.1, growth stage had an effect on DM production, but only the interaction between treatments and growth stages was significant. There were no significant differences in DM production before or after water stress was applied. However, during anthesis stage, there was a rapid increase in DM production in the control

treatment as well as a slight increase in the medium water stress treatment. When conditions are favourable as with the control treatment, the plant will keep on increasing its above-ground biomass as the growing season progresses. As for the medium water stress treatment where water was gradually increased throughout the crop's growing season, the above-ground biomass also tended to increase. The number of leaves (Figure 7.3) corresponds well with the DM production (Figure 7.5), which supports the findings of Lawlor et al. (1981).



	Tillering	Flag leaf	Anthesis
Control	0.2	1.4	2.5
Low stress	0.2	1.5	1.7
Medium stress	0.2	1.2	1.7
High stress	0.2	1.5	1.3

Figure 7.5: The average dry matter production of treatments control, low, medium and high water stress at tillering, flag leaf and anthesis stage. Vertical bars indicate 95% confidence intervals. Bars indicated with different letters differed significantly at p = 0.05.

7.2 Reproductive and quality parameters

Table 7.2 summarises the results of an Analysis of Variance (ANOVA) of reproductive and quality parameters of the water study. No significant interactions were found between cultivar and treatment for all reproductive and quality parameters tested (Table 7.2).

Table 7.2: ANOVA table of main effects (cultivar and treatment) and their interactions in terms of the number of tillers (T) and spikes (S), total number of grains (TNG), grain per spike (G/S), grain mass (GM), biomass (B), harvest index (HI), thousand grain mass (TGM), visual test (VT) and electrical conductivity (EC) test

	T	S	TNG	G/S	GM	B	HI	TGM	VT	EC
Cultivar	*	*	*	*	ns	ns	*	ns	*	*
Treatment	*	*	*	ns	*	*	*	*	*	*
C*T	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

ns = not significant

* = significant at 0.05%

Table 7.3: Means from the analysis of variance for effect of water stress and cultivar on the number of tillers (T) and spikes (S), the total number of grains (TNG), harvest index (HI), visual test (VT) (% split) and electrical conductivity (EC) test

Treatments	T	S	TNG	HI	VT (% split)	EC (µS/cm)
Water application						
Control	10.8a	9.3a	123.8a	0.49a	4.4a	236.3b
Low stress	10.5a	8.5a	121.1a	0.52a	5.9a	259.4a
Medium stress	7.9b	6.9b	101.9b	0.51a	1.0b	225.0b
High stress	7.3b	4.5c	72.9c	0.42b	0.0b	276.5a
Cultivars						
Overture	11.0a	7.8a	99.1b	0.47b	4.4a	255.3a
Cristalia	7.2b	6.8b	110.8a	0.50a	1.3b	243.3b

Means followed by the same letters within the same column within a treatment are not significantly different at the 5% probability level.

Tillers

There was a significant difference ($p < 0.05$) within cultivars and treatments (Table 7.2). Regardless of the treatment, on average Overture produced more tillers (11) than Cristalia (7) (Table 7.3). Treatments control and low-stress were similar to each other and had a significantly higher amount of tillers than both the medium and high-

stress treatments. The number of tillers at harvest indicated that water stress (drought) had a clear influence on the production of tillers, with fewer tillers being produced as the water supply was decreased with the different treatments. McDonald (1984) noted that plants reached a maximum number of tillers while receiving irrigation more frequently, which supports the results shown in Table 7.2.

Spikes

There were significant differences within cultivars and treatments (Table 7.2). Comparing all of the treatments, Overture had produced on average more spikes than Cristalia (Table 7.3). Regarding treatments, the control and low-stress treatments were similar to each other and resulted in a significantly higher amount of spikes than both the medium and high-stress treatments. The medium and high-stress treatments were also significantly different from each other with the high-stress treatment producing much fewer spikes than the medium stress treatment. Samarah (2005) found in a study that water-stressed plants produced fewer tillers and consequently less fertile spikes and grains compared to fully watered plants. Fertile tillers are an important factor influencing the final grain yield (Akram 2011). Results from Table 7.2 shows a clear relationship between the number of spikes and tillers produced, which supports the finding of Samarah (2005).

Total number of grains

There were significant ($p < 0.05$) differences within cultivars and treatments (Table 7.2). The cultivar Cristalia produce more grain than Overture regardless of the treatment (Table 7.3). This might be because of water stress at pollination or embryo survival.

Regarding treatments, the control and low-stress treatments were similar to each other and had a significantly higher amount of grain than both the medium and high-stress treatments (Table 7.3). The medium and high-stress treatments were also significantly different from each other with the high-stress treatment producing much less grain than the medium stress treatment. The decrease in total grain number observed here is clearly the result of fewer tillers and spikes formed as water were reduce with the different treatments. Water stress during anthesis (e.g. high water stress) reduces pollination causing a decrease in the number of grains formed, which results in lower grain yields (Ashraf, 1998). Sufficient water during or after anthesis

(e.g. control and low water stress) increase the photosynthesis rate of the plant and also allows more time for carbohydrates to be transferred to the grains which improve grain size and thus increase grain yield (Akram 2011).

Grain per spike

Only cultivars showed significant ($p < 0.05$) differences (Table 7.2) with Cristalia (17) producing more grain per spike than Overture (13) (Data is not shown).

Grain mass

Only treatment showed significant ($p < 0.05$) differences (Table 7.2) in terms of grain mass. The control (6.8 g) and low stress (6.4 g) treatment were similar to each other but significantly higher than both the medium (5.4 g) and high stress (3.1 g) treatments (Table 7.4). The high-stress treatment grain mass were also significantly lower than the medium stress treatment.

Table 7.4: Influence of water stress (control –no stress, low stress, medium stress and high stress) on grain mass, biomass and thousand grain mass (TGM) under controlled conditions at Welgevallen. Different letters after the values in a column indicate significant differences at $p = 0.05$

	Grain mass (g)	**Biomass (g)**	**TGM (g/1000)**
Control	6.8a	7.2a	54.7a
Low stress	6.4a	5.9b	53.2a
Medium stress	5.4b	5.3b	53.0a
High stress	3.1c	4.3c	43.6b

Studies have shown that post-anthesis drought (i.e. medium and high water stress) reduces the grain yield mostly through a decrease in grain mass (Barnabás et al. 2008). The two post-anthesis treatments, medium and high water stress, showed a clear reduction in grain mass (Table 7.4) which supports the findings of the researchers mentioned above. Studies found that early irrigation in the season followed by drought occurring throughout the grain filling period resulted in smaller grains being produced (Rajala et al. 2011). In other words, the late drought affects grain size.

Biomass

Only treatment showed significant ($p < 0.05$) differences (Table 7.2). In this case, the control treatment was significantly higher (7.2 g) than the rest of the treatments. The medium (5.9 g) and low stress (5.3 g) treatments were similar to each other but significantly higher than the high stress (4.3 g) treatment (Table 7.4).

Harvest index

There was no significant interaction ($p > 0.05$) between cultivars and treatments (Table 7.2). There were however significant ($p < 0.05$) differences within cultivars and treatments. The Cristalia (0.50) cultivar had a significantly higher harvest index than Overture (0.46), regardless of the treatment. Regarding treatments, the high-stress treatment had a significantly lower harvest index than all of the other treatments. Harvest index is generally higher for short duration cultivars (Cristalia) in comparison to long duration cultivars (Overture) (Donald and Hamblin 1976). It was noted by Passioura (1983) that harvest index (grain/above-ground biomass) clearly depended on the pattern of water application and not on the amount. These results is similar to what was reported by Passioura (1983), who found that the high water stress treatment had a lower harvest index, due to different water patterns throughout the growing season.

Thousand grain mass (TGM)

Only treatment showed significant ($p < 0.05$) differences (Table 7.2). The high stress (43.6 g) treatment had a significantly lower TGM than the rest of the treatments (Table 7.4). During early periods of crop development, a shortage of water can reduce yield and leave the grain wrinkled and shrunken when ripe (Morgan and Riggs 1981). Therefore, the high water stress treatment resulted in much lower TGM, because of a lack of water at a crucial stage of grain filling. Severely stressed plants reach maximum grain mass much earlier than fully watered plants, due to shorter duration of grain filling (Samarah 2005). They also have a lower individual grain mass, which contributes to TGM.

Visual test (% split)

There was no significant interaction ($p > 0.05$) between cultivars and treatments of the water experiment (Table 7.2), but if one compares the average percentage of splits over all the treatments, the Overture (4.39%) cultivar had a higher percentage of splits than Cristalia (1.25%) (Table 7.3). This means that regardless of the level of water stress, Overture had more splits than Cristalia. It was expected from a high risk for split cultivar. Regardless of the cultivar, the control and low-stress treatments were similar and had a significantly higher percentage of split than the high and medium stress treatment. Kai et al. (2003) stated that water deficiencies during the development of the husk at grain filling can slow down the growth of the husk, increasing the risk of splitting. Applying water at field capacity daily (control and low water stress treatments) during grain filling period, resulted in a longer developing husk with excessive filling causing the grain to split open.

Electrical conductivity (EC) test

There was no significant ($p > 0.05$) interaction between cultivars and treatments for the water experiment (Table 7.2). However, regardless of the different treatments, the Overture cultivar had significantly ($p < 0.05$) higher EC-value (splits) than Cristalia. As mentioned earlier it was expected from a high risk for split cultivar. The high water stress (276.5) treatment had the highest EC-value of all the treatments, regardless of the cultivar. Medium water stress had a similar EC-value than low water stress and control treatments. However, low water stress treatment had a higher EC-value than the control treatment. Comparing the results of the EC and visual test they were similar in the sense that Overture had split more than Cristalia. However, for the treatments, the results were different, which means that the visual test was not accurate.

Grain nitrogen content

Savin (1996) found that fully watered plants produced grain with a higher protein content per grain and a lower protein concentration than water-stressed plants, which were applied from early to the middle of the exponental growth. Results from Table 7.5 shows a slight increase in the percentage of grain protein concentration with increased stress levels. The increase in grain protein caused by water stress can affect the malting quality by reducing the amount of grain suitable for the malting

proses as well as reducing the malt extract without clear impact on wort viscosity (Paynter and Young 2004).

Table 7.5: The grain nitrogen content of cultivars Overture and Cristalia, as a result of no stress (control), low, medium and high water stress

Cultivar	Grain nitrogen content (%)			
	Control	Low stress	Medium stress	High stress
Overture	1.7	1.5	1.8	2.8
Cristalia	1.9	2.0	1.9	2.2

7.3 Conclusion

Water stress during the earlier and later stage of crop development has a definite impact on the growth of the barley plant. During the vegetative growth stage water deficits reduced the plant's growth parameters slightly. The higher rates of water shortage during the reproductive phase had a major impact on the growth of the barley plant, which resulted in lower yields. The influence of water stress on the splitting of barley grain was minor when treated alone under normal growing conditions. Further research is required to enable more accurate results when water deficiency is combined with other environmental factors.

CHAPTER 8

8. Results and discussion – Temperature and Nitrogen

The influence of temperature and nitrogen rates during grain filling on splitting of malting spring barley (*Hordeum vulgare L.*) grain

8.1 Vegetative growth parameters

Table 8.1 Means from the analysis of variances for the effect of growth stage on the number of tillers and leaves and plant height

	Tillers	Leaves	Plant height (cm)
Stages			
Tillering	4.4b	13.5c	38.3c
Flag leaf	10.3a	46.7b	69.9b
Anthesis	9.7a	53.9a	80.5a

Means followed by the same letters within the same column are not significantly different at the 5% probability level.

The temperature and N treatments for this experiment were introduced after anthesis, so there were no significant ($p > 0.05$) differences for the vegetative growth parameters until anthesis. There were, however, significant ($p < 0.05$) differences between growth stages for the number of tillers and leaves and plant height (Table 8.1). Regarding the number of tillers, both flag leaf and anthesis had produced a significantly higher number of tillers than at the tillering stage. The slight decrease in tiller number at anthesis was due to the lack of secondary tillers developing from the base of primary tillers (Dabbert et al. 2010). There was for both the number of leaves and plant height a significant difference between tillering, flag leaf and anthesis (Table 8.1). Plant size normally tends to increase from tillering to anthesis as the growing season progresses.

8.2 Reproductive and quality parameters

Table 8.2 summarises the results of an Analysis of Variance (ANOVA) of reproductive and quality parameters of the combined temperature and nitrogen study.

Table 8.2: ANOVA table of main effects (temperature and nitrogen) and their interactions in terms of number of tillers (T) and spikes (S), total number of grains (TNG), grain per spike (G/S), grain mass (GM), biomass (B), harvest index (HI), thousand grain mass (TGM), visual test (VT) and electrical conductivity (EC) test.

	T	S	TNG	G/S	GM	B	HI	TGM	VT	EC
Temperature	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
Nitrogen	ns	ns	ns	ns	ns	ns	ns	ns	*	*
T*N	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

ns = not significant

* = significant at 0.05%

There were no significant ($p > 0.05$) differences within temperature and N as well as no interaction between temperature and N for all of the reproductive and quality parameters at harvest, except for the effect of N treatments on the visual test and EC test of splitting grains (Table 8.2). The reason for no significant effects for this year's trial (2019) compared to the previous year's (2018) trial, can be due to differences between the treatments of the two years.

During the temperature and N experiments of 2018, significant differences occurred at harvest time for most of the reproductive parameters. (See Chapters 4 and 5). The temperature treatment of the 2018 trial was introduced at grain filling for a duration of a **week** to simulate the passing of a cold front, while the 2019 temperature treatment was implemented at grain filling as a **once-off**, cold shock for only three hours. Therefore, the cold stress period of the 2019 trials was not long enough to have an effect on the vegetative growth and quality parameters of the plant.

In 2018, the different N rates for the trial was applied at **planting, six weeks after planting and at grain filling respectively**, while it was **only introduced at grain filling stage** in the 2019 trial. Therefore, the 2019 trial did not have an effect because the reproductive growth is usually completed when reaching the grain filling stage.

However, according to Table 8.4, although it was not shown as significant ($p > 0.05$) in the ANOVA table (Table 8.3), there were differences shown for tillers and biomass. There were significant ($p < 0.05$) differences detected for the visual and EC tests of the quality parameters.

Tillers

Temperatures resulted in differences regarding the number of tillers formed, with a drop of 15°C treatment producing much more tillers than the control treatment (Table 8.4). The cold shock appeared to stimulate reproductive growth since the number of spikes and the total grain mass showed an increasing trend where the cold shocks were applied, though the increases were not significant.

Biomass

Nitrogen showed differences regarding the vegetative plant mass with the 40 kg N ha^{-1} treatment producing a much higher plant mass than the control treatment (no N applied) (Table 8.4).

Visual test (% split)

Only the rate of applied N had a significant ($p < 0.05$) effect on the visual tests (Table 8.1). Results from Table 8.4 show that the 40, 50 and 60 kg N ha^{-1} treatments had produced more splits than the control and 30 kg N ha^{-1} treatments. Hoad et al. (2003) found in a study that high rates of N fertiliser late in the growing season, during grain filling, resulted in the grain to split. This result supports the findings of Hoad et al. (2003), that higher N rates produced more splits.

Electrical conductivity (EC) test

Only N had a significant ($p < 0.05$) effect on the EC test (Table 8.1). Results from Table 8.4 show that rates of 40 kg N ha^{-1} had produced a higher EC-value than both the control and 30 kg N ha^{-1}. This means that rates of 40 kg N ha^{-1} at grain filling increased the occurrence of splitting. Comparing these results with those of the visual test showed similar results, which means both tests was a suitable method to use for identifying splits.

Grain nitrogen content

There are a number of environmental factors such as temperature, fertiliser, precipitation etc., that have an effect on the protein composition of barley (Zhang et al. 2001). Andersson and Holm (2011) found in a study that post-anthesis low-temperature treatments resulted in lower grain N concentration, which contributes to

protein composition. Results from Table 8.3 show a higher percentage of N in the grain for the control temperature treatment at 60 kg N ha^{-1} as well as for the 10°C drop temperature treatment at 30 and 50 kg N ha^{-1}. The 15°C drop temperature treatment shows a slight increase in grain percentage N as the N rate increases. Generally, the increase in N application rates is correlated with a linear or quadratic increase in grain protein concentration (Le Bail and Meynard 2003).

Table 8.3: The grain nitrogen content of the temperature control, 10°C drop and 15°C drop, as a result of 0 (control), 30,40, 50 and 60 kg N ha^{-1}

Temperature	Grain nitrogen content (%)				
	Control	**30 kg N ha^{-1}**	**40 kg N ha^{-1}**	**50 kg N ha^{-1}**	**60 kg N ha^{-1}**
Control	2.2	2.4	2.3	2.2	2.8
10°C drop	2.2	2.6	2.2	2.5	2.1
15°C drop	2.0	2.0	2.3	2.4	2.6

8.3 Conclusion

The effect of a decrease in temperatures and different N rates during the period of grain filling had no effect on the vegetative growth of the barley plants. This may be due to the plants usually completing their vegetative growth before reaching the grain filling phase. Temperatures had no clear effect on splitting, probably due to the short period exposed to the different temperature levels. On the other hand, N had a significant impact on splitting when the application of N was higher than 40 kg N ha^{-1}.

Table 8.4: Means from the analysis of variance for effect of temperature and nitrogen on the reproductive growth and quality parameters at harvest (tillers (T), spikes (S), total number of grains (TNG), grain per spike (G/S), grain mass (GM), biomass (B), harvest index (HI), thousand grain mass (TGM), visual test (VT) (% split) and electrical conductivity (EC) test)

Treatments	T	S	TNG	G/S	GM (g)	B (g)	HI	TGM (g/1000)	VT (% split)	EC (µS/cm)
Temperature										
Control	**17.8b**	14.4a	158a	11.0a	7.7a	33.9a	0.19a	48.6a	6.9a	152.3a
10℃ drop	**19.5ab**	16.0a	168a	10.7a	8.0a	35.0a	0.19a	47.3a	6.8a	143.8a
15℃ drop	**20.2a**	16.2a	170a	10.8a	8.2a	34.0a	0.19a	47.7a	5.4a	146.3a
Nitrogen										
Control	18.2a	14.6a	159a	11.1a	7.6a	**32.2b**	0.19a	48.2a	**5.0b**	**133.4c**
30 kg N ha^{-1}	19.4a	15.4a	165a	10.9a	8.0a	**34.8ab**	0.19a	48.3a	**5.0b**	**139.1bc**
40 kg N ha^{-1}	20.1a	16.1a	163a	10.5a	7.8a	**36.1a**	0.18a	47.6a	**6.9a**	**160.3a**
50 kg N ha^{-1}	19.5a	16.2a	182a	11.3a	8.7a	**35.3ab**	0.20a	47.6a	**7.5a**	**153.4ab**
60 kg N ha^{-1}	18.5a	15.3a	158a	10.4a	7.6a	**33.1ab**	0.18a	47.7a	**7.1a**	**151.0ab**

Means followed by the same letters within the same column are not significantly different at the 5% probability level.

CHAPTER 9

9. General conclusions and recommendations

9.1 Synopsis

Splitting was recently found in barley produced in the Northern Cape (Figure 9.1, A), which has a major negative impact on the malting industry. “Splitting” is the term used to describe a condition where the grain is cracked open through its pericarp, aleurone or testa, exposing the starchy endosperm (Hoad et al. 2003). This affects the malting process due to water entering through the open area and saturating the starchy endosperm, which leads to uneven germination, the formation of foam in steep tanks or possible fungal growth (ARC 2018).

Therefore, because of the lack of research in splitting of malting barley in South Africa particulary on the causes of splitting, glasshouse studies were conducted during 2018 (Figure 9.1, B) and 2019 (Figure 9.1, C) on the Welgevallen Experimental Farm in Stellenbosch, where certain climatic conditions were simulated to determine its effect on barley grain. The aim of this study was to identify such factors that might lead to splitting of malting spring barley by investigating the influence of temperature, water deficiency, different levels of light intensity, and the rate of N application on a low-risk (*Cristalia*) and a high-risk (*Overture*) cultivar for splitting during different growth stages.

Figure 9.1: Split grain from 2014 harvest (A), 2018 (B) and 2019 (C).

9.1.1 Objective 1: Evaluate the effect of lower temperatures during the period of grain filling on splitting of barley grain.

Plants were grown in a glasshouse with day/night temperatures maintained at 25 °C/20 °C for a 12 h/day. When reaching grain filling, half of the plants were exposedto a temperature drop of 10°C for a week, which simulated the passing of a cold front. It was expected that there would be no differences ($p > 0.05$) for the vegetative growth parameters at the different growth stages due to late exposure of lower temperature at grain filling. The lower temperature also did not affect the reproductive growth parameters for they were already established when the temperature was introduced. Exposing the plants to a lower temperature of 10°C for a week at grain filling, had a major impact on the quality of the grain, which led to an increase ($p < 0.05$) in split-grain. The temperature experiment produced an average of 13% split-grain. The cultivar Overture produced a higher yield than Cristalia, which was the result of an increase in yield components such as the number of fertile spikes, the total amount of grains, grain mass and thousand grain mass. As expected, Overture the cultivar that has high risk for split produced more ($p < 0.05$) split-grain than Cristalia the cultivar that has low risk for split.

9.1.2 Objective 2: Evaluate the effect of different rates of nitrogen applications on splitting of barley grain.

Nitrogen was applied in three stages; at planting, six weeks after planting and grain filling. Three rates of total N were investigated: low (100 kg N ha^{-1}), control (150 kg N ha^{-1}) and high (200 kg N ha^{-1}) rates. These rates were evenly divided and applied over the respective growth stages. Higher rates of N application was expected to increase the size of the barley plant, which includes the number of tillers and leaves, leaf area, plant height and dry matter production. The higher rates of N application at grain filling consistently increased most of the yield components such as the number of fertile spikes, the total amount of grains and the grain mass as well as the vegetative plant mass. Nitrogen rates and cultivar had a major effect on splitting, however, the methods used (Visual and EC test) to identify split-grain was inconsistent. On average, the N experiment produced an amount of 23% split-grain.

9.1.3 Objective 3: Evaluate the effect of reduced light intensity on splitting of barley grain.

The light intensity study was conducted in a glasshouse where the effect of two levels of shading (40 and 60%) was compared to no-shading (control) of two cultivars (*Overture* and *Cristalia*). A reduction in light intensity had a less profound effect on the vegetative growth of the barley plant and only plant height and leaf area increased as light intensity decreased. The cultivar Cristalia was the most affected by reduced light intensity. Lower yields were recorded at harvest under the shaded treatments making it clear that light intensity has a great effect on the reproductive growth of the plant. From the results, it became clear that reducing light intensity by 40% had the greatest effect on the quality of the grain (splitting) for only Overture the cultivar that has high risk for split. The light intensity experiment produced an average of 6% split-grain.

9.1.4 Objective 4: Evaluate the effect of water deficiency (stress) during different growth stages on splitting of barley grain.

Plants received normal irrigation daily by means of drip irrigation. At the start of stem elongation, water stress was introduced as low, medium and high water stress treatments, which were compared to a fully watered control treatment. Stressing the plants in the early stages of crop development, slightly decreased the plant's growth parameters (tillers, leaves, leaf area, plant height and dry matter). During the reproductive growth, water deficiency reduced the yield components, which resulted in lower yield potential of the plant. The effect of water deficiency on the quality (splitting) of the grain was negligible, producing an average of 3% split-grain.

9.1.5 Objective 5: Evaluate the effect of the combined influence of temperature and nitrogen during grain filling on splitting of barley grain.

Plants were grown in a glasshouse with day/night temperatures maintained at 30 °C/20 °C for a 12 h/day until ear emergence. During grain filling, the start of the hard dough stage, a third of the plants were exposed to 15°C and 10°C respectively for a duration of three hours to simulate a cold shock. The same amount of N was applied to all the treatments at planting and four to six weeks after planting. During grain

filling at soft dough stage, five different rates (0, 30, 40, 50 and 60 kg N ha^{-1}) were applied. No differences were expected for the vegetative and reproductive growth parameters due to the late introduction of the temperature and N treatments. Temperatures did not influence ($p > 0.05$) the quality of the grain. However, N had a clear effect on the quality of the grain with rates above 40 kg N ha^{-1} which resulted in more split grain. The combined influence of temperature and N produced an average of 6% split-grain.

9.2 Limitations of the research

The study was conducted in pots in a glasshouse which meant that space was limited and the number of barley plants that could be grown was restricted to a few per pot. Therefore, the number of grains produced was limited and not enough for additional measurements such as hectolitre mass and N concentration. It would, however, be impractical to do such a study as a field trial, because it would be impossible to simulate temperature.

9.3 Recommendations for future research

There is certainly a need for further studies on factors that can result in the splitting of barley grain. The effect of different N rates and timing of application; the combined effect of temperatures and N with longer exposure to lower temperatures and different application times of different rates of N fertiliser, testing the effect of splitting on a wide variety of cultivars and studies where analysing the weather patterns in areas where splitting of barley is a possibility, might all aid in identifying such factors. Results from these studies can help to develop methods to prevent or limit the splitting of barley grain.

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