THE INFLUENCE OF ENVIRONMENTAL FACTORS AND AGRICULTURAL PRACTICES ON WHEAT FALLING NUMBER

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BLOEMFONTEIN

In loving memory of my mother, Marietjie

Contents

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Summery

Various environmental factors together with agricultural practices by producers that might contribute to reduced Hagberg Falling Numbers (HFN) of wheat, Triticum aestivum, in the absence of pre-harvest sprouting were simulated to determine whether HFN could be managed through specific management practices. Sufficient data have been generated to recommend that wheat should be allowed to dry to acceptable levels before they are harvested. *Alpha*-amylase activity could, however, not be successfully linked to reduced HFN at high kernel moisture content (KMC). Glyphosate treatments, administered at soft and hard dough stages to induce dry down of kernels at various KMC, produced more stable HFN for two of the three cultivars evaluated, but the optimal physiological growth stage resulting in the most stable HFN varied over seasons. Fifteen South African wheat cultivars were subjected to evaluation for their HFN response to various degrees of fertilizer application. No statistically significant effect on the HFN of wheat in general could be made. Cultivar differences did, however, occur that allowed for the individual effect of fertilizer on the HFN of these cultivars to be identified. This allowed for the grouping of cultivars into four response groups namely low, low to medium, medium and high response cultivars. Classification was refined with the use of a CVA (canonical variate analysis) that included the HFN, yield and protein response to fertilizer application. Recommendations regarding cultivar choice in areas prone to leaching can therefore be limited to cultivars that fall into the low and low to medium response groups identified in this study. Moderately high temperature exposure (32°C) during various grain filling stages of wheat resulted in reduced HFNs being measured. The physiological growth stage most affected by such temperatures, however, varied between cultivars. Further studies are suggested. In addition, farmers in areas that are known for their late frost should avoid planting early, as a study into the effect of a single night of low temperatures (–4°C) at late milk stage indicated that HFN could, as a result, be significantly reduced. A screening protocol was accordingly created to screen all cultivated varieties for such reactions, so that recommendations could be made as to which cultivars would be more tolerant to such conditions.

Key words: Hagberg Falling Number, kernel moisture content, glyphosate, fertilizer application, frost, moderately high temperatures.

Opsomming

Verskeie klimaatsfaktore asook landboukundige-praktyke deur produseerders is ondersoek om die moontlike oorsake van lae Hagberg Valgetalle (HVGe) in die afwesigheid van uitloop te verklaar, asook om te bepaal of HVG sodoende deur spesifieke bestuurspraktyke gemanipuleer kan word om die beste moontlike HVG binne ‘n gegewe seisoen te verseker. Voldoende data is gegenereer om die aanbeveling te maak dat koring toegelaat moet word om natuurlik op die lande af te droog. Die effek van *alfa*-amilase aktiwiteit by hoë vogpersentasies kon egter nie gekoppel word aan verlaagde HVGe wat waargeneem is nie. Glufosaatbehandelings, toegedien op sagte- en hardedeeg-stadia, was in staat om ‘n meer stabile HVG te lewer in twee van die drie cultivars wat geëvalueer is. Die spesifieke groeistadia waarop die behandelings gedoen is wat tot groter stabiliteit gelei het, het gewissel tussen seisoene. Vyftien Suid-Afrikaanse cultivars is ondersoek rakende hul HVG reaksie by verskillende bemestingsvlakke. Geen statistiese betekenisvolle effek kon egter aan bemesting en HVG gekoppel word as ‘n geheel nie. Cultivareffek het egter voorgekom, wat die indeling van die onderskeie cultivars in reaksiegroepe (laag, laag tot medium, medium en hoog) moontlik gemaak het. Klassifikasie is verfyn deur die gebruik van Kanoniese Variant Analiese (KVA) wat HVG, opbrengs asook proteïen reaksies op die verskillende bemestingsvlakke ingesluit het. Die groepering van die cultivars binne reaksiegroepe, skep egter die geleentheid om cultivaraanbevelings te maak in omgewings wat onderhewig is aan loging. Matige hoë temperature (32°C) gedurende die graanvullingstadia van koring het gelei tot betekenisvolle laer HVGe. Die fisiologiesegroeistadia wat die mees sensitief was, het tussen cultivars verskil. Addisonele studies in hierdie verband is egter noodsaaklik. Boere in gebiede wat bekend is vir laat ryp moet laat aanplantings vermy, aangesien ‘n enkele nag van lae temperature (-4°C) op laat melkstadia in staat is om HVG te verlaag. ‘n Protokol is saamgestel vir die evaluasie van cultivars vir rypsensitiwiteit sodat aanbevelings gemaak kan word rakende die graad van cultivartoleransie onder ryptoestande.

Chapter 1
General introduction

The physiological process of germination is associated with the synthesis of large amounts of *alpha*-amylase enzymes that are responsible for the breakdown of starch to a mixture of glucose and maltose. The release of sugars by *alpha*-amylase activity aids the fermentation process of bread (Gooding and Davies, 1997). The presence of these sugars results in sticky crumb of poor resilience and texture. The sticky crumb also results in difficulties with mechanical cutting, as loaves are deformed as they pass through the slicer and slice thickness becomes irregular and therefore unacceptable for the industry (Chamberlain *et al*., 1982). The elimination of sprouted wheat at delivery points therefore became a necessity.

The incorporation of the Hagberg Falling Number (HFN) test within the wheat industry (1960) was thought to enhance and elevate the grading of wheat with regard to the occurrence of pre-harvest sprouting. The test was designed as a method for the indirect measurement of *alpha*-amylase activity within a sample that might contain high levels of sprouted grain (Hagberg, 1960). Shortly after its establishment, however, it became clear that the HFN test is subject to deviation as it was possible to obtain different HFNs for the same level of *alpha*-amylase activity (Olered, 1967).

The Hagberg Falling Number (HFN) test was incorporated within the South African wheat grading regulations during June 1998, without prior testing or impact studies being performed (Anonymous, 2001). Before its incorporation, wheat was indirectly evaluated for low HFN through a visual screening test that required that a 25 g wheat sample should not contain more than 2 % sprouted wheat. Initially a 250 s HFN was required for grade. It soon, however, became obvious that various factors, other than sprouted wheat, had an influence on the HFN of wheat, as numerous reports of low HFN wheat without visual sprouting were received throughout the summer rainfall wheat producing areas of South Africa, resulting in enormous financial implications for the producers. Once the lack of stability of the test was realized, the required HFN for grade within the grading regulations was adjusted. The new quality regulations stipulated that a HFN minimum of 220 s was required to obtain grade B1 to B3, depending on the protein content and hectolitre mass. A HFN of 200 s is required for grade 4, with wheat being downgraded to utility grade with a

HFN of 150 s (Anonymous, 2003). Even with the new regulations in place, producers continued to experience problems with the test, which was voiced to the ARC- Small Grain Institute. Research into the various factors, aside from high *alpha*-amylase activity as a result of sprouting, that might contribute to low HFN was therefore necessitated.

The aim of the current study was to investigate various agricultural practices by producers that might explain the reduced HFNs that are obtained in the absence of pre-harvest sprouting, and accordingly to determine whether HFN could be managed through specific management practices.

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Chapter 2

Hagberg Falling Number of wheat - an overview

1. Introduction

The incorporation of the Hagberg Falling Number (HFN) test within the wheat industry was thought to enhance and elevate the grading of wheat with regard to the occurrence of pre-harvest sprouting. Shortly after Hagberg (1960) reported on the new test, Olered (1967a), indicated that the HFN test is subject to deviation. He indicated that it is possible to obtain different HFNs for the same level of *alpha*-amylase activity that are primarily attributed to differences in the amount of starch damage. Later Olered and Jönsson (1970) indicated that the HFN method in its actual form is not the same over the entire range of variation of amylase activity. Since the incorporation of the test within the South African wheat grading regulations (1998), various complaints with regard to the HFN test and its consistency have been reported to the Agricultural Research Council – Small Grain Institute (Bethlehem).

2. South African wheat industry

During the 2004/05 season, an estimated total area of 830 000 ha of South African soil were under wheat production, that produced an estimated yield of 1.68 million tons. The major wheat producing areas in South Africa are the Western Cape and the Free State with each having more than 350 000 ha under mostly (>90 %) dryland production (Anonymous, 2006). Producers are currently compensated for their produce in accordance with the Wheat Grading Regulations as stipulated in the Agricultural Product Standards Act of 1990 (Act no. 119) as amended (Anonymous, 2003). Accordingly, a HFN of at least 220 s is required for Grade B1, B2 and B3. Should a producer have sufficient levels of protein and hectolitre mass to obtain grade B1, but the wheat is downgraded to grade B4 due to a HFN of below 220 s, a loss of more than R 180 (± US $29) per ton could be expected (personal communication, N van der Merwe, Small Grain Institute; 2005/06 season prices).

3. Hagberg Falling Number: Principle and implication within the wheat industry

Hagberg (1960) first reported on a reliable viscometer method known as the 'falling number'. This test, hereafter referred to as the Hagberg Falling Number (HFN), indirectly measured the amount of *alpha*-amylase present within a ground wheat sample, through viscosity determinations of samples that consisted of a mixture of water and ground wheat. *Alpha*-amylase is responsible for the breakdown of starch to a mixture of glucose and maltose, resulting in a reduction of viscosity that is quantified by the HFN test as the time in seconds required for a stirrer to fall through the hot viscose medium consisting of ground wheat placed in a tube and incubated in a 100°C water bath (Gooding and Davies, 1997). Gelatinized starch (55-65°C) is therefore being measured after it has been degraded by *alpha*-amylase during a 30-40 s period before the reaction mixture exceeds the temperature when the enzyme is denatured (75-80°C; Vaidyanathan, 1987). According to Perten (1964), the starch passes the critical temperature zone for *alpha*-amylase activity in nearly the same period of time as during baking.

The relationship between HFN and *alpha*-amylase activity is curvilinear. Relatively large amounts of wheat with high HFN therefore need to be added to wheat with low HFN to achieve a satisfactory level. This non-linear relationship is described by the liquification number (LN = 6000/HFN - 50). For this equation 6 000 is a constant, with 50 corresponding to the time in seconds required for the flour starch to gelatinize sufficiently (Perten, 1964).

The release of sugars by *alpha*-amylase activity aids the fermentation process of bread. Excess presence of these sugars results in sticky crumb of poor resilience and texture. Caramelization furthermore results in dark crusts (Gooding and Davies, 1997). As sugars combine with some amino-acids by the Maillard reaction, the crumb is turned brown (Kent and Evers, 1994). The sticky crumb also results in difficulties with mechanical cutting, as loaves are deformed as they pass through the slicer and slice thickness becomes irregular (Chamberlain *et al.,* 1982).

Bakery-type wheat flour generally has a HFN between 200 and 250 s. Below 150 s the bread crumb becomes sticky. Above 350 s, bread volume is diminished and a dry crumb results, unless the defect is balanced by the addition of malt (Perten, 1964).

4. *Alpha*-amylase

High *alpha*-amylase activity is responsible for low HFN (Chamberlain *et al.*, 1981). *Alpha*-amylase enzymes are examples of endo-enzymes i.e. enzymes that attack linkages within the molecular structure of macromolecules. Wheat as well as barley *alpha*-amylase does not attack intact starch granules. This enzyme group slowly hydrolyzes damaged starch granules, while it gelatinizes starch at a fast rate. Hydrolization by *alpha*-amylase is mainly limited to random 1,4-alpha glucosidic linkages within starch paste. With continued hydrolysis, glucose, maltose and low-molecular weight (MW) polysaccharides with both amylose or amylopectin as substrates, are produced (Reed and Thorn, 1971).

Three different *alpha*-amylase isozyme families are expressed during grain development, namely *alpha-AMY-1*, *alpha-AMY-2* and *alpha-AMY-3* (Gale and Ainsworth, 1984; Daussant and Renard, 1987). These iso-enzyme groups have different immunochemical properties (Daussant and Renard, 1972), can be separated by iso-electric focusing (Sargeant and Walker, 1978) and are controlled by different sets of triplicate loci (Nishikawa and Nobuhara, 1971).

During germination or sprouting the *alpha-AMY-1* isozyme (also referred to as 'malt', high pI, Group 1 and GIII, Marchylo *et al.,* 1980), with *alpha-AMY-2* to a lesser extent, is produced (Sargeant, 1980). The *alpha-AMY-2* isozyme, also referred to as 'green', low pI, Group 2, GI and GII (Marchylo *et al.,* 1980; Sargeant, 1980), appears shortly after anthesis (Kruger, 1972) and is expressed in high concentrations in the pericarp of immature, green grains (Gale, 1989). According to Olered and Jönsson (1970) *alpha-AMY*-2 originates as a result of synthesis during the growing period of the grain, with the extent of the activation depending on the moisture equilibrium in the grain, which is not identical to the moisture content. This may result in a temporary increase in *alpha*-amylase activity stimulated by an increase in humidity at any level of development of the wheat. Under normal growing conditions, this enzyme can be continuously activated and inactivated in ripening grain.

As the pericarp is degraded during grain development, the pericarp *alpha*-amylases are degraded to the extent that they are usually completely absent by the time that the grain is ripe for harvest (Olered and Jönsson, 1970). Lunn *et al.* (2001a) confirmed that the presence of pericarp isozymes do have an effect on the HFN of wheat but also concluded that more *alpha-AMY-2* activity is required to reduce HFN compared to the *alpha-AMY-1* activity due to the different adsorbent properties of *alpha-AMY*-1 and *alpha-AMY-2*.

Olered and Jönsson (1970) also concluded that the inclusion of a small sample of green kernel could result in the deterioration to the quality of the general sample, irrespective of the fact that no sprouting is observed. The last of the three isozymes, *alpha-AMY-3,* is transiently expressed (Daussant and Renard, 1987).

4.1. Mechanisms of *alpha*-amylase accumulation

With the clarification of the number of *alpha*-AMY isozymes present during grain development and the function of each within grain morphology, deviations from the normal functions or termination of functions of some of the isozymes have been detected. Accordingly, Lunn *et al.* (2001b) identified four types of enzyme activity due to various mechanisms of *alpha*-amylase accumulation.

a) *Retained pericarp alpha-amylase activity (RPAA).*

With their studies on barley, Allison *et al*. (1974) indicated that half of the total *alpha*-amylase and phosphorylase activity was localized in the pericarp, which showed high activity in the early stages of grain development. They further suggested that this pericarp enzyme activity might directly influence starch type and content in the mature grain. Hill *et al*. (1974) indicated that *alpha*-amylase activity in selected triticale cultivars reached a maximum within the pericarp at approximately 12-15 days after anthesis and declined to a minimum at approximately 20 days. In addition, aleurone and endosperm *alpha*-amylase increased from day 20 to a maximum at 28-31 days in most of the cultivars included in the study.

As previously mentioned, *alpha-AMY-*2 activity generally decreases as the grain matures (Olered and Jönsson, 1970). Lunn *et al.* (2001b), however, demonstrated that some *alpha-AMY-2* activity remains after green colour has been lost. This *alpha*-amylase activity within the pericarp, that remains in grains that already lost their green colour, is referred to as 'retained pericarp *alpha*-amylase activity' (RPAA). The whole mechanism associated with RPAA is not well understood. It appears to be associated with environmental conditions such as frost, low temperature, low light intensity or with conditions that interfere with the normal course of grain development and ripening. They concluded that RPAA could be a problem in non-uniform crops containing a sub-population of later-developed wheat grains when the main part of the crops is ripe for harvest.

Previously, pericarp amylases have not been thought to be important as they were assumed to be inactivated in the high-temperature HFN test due to a greater thermal

instability than *alpha-AMY-1* isozymes (Kruger, 1972; Marchylo *et al*., 1976). Lunn *et al.* (2001a), however, demonstrated that *alpha-AMY-2* activity is capable of lowering HFN.

b) *Late maturity alpha-amylase (LMA)*

Also known as pre-maturity amylase (PMA: Lunn *et al.,* 2001a), LMA refers to the synthesis of high pI *alpha*-amylase isozymes throughout the entire aleurone layer and its deposition in the endosperm cavity of the grain (Mrva and Mares, 1996a). This differs from the pattern of enzyme production during germination or sprouting where initial enzyme synthesis is concentrated at the embryo end of the grains (Mares *et al.,* 1994; Mrva and Mares, 1996a). LMA appears to be limited to specific genotypes under certain environmental conditions, is completely independent of pre-harvest sprouting and can be expressed in sprouting tolerant and dormant genotypes (Lunn *et al.,* 2001a). Genotype (Gale *et al.,* 1983) and temperature shocks (Mrva and Mares, 1996b) have been listed as some of the factors that induce the phenomenon. According to Mares *et al*. (1994) BD 159 only develops LMA if cool temperatures are experienced during grain development, compared to Spica and Lerma 52 that produced LMA in all of the environments examined, although the amount of enzyme synthesized was less at higher temperatures (Mares and Gale, 1990). Mrva and Mares (2001a) indicate that cool treatment resulted in high enzyme levels between 26 and 35 days after anthesis. They also indicated that cultivars that maintain a low grain amylase across environments might produce a small response to cool temperatures. The duration of cool temperatures required to evoke a response is, however, uncertain. According to Mrva and Mares (2001a) a temperature range of 13˚C night and 17˚C day successfully stimulated amylase production in BD 159. Other factors, other than temperature shocks, that are thought to induce or modify the phenomenon have been reported by international literature that includes grain size (Evers *et al.,* 1995) as well as drying rate (Kettlewell and Cashman, 1997). It is speculated that grains above a certain threshold size have certain anatomical abnormalities that can result in a failure to effectively control mechanisms operating in smaller grains. According to Kettlewell (1999) the hypothesis of Evers *et al*. (1995) regarding the positive relationship between LMA and grain size is a consequence of the location of LMA in the crease of grain and therefore that larger grains will dry more slowly. Any relationship between grain size and LMA accordingly reflects the differences in drying rate. Research by Kettlewell (1999) indicated that the effect of nitrogen on increased HFN resulted from a reduced *alpha*-amylase activity rather than an effect on starch properties. He also concluded that the increased

alpha-amylase activity at low nitrogen application was LMA and that LMA was associated with slower grain drying.

The LMA phenomenon is reported to be wide spread in Australian wheat breeding programmes (Mrva and Mares, 2001a). In this regard, fifteen South African wheat cultivars were evaluated for their inherent LMA activity at the University of Adelaide, Australia during the 2003/04 season (Anonymous, 2004). According to the results obtained, only three cultivars produced low levels of LMA (SST 363, Tugela-DN and SST 124). In addition 100 % of the kernels evaluated with Caledon, Gariep, Karee, Limpopo and PAN 3377 were affected by LMA production. Approximately 80 % of the fifteen cultivars subjected to the test produced unacceptable high levels (>38 %) of LMA production (Table 1).

Table 1. Late maturity *alpha*-amylase status of fifteen South African cultivars (Anonymous, 2004).

Cultivar	*Percentage AA grains	Cultivar	*Percentage AA grains
Betta-DN	65.5	PAN 3364	87.5
Caledon	100.0	PAN3349	50.0
Elands	87.5	PAN3377	100.0
Gariep	100.0	SST 124	37.5
Karee	100.0	SST 363	0.0
Limpopo	100.0	SST 367	75.0
Tugela-DN	12.5	SST 936	62.5
PAN 3211	50.0		

* - Percentage of grains out of 40 grains affected with high *alpha*-amylase activity

c) *Pre-maturity sprouting (PrMS)*

This process involves germination early in the development of the wheat grain, when kernel moisture content is still high (>35 %) and is affected or influenced by orange blossom midge (*Sitodiplosis mosellana)* larvae as well as unseasonal weather conditions (Lunn *et al.,* 2001a). This phenomenon has been observed in the United Kingdom (Kettlewell and Cashman, 1997). Lunn *et al.* (1995) demonstrated, through iso-electric focussing, that secretion of midge *alpha* -amylase enzymes occurred during feeding but that the activity was absent from mature grains. From this, they concluded that effects of midge enzymes on HFN are likely to be minor. They further concluded that the induction of germination is mostly due to the interaction of midge damage and weather conditions.

Nakatsu *et al*. (1996) also observed the phenomenon in grains that showed symptoms of black point, in trials conducted in northern Japan.

d) *Post-maturity sprouting (PoMS)*

The term post-maturity sprouting (PoMS, Lunn *et al.,* 2001a) refers to the germination of grains still on the ear, when wet conditions occur before harvest. This phenomenon is also referred to as pre-harvest sprouting (PHS; Flintham and Gale, 1988) and is generally associated with *alpha-AMY-1* (Sargeant, 1980).

Lunn *et al.* (2001b) concluded that even though PMAA occurred more frequently in the UK than PoMS, PoMS is still considered to be the most important cause of low HFN. PrMS was third in frequency of occurrence followed by RPAA.

5. Physiology of starch and protein deposition during grain development.

Jenner *et al.* (1990) distinguishes between two stages of grain development i.e. grain enlargement and grain filling.

5.1. Grain enlargement

Grain enlargement almost immediately follows fertilization. This stage involves the division of endosperm nuclei, the enlargement of the structure resulting from the influx of water, and the formation of organelles and the biosynthetic mechanisms required for starch and protein synthesis (Jenner *et al.,* 1990).

Endosperm nucleus division begins within a few hours of fertilization (Bennett *et al,* 1975), with the first amyloplast (A-type) appearing a few days after anthesis (Briarty *et al.,* 1979). Starch is deposited in the amyloplasts as granules with an ordered crystalline structure (Briarty *et al*., 1979). Starch deposition does not reach maximum rate until endosperm cell division and granule initiation has almost finished (Evers and Lindley, 1977). The first B-type granules only appear at about 18 days after anthesis, but are produced at such an extent that they eventually outnumber the A-type starch 10 to 1 (Jenner *et al*., 1990).

Approximately 10 days after anthesis, storage proteins start to appear within membrane bound spherical bodies (Barlow *et al*., 1974). Twenty days after anthesis nearly 50 % of the final amount of storage protein has been synthesized (Donovan *et al.,* 1977). Hereafter, synthesized protein is transferred across the membrane into the lumen. The endoplasmic reticulum swells and becomes distended as the protein is deposited. Due to the fusion of protein bodies in the final stages of grain filling, a continuous, highly compressed protein matrix is formed, in which the starch granules are embedded (Campbell *et al.,* 1981).

The total number of cells produced is little affected by temperature, even though the rate of cell division is influenced. It is speculated that the increased rate at high temperature is counterbalanced by a reduction in the duration of cell division. As elevated temperature during the cell division phase results in lower grain weight at maturity, it is assumed that cell weight is the attribute factor (Jenner *et al.,* 1990).

5.2. Grain filling

The grain filling stage is mainly dominated by starch and protein synthesis. Rate and duration are the two variable components of grain filling that display genetic and environmental influences. According to Jenner *et al.* (1990) grain filling starts at about 10 to 15 days after anthesis and occupies the last 20 to 30 days of the grain's development until it ripens.

The precursors for starch and protein synthesis (i.e. sucrose for starch and amino acids for proteins) are supplied by the rest of the plant and are transported into the grain in the phloem during grain filling (Jenner *et al.,* 1990). According to Jenner (1970), the pool of precursors in the grain for starch synthesis is less than required for one day's grain filling at any point in time, whereas enough amino acid is present to provide for one to two day's protein synthesis (Ugalde and Jenner, 1990). The supply of these precursors to the grain that regulate the rate of deposition of dry matter differs for starch and protein (Jenner *et al.,* 1990).

Most of the carbohydrate deposited in the grain is derived form CO_2, fixed during the grain filling period (Evans *et al.,* 1975). The rate of starch deposition is influenced mainly by sink-limited factors i.e. the capacity of the grain to utilize the substrate (Jenner *et al.,*

1990). Approximately 35 days after anthesis, starch synthesis ceases (Kumar and Singh, 1980).

According to Sofield *et al,* (1977b) protein is deposited slightly faster than starch. Assimilated nitrogen is stored throughout the plant, either as vacuolar nitrate or as protein. It is remobilised later to provide nitrogen for deposition of protein in the grain (Austin and Nair, 1963). The deposition of grain protein is mainly a source-limited process (Perez *et al.,*1989) i.e. an increase in nitrogen supply causes a direct increase in deposition of grain protein**.** Most N is absorbed as nitrate from the soil, where the bulk is transported to the leaves. Here it's transformed to glutamate (utilised in the synthesis op protein) in the chloroplast (Dalling, 1985). As the older leaves senesce, their protein is mobilised and utilised for protein synthesis in younger leaves (Leopold, 1980).

During stress periods such as drought (Spiertz and Van de Haar, 1978) and leaf senescence (Blacklow *et al.,* 1984), limitations are placed to photosynthetic supply. During such periods, soluble carbohydrates in the internodes of wheat can be mobilized to sustain growth (Jenner *et al.,* 1990).

Increased temperature leads to an increase in the senescence rate, which may reduce the accumulation of carbohydrates more than the accumulation of nitrogen. The number and size of starch granules in the endosperm is also reduced (Tester *et al.,* 1995). During grain filling, higher temperatures reduce the duration of grain growth and limit the maximum size of the grain. As nitrogen translocation is less affected, crude protein concentration would be increased (Evans *et al.,* 1975).

5.3. Starch

According to Kumar and Singh (1980), grain size and therefore yield is determined by starch accumulation in the cereal grain. They also concluded that yield was only dependant upon the sink size.

Damage to starch during the milling of wheat flour affects the properties of the dough and the baked loaves. A moderate amount of damaged starch is beneficial with excessive damage undesirable. Undamaged starch granules swell only slightly (approximately 30%) at the temperatures that prevail during mixing and fermentation, whereas damaged granules gelatinised partly to completely (Sandstedt, 1955).

5.4. Protein composition

Huebner and Wall (1976) and Bottomley *et al.* (1982) separated total flour protein into four main fractions of decreasing size range. These fractions include high molecular weight (HMW) glutenin, low molecular weight (LMW) glutenin, gliadin and albumin/globulin. The two main components of wheat gluten, gliadin and glutenin, are each composed of many different molecular species, with the viscoelastic properties of dough thought to arise from both the structure and interactions of these proteins (Bietz and Wall, 1972). Kim *et al.* (1988) demonstrated that the gluten quality of wheat could be modified by altering the gliadin-glutenin ratio (22-56 % gliadin), leading to a 20-fold variation in resistance and a 2.5-fold variation in extensibility. According to Singh *et al*., (1990) the classic reconstitution studies suggest that the physical properties of doughs are primarily determined by the balance between the gliadin and glutenin proteins.

Singh *et al.* (1990) also indicated a very strong negative correlation between relative quantity of albumin/globulin and flour protein content. Absolute quantity of glutenin were also strongly correlated with quality attributes, such as extensibility, farinograph dough development time and dough breakdown.

6. Factors affecting Hagberg Falling Number

6.1. Gene composition

Rht 1, Rht 2 and Rht 3 are dwarf genes speculated to regulate *alpha*–amylase activity. According to Mrva and Mares (1996c) *Rht 3* strongly inhibited LMA production, with *Rht1* and *Rht 2* having a less pronounced effect. *Rht 3*, however, generally results in excessive dwarfing (Gooding and Davies, 1997). Mares *et al.* (1983) tested a wide rage of dwarf and semi dwarf wheats with the various dwarf genes with regard to their response to pre-harvest rain and concluded that the subsequent HFN and *alpha*-amylase activity varied between environments and genotype irrespective of the dwarfing genes. Research by Mrva and Mares (2001b, 2002) on double haploid populations derived from wheat cultivars Cranbrook (the LMA source) and Halbert (non-LMA), indicated QTLs controlling the expression of LMA in wheat on the long arm of chromosome 7B and 3B.

Various other research studies have attempted to link HFN to specific genes. Early in the 1980's, Nalepa *et al.* (1981) indicated that high HFN was governed by genes situated on

1B and 6D, with genes on 3A, 4A and 7D also having some influence. With their genetic analysis of characteristics associated with grain quality in winter wheat, Jedynski and Zalewski (2004) found that dominant genes governed HFN. Law *et al*. (2005) reported on a likely single gene on the long arm of chromosome 3A that may be responsible for controlling HFN, with Shi and Tian (2005) indicating that the inheritance of HFN is conditioned by the cell nucleus.

6.2. Morphological characteristics

Morphological characteristic that are speculated to be involved in enhanced or reduced HFN are mostly associated with characteristics associated with pre-harvest sprouting, as the production of *alpha*-amylase enzymes associated with germination will ultimately result in a reduced HFN.

According to King and Wettstein-Knowes (2000) morphological features of the cereal ear alter pre-harvest sprouting damage by changing the rate of water absorption during rainfall. King and Richards (1984) indicated that the presence of awns resulted in 30 % more water uptake, with sprouting within the ear increasing to 40 %. Unpublished data by the Small Grain Institute (Bethlehem), however, indicated that a cultivar with high pre-harvest sprouting resistance (Betta-DN) did not lose its resistance when the awns were removed. Cultivars that are prone to lodging, will inevitably also be subject to reduced HFN, as lodging results in a more moist microclimate in flattened crops (Gooding and Davies, 1997) enhancing pre-harvest sprouting. Seed dormancy is also closely linked to pre-harvest sprouting and therefore *alpha*-amylase activity. Within wheat, red seed coat color is associated with strong dormancy, with white seed coats being non-dormant or weakly dormant and therefore more prone to pre-harvest sprouting damage (Gfeller and Svejda, 1960; Mares, 1994). King and Wettstein-Knowes (2000) indicated that the ears of glaucous wheat and barley lines showed a clear reduction of wetting (20-30 % less) under simulated rain and, after 72 h of wetting, the in-ear sprouting observed within the various lines was reduced by 50 to 65 %. They also indicated that pre-wet, glaucous ears also shed water more readily.

6.3. Kernel moisture content (physiological growth stage)

According to Olered (1967b), irregular amplitude or range of approximately 50 s is often observed even in sound wheat throughout the usual harvest period. From this study it was

also concluded that an increase in *alpha*-amylase activity does not necessarily presuppose a heavy rainfall, but that it can be induced by changes in environmental conditions that retard moisture evaporation from the grain, and disappears again as the drying process continues.

Due to late tillering, a crop can contain both mature grain with negligible *alpha-AMY-2* activity and an immature population with retained *alpha-AMY-2* activity. Previous research indicated that due to its temperature sensitive nature, the *alpha-AMY-2* enzyme does not have an effect on HFN as it denaturates at 100°C or has different absorption properties (Kruger and Marchylo, 1985; Sargeant and Walker, 1978). Lunn *et al.* (2001a), however, confirmed research by Olered and Jonsson (1970) and Olered (1975) that the presence of pericarp isozymes does have an effect on the HFN of wheat but also concluded that more *alpha-AMY-2* activity is required to reduce HFN compared to the *alpha-AMY-1* activity due to the different adsorbent properties of *alpha-AMY*-1 and *alpha-AMY*-2. They further speculated that the reduction in *alpha*-amylase activity observed by Vaidyanathan (1987) with prolonged storage, could be explained by the inactivation of the enzyme during drying, but that the effect would be determined by the enzyme activity retained as well as the dilution of immature grains by mature grains.

6.4. Fertilizer application

The rate and timing of nitrogenous fertilizer, as well as the level and form of soil nitrogen application have a varying influence on the grain protein percentage. Finney *et al.* (1975) as well as Pushmann and Bingham (1976) proved that the later the nitrogen application, the greater the influence observed on protein percentage and the less the influence on yield. Rainfall prior to grain filling may exacerbate nitrogen leaching and other nitrogen loss. Powlson *et al*. (1992) for example, found a negative relationship between rainfall in the three weeks following nitrogen application and nitrogen availability to the crop.

Literature is not clear on the effect of nitrogen (fertiliser) application to the HFN of wheat. According to Brun (1982), high nitrogen fertilizer application can lead to lodging and can decrease HFN, possibly due to damp conditions around the ear that encourage germination and therefore increased *alpha*-amylase activity (Stewart, 1984). Jönsson (1966) demonstrated with glasshouse trials that applied nitrogen led to a reduction in *alpha*-amylase activity. Tabl and Kiss (1983) as well as Oskarsen (1989) reported a negative association between nitrogen application and HFN. Hook *et al.* (1989) and Webb

and Sylvester-Bradley (1995) reported no visible effect, with Gooding *et al.* (1986) and Kettlewell (1999) reporting a positive linear association between nitrogen application and HFN, possibly due to delayed maturity. Clarke *et al.* (2004) reported that nitrogen application resulted in an increase in HFN with the first year of evaluation, but a decrease with the second. It is, however, also possible that a cultivar effect might be associated with HFN and fertilizer application. With their study, Gooding *et al.* (1986) demonstrated that cv 'Avalon' and 'Brimstone' showed a larger increase in HFN with increasing nitrogen application than cv ' Mission'. With their study into different cropping systems, Hanell *et al*. (2004) reported that the HFN was significantly higher under an organic cropping system than under a conventional system, and speculated that this might be as a result of a heavier crop stand with conventional systems that caused more difficult drying conditions.

According to Ringlund (1983), HFN can also be affected by starch properties, while it is also possible that nitrogen may affect HFN through starch properties without influencing *alpha*-amylase activity. Another alternative to the effect of nitrogen on HFN is its effect on pre-harvest sprouting capability, thus indirectly affecting HFN. As previously discussed under the LMA section, Kettlewell (1999), however, indicated that the effect of nitrogen on increased HFN resulted from a reduced *alpha*amylase activity rather than an effect on starch properties. He also concluded that the increased *alpha*-amylase activity at low nitrogen application was LMA and that LMA was associated with slower grain drying.
Morris and Paulsen (1985) speculated that nitrogen applications would not affect pre-harvest sprouting of genotypes with strong resistance to sprouting, but would probably have an influence in genotypes with moderate to low levels of sprouting resistance due to increased sprouting.

Rainfall prior to grain filling encourages dilution of early nitrogen reserves by vegetative proliferation it also increases leaching and other forms of soil nitrogen loss. The soil moisture reserves might also be augmented so that leaf life is extended during grain growth, favoring carbohydrate assimilation and translocation more than that of nitrogen (Schlehuber and Tucker, 1959; Hopkins, 1968; Taylor and Gilmour, 1971).

6.5. Frost stress injury

When plants are exposed to low non-freezing temperatures for a few hours per day, new proteins are synthesised and these plants develop the capacity to adapt to subsequent chilling or freezing temperatures. Such a mechanism of adaptation is known as cold

acclimation (CA). CA is also referred to as frost hardening or cold hardening (Kozlowski, 1972). Generally, temperatures of 4 to 15°C are considered to be chilling, whereas a temperature below 4°C is considered to be freezing (Artlip and Funkhouser, 1995). CA results in altered gene expression leading to synthesis of specific proteins and certain enzymes that are responsible for the development of freezing tolerance (Antikainen and Griffith, 1997). In freezing tolerant cereal plants, such as rice, wheat, and barley, antifreeze proteins are synthesised during CA, which play a significant role in increasing freezing tolerance (Antikainen and Griffith, 1997). With their studies into the effect of hypothermia on the 310 kD stress protein of rye and wheat seedlings, Borovskii *et al.* (1999) indicated that the largest increase in the content of the 310 kD protein was at 3°C. They therefore concluded that this protein was associated with the processes of plant hardening. There does not appear to be any uniform pattern of protein synthesis among various plant species during CA. This implies that CA-induced proteins are not highly conserved as heat shock proteins. A characteristic feature of CA-induced proteins is that some of the synthesised proteins are transient, whereas others are stable, the synthesis of which continues for weeks (Guy and Haskell, 1987). In certain plants, an increase in the endogenous ABA level is observed following CA (Artlip and Funkhouser, 1995).

It is a known fact that frost damage has an influence on the quality of wheat. These quality defects are dependant on the temperature of the frost, severity, duration and to the growth stage of the plant, all of which will influence the amount of damage to the seeds in the emerged ear (Single, 1985). As the visual degree of frost damage increased, ash and colour increased, while loaf volume decreased and crumb and crust characteristics became progressively poorer. In addition, physical dough properties weaken, flour starch damage increases and farinograph absorption increases. The starch damage that occurs is the result of increased kernel hardness as frost damage becomes more severe (Dexter *et al.,* 1985).

The effect of frost on quality test results is much more evident during the early maturity stages. Very high starch damage was reported above 37 % moisture, but little change in starch below 37 %. According to Tottman (1987) the soft dough stage contains approximately 50 % moisture and the hard dough stage approximately 30 % moisture. The critical stages will therefore be during the late milk, early dough, and soft dough stages, with the early hard dough stages also being subject to frost damage. A

temperature below approximately –3°C is required to bring out this response and maximum response is attained over a narrow temperature range (Preston *et al.,* 1991).

With their study into the effects of frost during grain filling on wheat yield and grain structure, Cromey *et al.* (1998) concluded that the pericarp and testa were affected the most by frost damage. A reduced volume of starch, however, indicated that the starchy endosperm was also affected by frost. They speculate that this is probably due to reduced efficiency of uptake of photosynthate and/or a reduction in the number of living endosperm cells.

Preston *et al.* (1991) also concluded that frost caused a large significant decrease in wheat protein content during the most immature stages. Dexter *et al.* (1994) indicated that frost damaged wheat exhibited significantly lower proportions of gliadins. Kernel weight was also strongly affected by frost at the most immature stages. The possible reason for this is that the ice nucleation that occurs below –4°C causes disruption of immature seed cell membranes and tracheary elements of the rachis and rachilla, where translocation of nutrients from vegetative tissue to the growing seed would occur (Marcellos and Single, 1984; Single, 1985). Frost damage is therefore responsible for underdeveloped endosperm and may cause green *alpha*-amylase to still be present in the kernels. Factors that are therefore associated with the development of the endosperm are HLM and thousand kernel mass (TKM). Results obtained by an investigation into the effect of frost damage on HFN (Anonymous 2001, Table 2) indicated a negative correlation between frost damage and HLM in 'Inia' samples that produced low HFNs with less than 2 % sprouting. What should be noted, was that HLM appeared to be influenced by frost damage higher than 30 %, but that the HFN was already influenced by 11 % frost damage. It therefore appears as though more intensive frost damage is needed to reduce HLM than is the case with HFN. It should be kept in mind that Inia had a very high HLM potential for the specific season and locality investigated. So even though it appears as though its HLM was only influenced at frost damage higher then 30 %, the frost damage gradually reduced Inia's potential of high HLM.

It therefore appears as though frost damage does have an effect on HLM of wheat, but that it will not necessarily result in unacceptable low HLM, depending on the variety's general HLM for the specific season. The HLM will therefore not always reflect cold/frost damage, as is the case with the HFN test.

Karvonen *et al*. (1991) also reported the role of temperature on the HFN of wheat, as they concluded that HFN would be below 120 s if average maximum daily temperatures during grain filling were less than 13°C and relative humidity was above 80 %.

Table 2. Grading and quality test on frost damaged wheat (Anonymous, 2001).

No	Prot (%)	Moisture (%)	HLM (Kg)	HFN (s)	Frost damage (%)	Sprouted (%)	TKM (g)
1	12.5	11.5	80.0	98	13.7	0.0	36.9
2	13.2	11.5	78.0	64	25.3	0.5	38.4
3	13.4	11.6	78.4	65	22.6	0.6	36.0
4	13.0	10.4	62.6	62	42.6	0.9	23.2
5	12.7	11.5	78.7	64	13.6	1.2	32.8
6	12.3	10.3	63.9	62	45.3	1.1	23.4
7	11.1	10.7	80.6	69	19.3	0.8	38.4
8	12.0	11.7	82.3	202	11.5	2.0	41.2
9	11.9	11.1	83.8	261	3.0	0.2	41.4
10	11.9	11.0	79.7	62	17.0	0.8	36.6
11	10.5	11.2	83.8	209	5.3	1.7	42.7
12	12.2	10.6	79.4	189	25.9	1.2	37.6
13	13.5	10.8	70.4	62	34.1	1.3	26.6
14	12.3	11.3	80.3	114	13.6	0.6	36.0

6.6. Heat stress injury

According to Chowdhury and Wardlaw (1978), the optimum temperature for grain growth in wheat is about 15°C. For each 1°C rise above the optimum, the single grain weight is reduced by 3-5 % (Wiegand and Cuellar, 1981; Wardlaw *et al*., 1989). Very high temperatures during grain filling result in changes in mature protein composition (Blumenthal *et al.,* 1991; Graybosch *et al.,*1995; Stone and Nicolas, 1995).

Between 15 and 30°C, the proportion of protein relative to starch increases with an increase in temperature during grain filling (Kolderup, 1975; Sofield *et al.,* 1977a; Spiertz, 1977). Protein content is generally determined by the relative rates and duration of both protein and starch synthesis. An increase in percentage protein could therefore be achieved by an increase in absolute amount of protein per grain without much change in wheat starch, as the temperature increases from 15 to 21°C. Higher temperatures (30°C) result in reduction of protein and starch, with starch being reduced relatively more than protein (Sofield *et al.*, 1977a). Bhullar and Jenner (1985) concluded that starch and nitrogen accumulation in grain have differing susceptibilities to brief episodes of high temperature during grain filling. They also concluded that the accumulation of nitrogenous

material is enhanced relatively more than starch synthesis as a result of elevation in temperature, due to a reduction in starch content rather than a change in the quantity of nitrogen. The reduction in grain weight observed at high temperature is therefore the result of the effect of such temperatures on starch deposition, as the conversion of sucrose to starch is impaired at high temperature and therefore limits starch synthesis (Bhullar and Jenner, 1985). Stone and Nicolas (1996) indicated that the timing of heat stress exerted a significant influence on the accumulation of the protein fractions. They also indicated that both protein and dry matter accumulation were more sensitive to earlier stress, with the timing of heat stress being more pronounced for dry matter than protein accumulation. Their research, however, differed from Bhullar and Jenner (1985) as no increase in rate of protein accumulation during heat stress was measured. Both studies, however, found that cultivars differed in their response of mature protein accumulation during heat stress.

The effects of heat stress on wheat yield and quality are speculated to be the result of lengthy periods of above optimal temperatures i.e. chronic heat with daily maximum of 20°C to 32°C. The effect could also be attributed to short periods of heat shock i.e. a few days with maximum temperatures of over 32°C (Skylas *et al.,* 2002).

Extremely high temperatures, as is the case with heat stress, result in the production of heat shock proteins, with the response generally induced at between 4 and 10°C above normal growing temperatures (Key *et al*., 1985). Blumenthal *et al.* (1991) indicated an increase in the proportion of gliadin in gluten as a result of heat shock (21 days after flowering). Blumenthal *et al.* (1994) further confirmed weaker dough properties as a result of a lower proportion of large sized aggregates of glutenin. Grain weight was also effectively reduced by 33-40 % depending on cultivar sensitivity to high temperatures, when temperatures were increased from 20°C to 30°C post-anthesis. Differences between cultivars ranging in sensitivity to heat stress, were due to changes in the rate of grain filling at high temperatures (Zahedi *et al.*, 2003). According to Wardlaw and Moncur (1995) the most tolerant cultivars are those in which the rate of kernel filling is most enhanced by high temperature.

Heat shock proteins (HSPs) are classed according to their approximate molecular weights in kDa. HSP110, HSP90, HSP70, HSP60 and small HSPs (smHSPs) are included in the 15-30 kDa molecular mass rage (Vierling, 1991). HSPs are generally known to function as

chaperones, playing an important role in the folding and assembly of protein (Waters *et al.,* 1996; Boston *et al.,* 1996).

Earlier research has shown that the gluten components of flour have little effect on the HFN of wheat (Perten, 1964), but the effect of the so-called stress induced proteins on the HFN of wheat are to a large extent still unknown. Olered and Jonsson (1970) have proven that different starch properties can cause considerable variation within HFN.

6.7. Glyphosate application

Pre-harvest applications of glyphosate control weeds that interfere with mechanical harvest of wheat, accelerate wheat dry-down, which allows more timely harvest, and potentially reduces grain drying costs with no effect on grain quality (Clark, 1981). According to O'Keefe and Makepeace (1985) applications made between seven and 17 days prior to harvest did not affect the yield, 1000-seed weight, crude protein, HFN or germination of the cultivars evaluated. Yenish and Young (2000), however, indicated that the wheat stage of development during glyphosate harvest aid application, and not the herbicide rate, are critical to seed as well as seed quality. They demonstrated that yield, kernel weight and germination were significantly poorer than the control when glyphosate treatments were applied during milk stage and recommended that such treatments should be administered during hard dough development stage.

The use of chemicals to enhance the dry down of wheat at a certain stage is not very common in the South African wheat industry. Such a technique has enormous potential with regards to HFN. It could result in the avoidance of pre-harvest sprouting due to the fact that wheat is naturally dried off within days. Bovey *et al.* (1975) indicated with sorghum that grain moisture content was reduced from 20-40 % to below 13 % within seven days with glyphosate application. Darwent *et al.* (1994) found that glyphosate treatments applied at seed moisture content above 25 % slightly enhanced the drydown of wheat seed and foliage, with treatments below 25 % having no effect. It would secondly ensure that all wheat kernels are dry, with no green *alpha*-amylase present that could influence the HFN. It could therefore be possible to manage HFN better if the harvest date could be manipulated.

6.8. Fungicide application

Various research studies indicate that the application of fungicide sprays at flag leaf emergence as well as ear emergence results in extended flag leaf life (Bryson *et al.,* 1995; Gooding *et al.,* 2000) prolonged grain filling, increased final mean grain weight and therefore increased yield (Dimmock and Gooding, 2002a). Although the use of fungicides in some aspects help producers to deliver grain that adheres to some quality requirements such as reduced amounts of shrivelled grain and high specific weights, occasionally they have also been associated with negative effects (Dimmock and Gooding, 2002b). Kettlewell *et al.* (1987) indicated that yield and specific weight were increased by propiconazole, but grain protein content and HFN were reduced. The reduced HFN is generally attributed to LMA where fungicides have been used, but no sprouting has occurred. The reason for its accumulation is, however, not clear. Both Olered and Jönsson (1970) and Gale *et al*. (1983) reported that *alpha*-amylase activity increases when the drying of the grain during maturation is delayed, as is the case with fungicide application. Similarly Ruske *et al*. (2004) indicated that protein content, sulfur concentrations, HFN and loaf volumes were decreased with an increase in fungicide application amount. Ruske *et al.* (2003) reported that HFN was reduced with fungicide application as early as at the start of stem extension, but that the effect was small in comparison to the variation observed among the cultivars. Evers *et al.* (1995) proposed a second hypothesis for reduced HFN, in that larger grains have impaired control over LMA and that this may explain why larger grains within samples, between samples and between cultivars may have reduced HFN. Increased ear weight as a result of effective disease management may result in lodging and subsequent low HFN (Gooding and Davies, 1997).

7. Conclusions

If accepted that South African cultivars have high LMA producing capability, the possibility that HFN could be managed has not been investigated as of yet. From international research the conclusion could be made that knowledge of the quality and characteristics of the cultivar together with the implementation of correct or required agricultural practices, would allow management of HFN within the seasonal limitations set. Under extreme wet conditions, little can be done aside from planting pre-harvest sprouting tolerant material to assure a sound HFN. Even with highly tolerant material, however, pre-harvest sprouting is still a possibility, as any cultivar will germinate if favourable conditions prevail. The same could be said for the LMA producing capability of South African cultivars. Under cool

weather conditions correctly timed to fall with the most sensitive grain filing stages for LMA production, little can be done aside for selecting cultivars that have low LMA producing capability. It should, however, be noted that cool weather conditions are apparently not the only factors inducing LMA, as can be seen in the discussion on fertilizer as well as fungicide applications. In addition, international literature has indicated that not all low HFN can be attributed to LMA production (i.e. RPAA and the possible contributions of protein compositions). It is, therefore, important to understand why and under what circumstances certain cultivars produce low HFN in the absence of pre-harvest sprouting. Once this is understood, agricultural production practices together with cultivar choice can be utilized to ensure that the optimum HFN could be obtained within seasonal specifications.

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Chapter 3
An investigation into the management of Hagberg Falling Number through harvest date

Introduction

The incorporation of the Hagberg Falling Number (HFN) test within the South African wheat grading regulations (June, 1998), had enormous financial implications for the producers within the wheat industry. The negative perception surrounding HFN was enhanced when numerous reports of low HFN wheat without visual sprouting were received in the following seasons throughout the summer rainfall wheat producing areas of South Africa. Due to the association between sprouted wheat and HFN, producers try to minimize the risk of low HFN by harvesting their wheat early (at high kernel moisture content) to avoid possible preharvest sprouting. Within the current South African wheat system, producers are allowed to deliver wheat with kernel moisture content (KMC) ranging from 18-16 %, depending on infrastructure, silo and company policy. With the delivery of the wheat, producers are compensated for their product according to various grading regulations. HFN is therefore determined at the moisture content of the wheat with delivery and the producer compensated accordingly. With the low HFN storage program currently employed by selected Cooperations, wheat is stored for a set period and the HFN determined at various intervals until the wheat has improved. The producer is then compensated according to the latest HFN achieved. This, however, only applies to wheat with low KMC, as the management of low HFN wheat with high KMC is very difficult (Personal communication Dr S. Ybema).

High *alpha*-amylase activity is responsible for low HFN (Chamberlain *et al.*, 1981). Three different *alpha*-amylase isozyme families are expressed during grain development, namely *alpha-AMY-1*, *alpha-AMY-2* and *alpha-AMY-3* (Gale and Ainsworth, 1984; Daussant and Renard, 1987). During germination or sprouting the *alpha-AMY-1* isozyme (also referred to as 'malt', high pI, Group 1 and GIII, Marchylo *et al.,* 1980), with *alpha-AMY-2* to a lesser extent, is produced (Sargeant, 1980). The *alpha-AMY-2* isozyme [also referred to as 'green', low pI, Group 2 (Sargeant, 1980), GI and GII (Marchylo *et al.,* 1980)] is expressed in high concentrations in the pericarp of immature, green grains (Gale, 1989). The *alpha-AMY-3* isozyme is also transiently expressed (Daussant and Renard, 1987). As the pericarp is degraded during grain development, the pericarp *alpha*-amylase is degraded to

the extent that it is usually completely absent by the time that the grain is ripe for harvest (Olered and Jönsson, 1970). Lunn *et al.* (2001) confirmed that the presence of pericarp isozymes do have an effect on the HFN of wheat but also concluded that more *alpha-AMY-2* activity is required to reduce HFN compared to the *alpha-AMY-1* activity due to the different adsorbent properties of *alpha-AMY*-1 and *alpha-AMY*-2.

The aim of this study was 1) to simulate current harvest practises followed by wheat producers to determine whether kernel moisture content with harvest might have an influence on the HFN of wheat and 2) to determine the role of *alpha*-amylase activity in the HFNs obtained.

Materials and methods

Field experiments

The trial was planted over a four year period (2001-2005) at the ARC-Small Grain Institute (Bethlehem, Eastern Free State) under rainfed conditions. Fertilizer application was annually done in accordance to the soil status as indicated by soil analysis. Rainfall was recorded approximately 1.2 km from the trial site. Three South African bread wheat cultivars (Gariep, Tugela-DN and Elands) were included with the initiation of the trial (2001/02). These cultivars were chosen due to their general pre-harvest sprouting (PHS) resistance (Elands – excellent PHS resistance, Gariep – good PHS resistance and Tugela-DN – poor PHS resistance). The selected cultivars also varied with regard to their capability to produce late maturity *alpha*-amylase (LMA), with Tugela-DN having the lowest capability (12.5 %) and Gariep the highest (100 %). Elands demonstrated a 87.5 % capability to produce LMA (Anonymous, 2004). During the 2003/04 season SST 966, PAN 3235 and Limpopo were also included, with PAN 3349, SST 367 and PAN 3235 added in the 2004/05 season. A total of nine cultivars were therefore included in the final season (2004/05). The trial consisted of three replications planted in a randomised complete plot design. Plot size in all the years was 2.4 m x 5 m with 40 cm interrow-spacing.

Sampling and laboratory analysis

The sampling of wheat was initiated when the KMC of the various cultivars were approximately 28 %. The average sampling period over the various seasons varied, but was generally limited to the second week of December to the first week of January. With each sampling, approximately 80 wheat ears for each replication of the different cultivars were harvested by hand and immediately threshed. The total grain obtained from the 80 wheat ears harvested for each sample did not produce the 300 g weight generally accepted as the required weight for HFN determinations as to diminish possible errors experience within the sampling process. Each sample was therefore run in duplicate and re-run when more than 10 s difference occurred in the duplicate runs. Once the sampling process was initiated, samples were gathered every day, with the cultivars rotated every second day. KMC of whole wheat kernels was determined with an Agromatic digital moisture meter. The flour sample moisture content was determined with a Brabender moisture meter (ICC 110/1, 1976) that is capable of determining high moisture within flour much more accurately than the Infralyzer that is currently used with normal HFN determinations. HFN determinations were performed according to the Brabender moisture content (BMC) on the same day as harvest.

Alpha-amylase determinations

Alpha-amylase activity was determined according to a revised method of Barnes and Blakeney (1974), for the seven wheat cultivars evaluated during the 2003/04 season and the nine cultivars in the 2004/05 season. A 5 g flour sample was shaken for 15 minutes in 30 ml NaCl (0.09 M) and filtered through Whatman no1 Filterpaper. After filtration, 5 ml filtrate and 3 ml NaCl (0.09 M) was added to centrifuge tubes, and placed in a 50°C water bath. As soon as the mixture within the tubes reached 50°C, the incubation period of 10 minutes was initiated. After incubation, one Phadebas tablet (a highly specific dye-labelled substrate) was added to each tube together with 1.0 ml acetate buffer at 1 minute intervals. These tablets consist of a substrate made by cross-linked partially hydrolyzed potato starch. The substrate is labelled with Cibacron blue by covalent bonds. Tubes were continuously stirred. The reaction was terminated after 15 minutes by adding 1 ml of NaOH (0.5 M). After centrifugation (10 minutes at 4 000 rpm), absorbency was determined at 620 nm as *alpha*-amylase hydrolyzes the blue starch polymer into a water-soluble blue dye that absorbs light at 620 nm.

Statistical analysis

Due to the unpredictable nature of the samples, difficulty was experienced in obtaining enough values per kernel moisture content (KMC) over the various seasons that would allow statistical analysis. In order to make comparative statistical analysis possible, Brabender moisture content (BMC) obtained by the cultivars at the various harvest dates were grouped into two moisture regimes (10-13.1 % and 13.2-16.9 %) as to ensure that sufficient replicates are available for each of the two KMC regimes for each of the cultivars included in the study. Statistical analyses were performed with the use of GenStat (GenStat, 2003). Due to the unbalanced nature of the data generated, each year was individually evaluated. Differences between entries, treatments and all interactions were tested with an analysis of variance. The data was acceptably normal with homogeneous variances. Means were separated using Fishers protected t-test least significant differences (LSD) at the 5 % level of significance (Snedecor and Cochran, 1980). F-probability for the ANOVA was significant at 5 %.

Three different types of data sets will be discussed.

Four year analysis (three cultivars)

The HFN performance of Gariep, Tugela-DN and Elands at various BMCs was evaluated over a four year period. The data generated was grouped into BMC regimes (10-13.1 % and 13.2-16.9 %) to make statistical analyses possible**.**

Two year analysis (six cultivars)

Two year data (2003/04 and 2004/05) of the HFN performance of six cultivars (i.e. Elands, Gariep, Tugela-DN, Limpopo, SST 966 and PAN 3235) was evaluated. *Alpha*-amylase activity was measured and analysis performed on data that was, similarly to the HFN analysis, grouped into the two BMC regimes (10-13.1 % and 13.2-16.9 %).

One year analysis (nine cultivars)

Nine cultivars were evaluated during the 2004/05 season (i.e. Elands, Gariep, Tugela-DN, Limopo, SST 966, PAN 3235, PAN 3349, SST 367 and Komati). *Alpha*-amylase activity of the nine cultivars at the various HFNs measured were determined and analysed.

Results and discussion

Rainfall

Figures 1 and 2 provide the total rainfall measured on a weekly basis for the months of December and January of the various seasons. As can be seen from both Figures 1 and 2, the degree of interference experienced with sampling varied from season to season. The 2003/04 season was on average the driest of the four seasons, followed by the 2001/02 season.

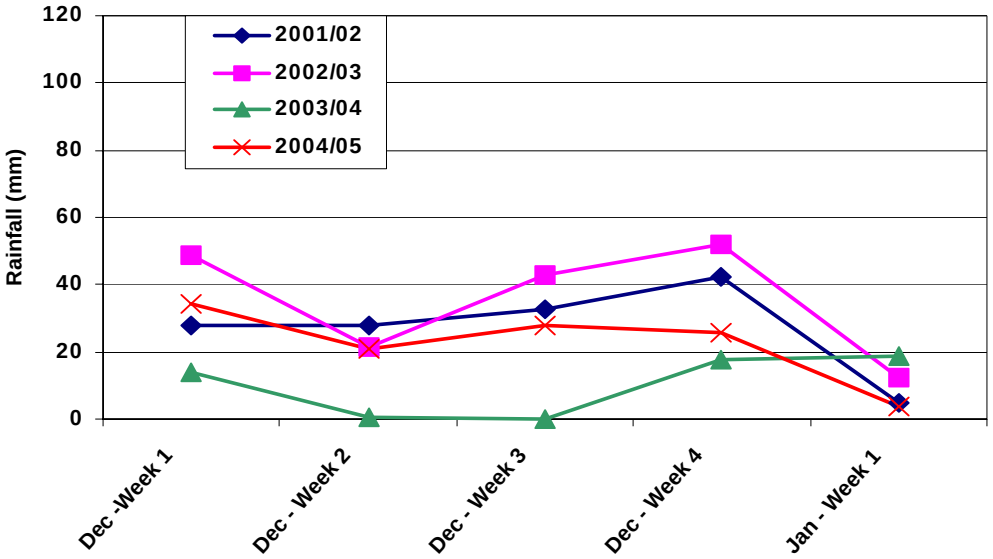

Figure 1. Rainfall measured on a weekly basis for the months of December and January for all four seasons of evaluations (2001/02 to 2004/05).

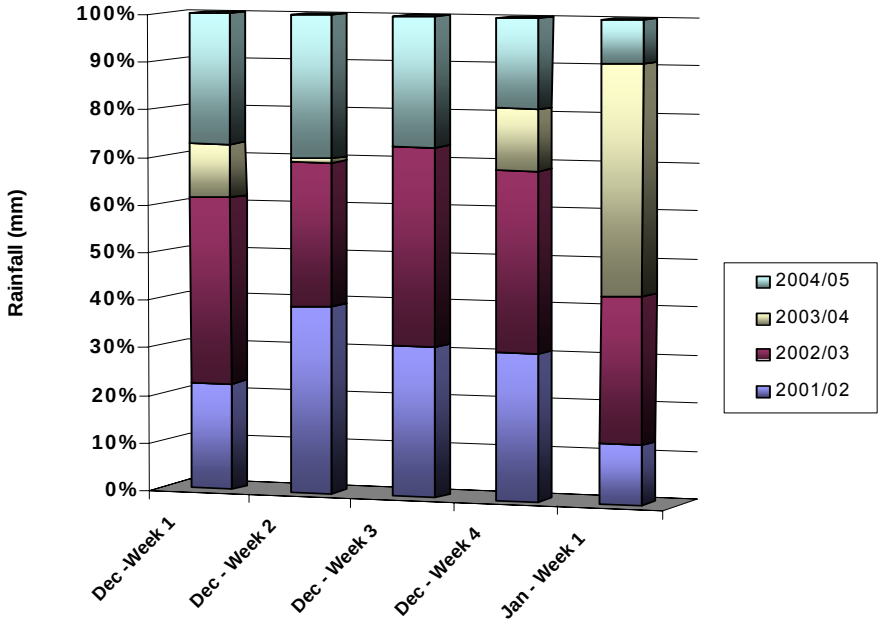

Figure 2. Percentage rainfall allocation as measured over the four seasons (2001/02 –2004/05) of evaluation.

Four year analysis (three cultivars)

The analyses obtained for the three cultivars over four years gives an indication of the long term evaluation results of a standard practice. The ANOVA of the first season of evaluation (2001/02) was very promising, as not only cultivars and KMC differed significantly, but also the interaction between cultivar and KMC (Table 1). From the t-test results (Table 2) from the same season, both Gariep and Tugela-DN produced significantly higher HFNs when the KMC was low (10-13.1 %). For the next three seasons, the same effect was not observed. Cultivar differences were only significant for the 2002/03 and 2004/05 seasons and KMC was only significant for the 2004/05 season. It is possible that rain that hampered the sampling process during the 2002/03 season might have resulted in wrongful classification of the various KMC regimes, and subsequent no difference in HFN being measured between the two regimes investigated. The lack of significant difference between the two regimes for the 2003/04 season is, however, not clear.

Tugela-DN once again obtained significantly higher HFN at lower KMC during the 2004/05 season. Elands was the only cultivar of the three evaluated, that maintained a constant HFN irrespective of the KMC or the season over the four years of evaluation. This analysis indicates that it is possible to harvest wheat early (at high KMC), without any reduction in HFN being measured, even in cultivars that are prone to reduced HFNs at higher KMCs (such as Tugela-DN). Should the seasonal specifications (characteristics), however, be favourable as to promote low HFN, producers that managed acceptable HFNs over several seasons with the early harvest practise with a specific cultivar, may suddenly have unexplained low HFN.

Table 1. Mean square values obtained from the ANOVAs over four seasons to investigate the influence of kernel moisture content on the Hagberg Falling Number of three wheat cultivars (Elands, Gariep and Tugela-DN).

	Mean square values			
	01/02	**02/03**	**03/04**	**04/05**
Cultivar	1733.2**	14255.1***	319.4	5494.5**
Kernel moisture content regime	11509.4***	626.1	3384.5	5760.7*
Cultivar–by-kernel moisture content	4501.4***	101.3	218.2	467.0
CV (%)	4.61	7.08	9.45	8.3

* P ≤ 0.05 ** P ≤0.01 *** P ≤ 0.001

From Table 2, two additional observations were made. In 50 % (six out of 12) of the cases tabulated, the average HFN measured at the low KMC regime (10-13.1 %) was 18 s or more higher than that of the high KMC regime (13.2-16.9 %) although not all were statistically significant. Secondly none of the HFNs measured at either high or low KMC were below the 220 s cut-off mark for grade.

Two year analysis (six cultivars)

With the two year analysis, three additional cultivars (Limpopo, PAN 3235 and SST 966) were included in the study in an attempt to screen more cultivars for possible reaction to early harvest. Both the cultivar and KMC regimes differed significantly over the two years of evaluation for the six cultivars included in the study. The cultivar-by-KMC regime did not differ significantly (Table 3) in any of the two years of evaluation.

Table 2. The t-test results for Hagberg Falling Numbers obtained over two measures of kernel moisture content for Elands, Gariep and Tugela-DN over four seasons.

	2001/02			2002/03			2003/04			2004/05		
	KMC 1	KMC 2	t	KMC 1	KMC 2	T	KMC 1	KMC 2	t	KMC 1	KMC 2	t
Elands	319.29	314.00	0.584	353.00	356.25	0.795	314.00	295.33	>0.05	367.67	336.37	0.117
Gariep	347.00	315.60	0.002*	309.2	323.11	0.322	322.67	289.4	>0.05	332.75	320.00	0.417
Tugela-DN	391.25	297.5	0.000*	386.71	390.75	0.609	301.67	288.00	>0.05	383.86	348.67	0.035*

KMC 1 – Average HFN obtained at kernel moisture content regime 1 (10-13.1 %)

KMC 2 – Average HFN obtained at kernel moisture content regime 2 (13.2-16.9 %)

t – t probabilities of pairwise differences

* - P≤0.05

Table 3. **Mean square values obtained from ANOVAs determined over two years on six wheat cultivars to investigate the effect of kernel moisture content on Hagberg Falling Number of wheat.**

	Mean square values	
	2003/04	**2004/05**
Cultivar	2425.3*	3149.8**
Kernel moisture content regime	4451.9*	4112.9*
Cultivar-by-kernel moisture content	391.8	628.6
CV (%)	9.74	8.21

* P ≤ 0.05 ** P ≤0.01 *** P ≤ 0.001

Of the six cultivars evaluated, Tugela-DN was the only cultivar that obtained significantly higher HFNs at the lower KMC regimes during the 2004/05 season (Table 4). Although the response observed in all the cultivars was not significant, generally higher HFNs were again observed for most of the cultivars evaluated at the lower KMCs.

Table 4. **The t-test results of the Hagberg Falling Numbers at two kernel moisture contents of six cultivars evaluated over two years.**

	2003/04			**2004/05**		
	KMC 1	**KMC 2**	**t**	**KMC 1**	**KMC 2**	**t**
Elands	314.00	295.33	0.290	367.67	336.70	0.074
Gariep	322.67	289.40	0.058	332.75	320.00	0.402
Limpopo	299.00	276.00	0.290	345.50	343.17	0.885
PAN 3235	282.2	260.00	0.213	329.00	321.80	0.726
SST 966	263.75	269.5	0.773	326.80	327.00	0.989
Tugela-DN	301.67	288.00	0.555	383.86	348.67	0.028*

KMC 1 – Average HFN obtained at Kernel moisture content regime 1 (10-13.1 %)

KMC 2 – Average HFN obtained at Kernel moisture content regime 2 (13.2-16.9 %)

t – t probabilities of pairwise differences

* - P≤0.05

During 2003/04, SST 966 became the first cultivar that produced a HFN below 220 s (at 15-15.9 % KMC) (Figure 4), indicating that the harvest of wheat at high KMC could result in low HFNs being measured. The effect was, however, lost with the creation of KMC regimes, resulting in a higher HFN average for the 13.2-16.9 % KMC regime. HFNs measured for the 2004/05 season were in general higher than that of the pervious season

for the six cultivars evaluated, supporting the hypothesis that low and high HFN seasons exist, and that the practice of early harvest may prove detrimental in a low HFN season, when the HFN potential as stipulated by the season, is already below average.

From the t-test results (Table 6) performed, only two cultivars (Gariep and PAN 3235) possessed significantly higher *alpha*-amylase activity at the higher KMC regimes during the 2003/04 season. This increased *alpha*-amylase activity was not high enough to have a statistically significant effect on the HFN of the two cultivars. Similarly the lower HFN measured for Tugela-DN at higher KMC was not associated with a significantly higher *alpha*-amylase activity. It should, however, be noted that according to the LMA production capability of South African cultivars as determined by the University of Adelaide, Australia (Anonymous, 2004), Tugela-DN ranked among the cultivars that produced acceptable levels of LMA production (12.5 %). The reduced HFN observed could therefore not be attributed to possible cold weather that might have resulted in the production of excess levels of *alpha*-AMY-1. Gariep and Elands, in contradiction to Tugela-DN, indicated high LMA production capability (100 % and 87 % individually, see Chapter 2). Any response therefore measured for these two cultivars might be attributed to LMA production. It should, however, be noted that Elands remained constant throughout the various seasons. Lastly it should be noted that if LMA was indeed responsible of the reduced HFN measured, the effect should have been evident in the lower KMC regimes as well.

Three possible reasons for lack of correlation between HFN and enzyme activity exist 1) an increased *alpha*-amylase activity may have occurred, but was lost in the creation of KMC regimes, as was the case with the low HFN measured for SST 966. This is, however, unlikely as Figure 3 clearly indicates that *alpha*-amylase activity of Tugela-DN remained relatively constant over the 2004/05 season compared to the 2003/04 season where a clear increase in activity was observed as the KMC increased. The second possibility is that *alpha*-amylase activity in the form of 'green-amylase' (*alpha*-AMY-2) is not the main reason for low HFN at high KMC. This would support research reported by Lunn *et al.* (2001) in that larger amounts of 'green'-amylase are required to have a visible effect on HFN. The last possibility is that late rain that fell during the harvest period influenced the KMC of the various samples, even with a 24h dry down period allowed. This may have resulted in high HFNs measured at high KMC of samples that are actually at a more mature stage (i.e. lower KMC), and would explain the constant *alpha*-amylase

activity at the various KMCs. Of the three possibilities, the latter appears to be the most probable explanation.

Table 5. Mean square values obtained from the ANOVAs determined over two years on six wheat cultivars to investigate the effect of kernel moisture content on *alpha*-amylase activity of wheat.

	Mean square values	
	2003/04	**2004/05**
Cultivar	18919**	3852
Kernel moisture content	28134*	8837
Cultivar-by-kernel moisture content	2947	2986
CV (%)	17.25	21.89

*- P ≤ 0.05 ** P ≤0.01 *** P ≤ 0.001

Table 6. The t-test results for *alpha*-amylase activity (U/l) at various kernel moisture contents for six cultivars evaluated over two years.

	2003/04			2004/05		
	KMC 1	**KMC 2**	**t**	**KMC 1**	**KMC 2**	**t**
Elands	220.9	254.1	0.540	237.1	298.2	>0.05
Gariep	287.6	368.2	0.03*	254.1	270.7	>0.05
Limpopo	314	331.9	0.691	205.5	261.0	>0.05
PAN 3235	322.5	409.2	0.024*	265.2	265.4	>0.05
SST 966	372.2	415.8	0.3	286.6	248.3	>0.05
Tugela-DN	371.6	355.7	0.742	232.7	256.9	>0.05

KMC 1 – Average HFN obtained at Kernel moisture content regime 1 (10-13.1 %)

KMC 2 – Average HFN obtained at Kernel moisture content regime 2 (13.2-16.9 %)

t – t probabilities of pairwise differences

* - P<0.05

One year analysis (nine cultivars)

Three more cultivars (Komati, PAN 3349 and SST 367) were added to the trial to bring the total cultivars included to nine. The ANOVA indicated that cultivar and KMC differed significantly for the nine cultivars evaluated over the 2004/05 season. Once again, the cultivar-by-KMC interaction was not significant (Table 7).

Table 7. Mean square values of the influence of kernel moisture content on the Hagberg Falling Number of nine wheat cultivars during the 2004/05 season.

	Mean square value
Cultivar	7165.2***
Kernel moisture content	6330.2**
Cultivar-by-kernel moisture content	488.4
CV (%)	7.97

* - P ≤ 0.05 ** - P ≤0.01 *** - P ≤ 0.001

The t-test results performed on the nine cultivars evaluated during the 2004/05 season indicated that two cultivars (Tugela-DN and SST 367) produced significantly higher HFN at the lower KMC regimes. This observation can, however, not be attributed to reduced *alpha*-amylase activity as none of the nine cultivars evaluated during the 2004/05 season indicated any significant response to increased KMC regimes (Table 10).

Table 8. The t-test results for the nine cultivars evaluated for Hagberg Falling Number performance at various kernel moisture contents during the 2004/05 season.

Cultivar	KMC 1	KMC 2	t
Elands	367.67	336.37	0.084
Gariep	332.75	320.00	0.375
Komati	356.00	346.67	0.619
Limpopo	345.50	343.17	0.879
PAN 3235	329.00	321.80	0.710
PAN 3349	318.40	306.29	0.436
SST 367	311.00	285.56	0.048*
SST 966	326.80	327.00	0.989
Tugela-DN	383.86	348.67	0.019*

KMC 1 – Average HFN obtained at Kernel moisture content regime 1 (10-13.1 %)

KMC 2 – Average HFN obtained at Kernel moisture content regime 2 (13.2-16.9 %)

t – t probabilities of pairwise differences

* - P≤0.05

Table 9. Mean square values of the influence of kernel moisture content on the *alpha*-amylase activity of nine wheat cultivars during the 2004/05 season.

	Means square value
Cultivar	18664***
Kernel moisture content	8922
Cultivar-by-kernel moisture content interaction	2567
CV (%)	19.53

* - P ≤ 0.05 ** - P ≤0.01 *** - P ≤ 0.001

Table 10. The t-test results for the nine cultivars evaluated for *alpha*-amylase content at various kernel moisture contents during the 2004/05 season.

Cultivar	KMC 1	KMC 2	t
Elands	235.1	307.4	0.053
Gariep	253.1	270.7	0.553
Komati	261.9	269.9	0.836
Limpopo	205.5	261.0	0.081
PAN 3235	265.2	265.4	0.991
PAN 3349	237.8	316.1	0.715
SST 367	334.9	367.2	0.376
SST 966	286.6	248.3	0.339
Tugela-DN	231.3	256.9	0.400

KMC 1 – Average HFN obtained at Kernel moisture content regime 1 (10-13.1 %)

KMC 2 – Average HFN obtained at Kernel moisture content regime 2 (13.2-16.9 %)

t – t probabilities of pairwise differences

Figures 3 to 5 give an indication as to why no *alpha*-amylase activity differences could be obtained for the 2004/05 season. With the exception of Elands and possibly SST 966 none of the cultivars showed a similar increase in activity with an increase in KMC as was observed in the previous season (2003/04). As discussed under the two-year analysis, this may be attributed to incorrect KMC being allocated to the various samples due to late rain that fell during the sampling period.

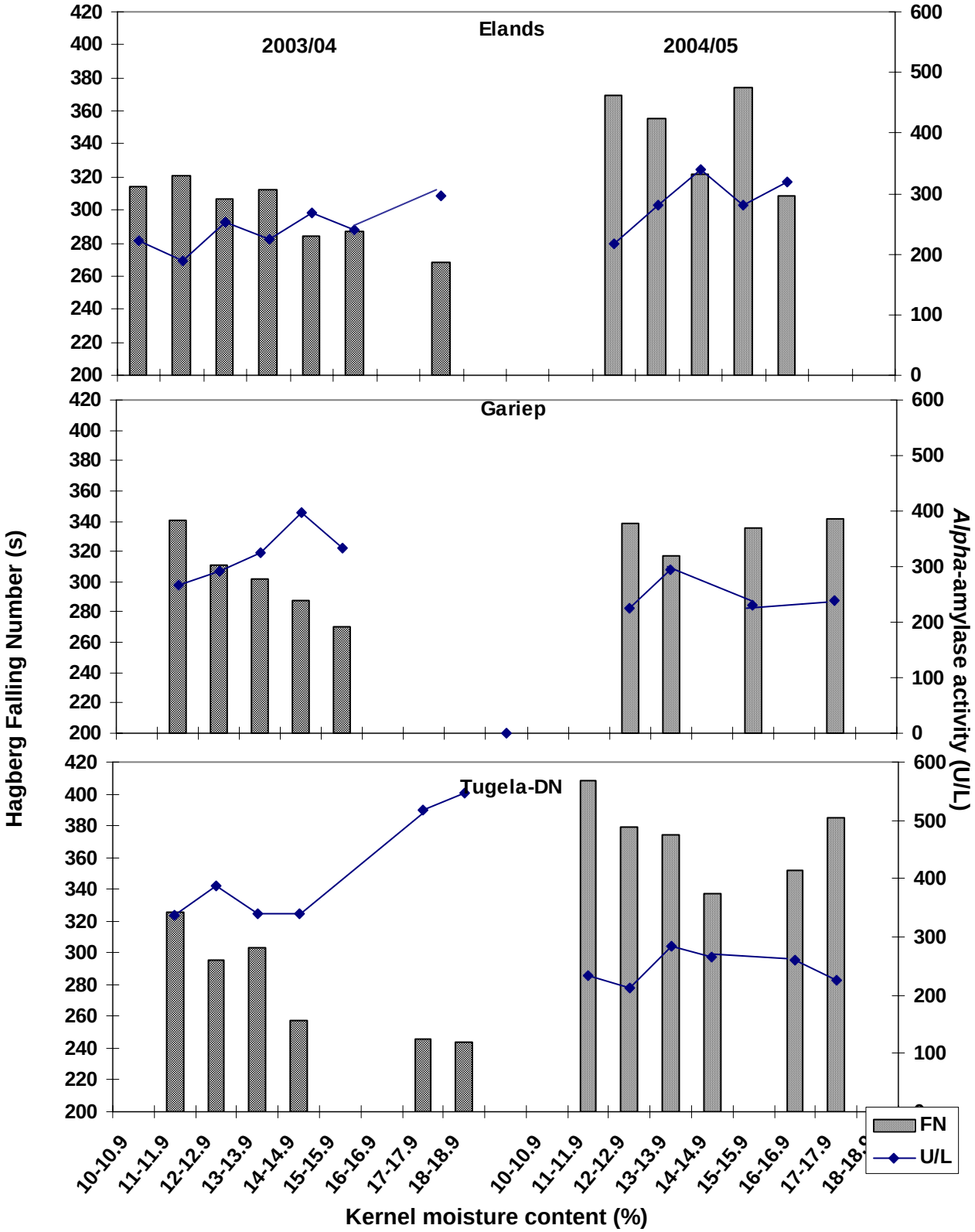

Figure 3. ***Alpha*-amylase activity and Hagberg Falling Number measured for three cultivars (Eland, Gariep and Tugela-DN) at various kernel moisture contents over two seasons (2003/04 and 2004/05).**

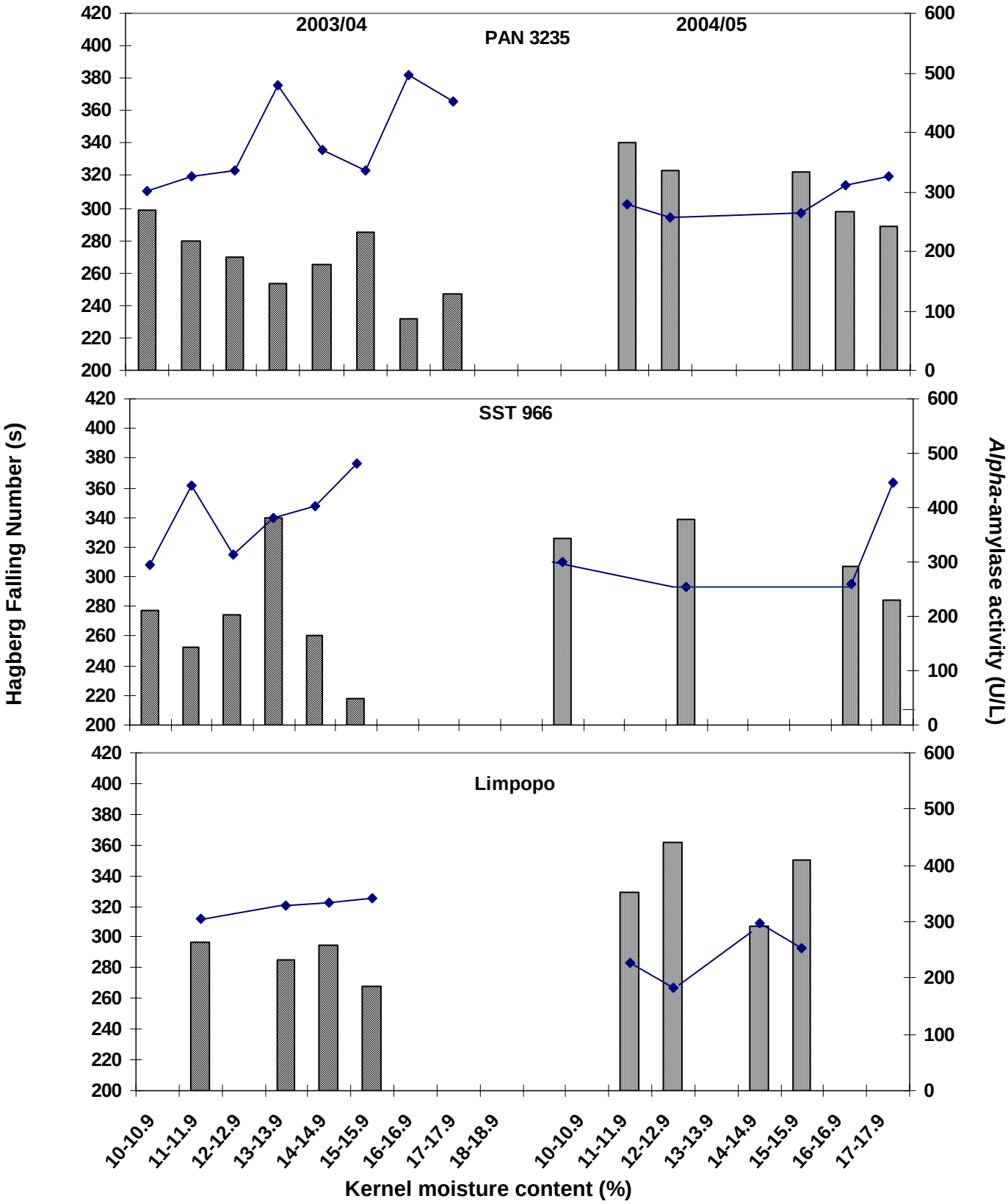

Figure 4. ***Alpha*-amylase activity and Hagberg Falling Number measured for three cultivars (PAN 3235, SST 966 and Limpopo) at various kernel moisture contents over two seasons. (2003/04 and 2004/05).**

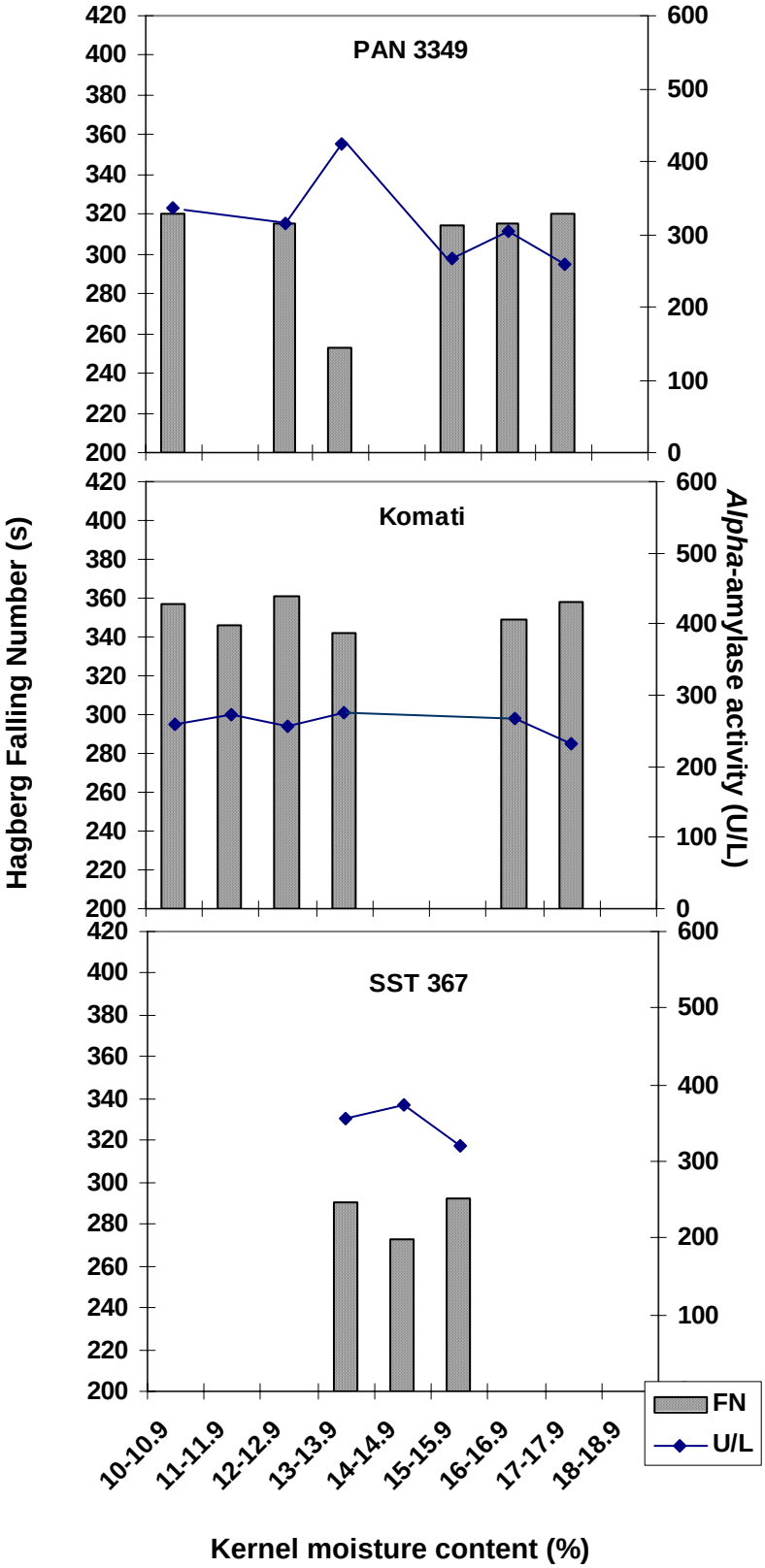

Figure 5. ***Alpha*-amylase activity and Hagberg Falling Number measured for three cultivars (PAN 3349, Komati and SST 367) at various kernel moisture contents during the 2004/05 season.**

Conclusions

Difficulty was experienced in obtaining sufficient replicates for the ideal KMC regimes (i.e. 10-10.9 %, 11-11.9 % etc). As a result, two KMC regimes were created (i.e. 10-13.1 % and 13.2-16.9 %) to accommodate statistical analyses. Data generated with three cultivars (Elands, Gariep and Tugela-DN) over a four year period indicated that the effect of KMC with harvest on the HFN of wheat was dependant on the season. Tugela-DN produced significantly higher HFN at the lower KMC regime (10-13.1 %) for two of the four years of evaluation (2001/02 and 2004/05). Gariep showed similar results for the 2001/02 season. Elands remained constant over the four years of evaluation with no HFN effect visible at the various KMCs.

With the inclusion of Limpopo, PAN 3235 and SST 966 during 2003/04 and 2004/05, the effect of KMC on HFN was investigated in a larger population that could be compared to three control cultivars (Elands, Gariep and Tugela-DN). Within the selected KMC regimes, none of the additional cultivars produced significantly higher HFN as the KMC decreased. Although not statistically significant, most of the cultivars produced higher HFNs at the lower KMC regime. During the 2003/04 evaluation SST 966 produced a HFN below the 220 s cut-off mark for grade when evaluated at 15-15.9 % KMC, confirming the danger associated with harvesting at early KMC. Gariep and PAN 3235 were the only cultivars that indicated significantly higher *alpha*-amylase activity at higher KMC (during the 2003/04 season). This increased activity did not, however, influence the HFN of the cultivars. With the inclusion of another three cultivars (SST 367, PAN 3349 and Komati) during the 2004/05 season, SST 367, in addition to the control Tugela-DN, indicated that KMC had a significant effect on the HFN, but not the *alpha*-amylase activity of the cultivars.

The grouping of data into two KMC regimes had a negative effect on the general investigation. In most cases a clear pattern of increased HFN with a decrease in KMC was observed, but the grouping of the data and the determination of an average for the regime resulted in a masking effect and subsequently no statistical significance was indicated. In addition, climatic conditions resulted in delayed or enhanced drying down, resulting in critical KMCs not being sampled. Rain during harvest also resulted in higher KMC with high HFNs, as experience during the 2004/05 season.

The current study indicated that within the limitations put on the generation of the data, some cultivars are prone to produce higher HFNs at lower KMCs. This is, however, also

limited to specific seasons. The current study also indicated that KMC can not be considered as an accurate indication of the physiological stage of the wheat, especially after a rainy period.

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Chapter 4
An investigation into the effect of glyphosate application on the Hagberg Falling Number of three South African wheat cultivars

Introduction

During February 2001, a single wheat sample was tested at 18 silos of four different agricultural companies within the wheat producing areas of South Africa as part of the ARC-Small Grain Institute's Falling Number Report (Anonymous, 2001). It was concluded that wheat was a commodity too heterogeneous to determine the Hagberg Falling Number (HFN) directly after harvest, as the enzyme activity differs from kernel to kernel. By implication, producers may suffer enormous losses due to wheat being down-graded at one silo or with a specific wheat sample whereas it may have obtained a good grade at another silo or wheat sample from the same load. A study was accordingly conducted at ARC-Small Grain Institute, Bethlehem to investigate the possibility of managing HFN by creating more homogenous wheat through glyphosate applications.

Pre-harvest applications of glyphosate control weeds that interfere with mechanical harvest of wheat. Such applications accelerate wheat dry-down, which allows more timely harvest as well as reduced grain drying costs with no effect on grain quality (Clark, 1981; Darwent *et al.,* 1994). According to O'Keefe and Makepeace (1985) applications made between seven and 17 days prior to harvest did not affect the yield, thousand kernel mass, crude protein, HFN or germination of the cultivars evaluated. Yenish and Young (2000) however, indicated that the wheat stage of development during glyphosate harvest aid application are critical to seed as well as seed quality and not herbicide rate. They demonstrated that yield, kernel weight and germination were significantly lower compared to the control when glyphosate treatments were applied during milk stage, and recommended that such treatments should be administered during hard dough development stage.

The use of chemicals to terminate wheat at a certain stage is, however, not very common in the South African wheat industry. Aside from the fact that little or no research on the subject is available, concern for chemical residue within treated wheat hampers the applicability of such a technique. Hypothetically speaking, such a technique could have

enormous potential with regard to HFN. It could result in the avoidance of pre-harvest sprouting due to the fact that wheat is naturally dried-down within days. Bovey *et al.* (1975) indicated with sorghum that grain moisture content was reduced from 20-40 % to below 13 % within seven days with glyphosate application. Darwent *et al.* (1994) found that glyphosate treatments applied at seed moisture content above 25 % slightly enhanced the drydown of wheat seed and foliage, with treatments below 25 % having no effect. It would also assure that all wheat kernels are dry, with little or no green *alpha*-amylase present, and would hopefully result in a more homogenous HFN. The aim of the current study was therefore to investigate the possible use of glyphosate application to obtain a more homogenous HFN as well as to determine the effect of such applications on wheat quality.

Materials and methods

Field trial

Three cultivars, Gariep, Tugela-DN and Elands were planted at the ARC-Small Grain Institute, Bethlehem, under rainfed conditions in a wheat-monoculture, during mid July for both the 2003/04 and 2004/05 seasons. The selected cultivars varies with regard to their capability to produce Late maturity *alpha*-amylase (LMA) as determined by the University of Adelaide, Australia, with Tugela-DN indicating the lowest capability (12.5 %) and Gariep the highest (100 %). Elands demonstrated an 87.5 % capability to produce LMA (Anonymous, 2004). Prior to planting, fields were prepared by conventional management for the area. The trial was planted as a strip split plot design, consisting of a control and two treatments. Each plot consisted of five 5 m rows spaced 0.45 m apart. The mid three rows were used for quality determinations. Plots were separated by an additional five rows of wheat that served as buffer to limit glyphosate drift to adjacent plots. Treatment one was sprayed with 3 l/ha glyphosate at soft dough stage (Zadoks' scale 85, Zadoks *et al.,* 1974) with the second treatment sprayed at hard dough stage (Zadoks' scale 87). Weather data were recorded, approximately 800 m from the trial site.

Moisture content determinations

Moisture determination for the two treatments was performed on wheat samples obtained from the side rows, according to the AACC Method 44-15A. Fifteen gram (15 g) of seed samples were put in tared moisture dishes and heated for 72h in oven regulated

temperatures 103°C (± 1°C). Moisture content was determined with the following equation:

% Moisture = (A/B)*100

where A = moisture loss in grams and B = original weight of sample

Drying rate

The dates of anthesis and harvest ripe for each of the cultivars at the various treatments were noted and the drying rate determined (i.e. days from anthesis to harvest – DH). Temperature was also taken into consideration by determining the growing degree days (GDD) for each of the treatments: [(max°C + min°C)/2 –0].

Quality parameters

Hagberg Falling Number (HFN: ICC no 107/1, 1995), flour protein content (FPC: Determined with NIR – Near Infrared Reflectance: AACC 39-10), thousand kernel mass (TKM) with SKCS (Sinlge Kernel Characterization System: AACC 55-31) and hectolitre mass (AACC 55-10) were determined within days after harvest. In addition to the general evaluation of HFN (HFN: ICC no 107/1, 1995), repeatability studies were conducted by generating 20 HFN measurements for each of the replicates of all the treatments and controls.

Pre-harvest sprouting

Selected wheat ears for all of the cultivars were labeled at anthesis within the side rows of each plot. When physiologically mature, the ears were harvested and allowed to dry for approximately 10 days before being subjected to 72h rain simulation at 15°C night/22-25°C day temperatures. After rain simulation, wheat ears were screened for pre-harvest sprouting on a scale of 1-8 (1 – no visible sprouting; 8 - heavily sprouted).

Alpha-amylase activity

Alpha-amylase activity was determined according to a revised method of Barnes and Blakeney (1974). A 5 g flour sample was shaken for 15 minutes in 30 ml NaCl (0.09 M) and filtered through Whatman no1 Filterpaper. After filtration, 5 ml filtrate and 3 ml NaCl (0.09 M) was added to centrifuge tubes, and placed in a 50°C water bath. As soon as the mixture within the tubes reached 50°C, the incubation period of 10 minutes was initiated. After incubation, one Phadebas tablet (a highly specific dye-labeled substrate) was added to each tube together with 1.0 ml Acetate buffer at 1 minute intervals. These tablets consist of a substrate made by cross-linked partially hydrolyzed potato starch. The substrate is labeled with Cibacron blue by covalent bonds. Tubes were continuously

stirred. The reaction was terminated after 15 minutes by adding 1 ml of NaOH (0.5 M). After centrifugation (10 minutes at 4 000 rpm), absorbency was determined at 620 nm as *alpha*-amylase hydrolyzes the blue starch polymer into a water-soluble blue dye that absorbs light at 620 nm.

Germination capability

Germination capability of all the cultivars at the different treatments was determined according to ISTA (International Seed Testing Association) regulations.

Statistical analyses

Statistical analyses were performed with the GenStat statistical program (GenStat, 2003). A strip split plot factorial analysis was performed on the various quality parameters evaluated over the two years. All measured parameters were separated by the LSD test at $P \leq 0.05$. Results obtained by the analysis of variance were presented separately for the two years of evaluations, due to the significant differences between years. In addition, a Canonical Variate Analysis (CVA) was used to determine which of the parameters discriminated most between the cultivar-by-treatment combinations over the two years combined. A CVA is often used when it is of more interest to show differences between groups (such as cultivar-by-treatment combinations) than between individuals (Digby and Kempton, 1987).

Repeatability studies were conducted by generating 20 HFN measurements for each of the replicates of all the treatments and controls. An AMMI (Additive Main effects and Multiplicative Interaction analysis) was performed on the data, as it is an important tool for explaining GxE interactions (Yau, 1995). The AMMI model combines an additive model (analysis of variance, AOV) for main effects and a multiplicative model (principal components analysis, PCA) for interaction effects. It is applicable to trials with at least three genotypes and three environments. AMMI has proven useful for understanding and visualizing complex GxE interactions, and gaining accuracy and thereby improving selections within breeding programmes (Gauch, 1992; 1993). By using the three cultivars and three treatments as genotypes (i.e. nine genotypes) with the 20 HFN values per replication as environments, the stability of the HFN obtained within each treatment could be determined.

Results and discussion

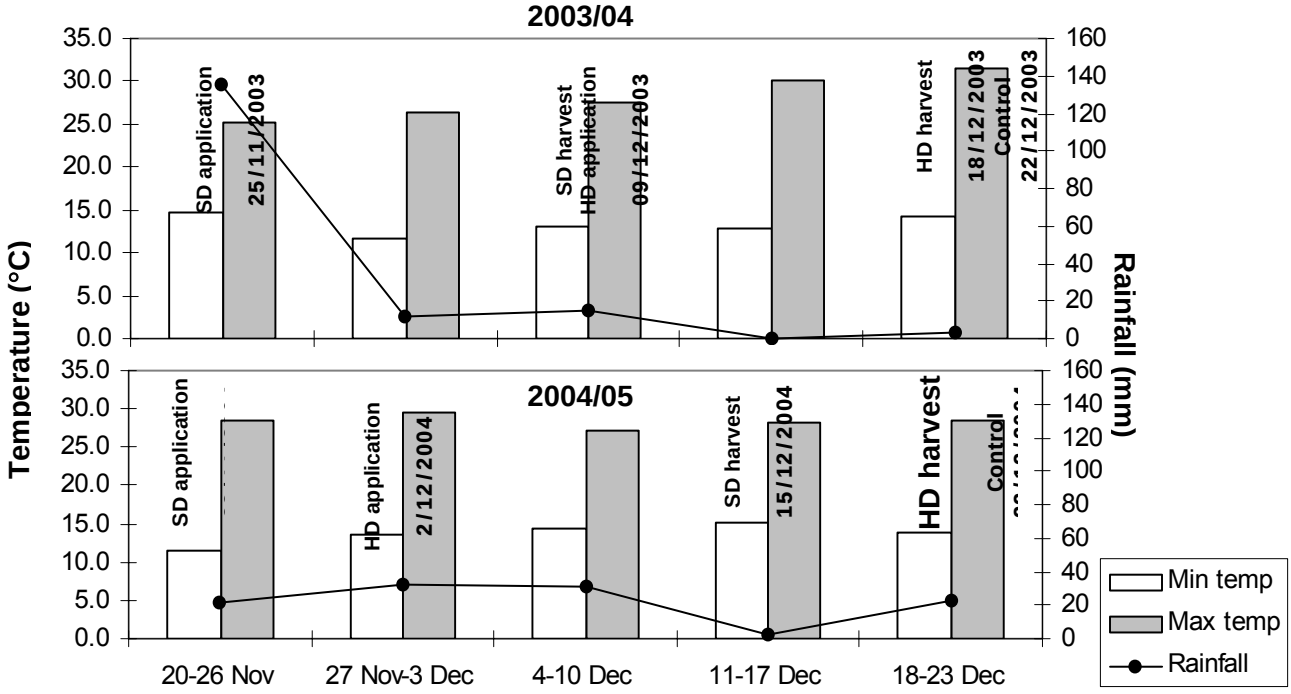


Figure 1. Weekly weather averages for glyphosate treatment periods for the 2003/04 and 2004/05 seasons. (SD – soft dough application, HD – hard dough application)

According to Tottman (1987), wheat contains approximately 50 % and 30 % moisture respectively at soft and hard dough stage. It was therefore attempted to apply the glyphosate at these kernel moisture contents. Table 1 indicates the kernel moisture content of Elands, Gariep and Tugela-DN at the time of treatments and with harvest.

Table 1. Kernel moisture content (%) of Elands, Gariep and Tugela-DN with treatment and harvest for the 2003/04 and 2004/05 seasons respectively.

	Control		Soft dough stage				Hard dough stage			
	KMC (hvst)		KMC (tmt)		KMC (hvst)		KMC (tmt)		KMC (hvst)	
	03/04	04/05	03/04	04/05	03/04	04/05	03/04	04/05	03/04	04/05
Elands	11.8	12.8	49.8	48.5	15.5	12.9	23.9	28.9	11.8	12.8
Gariep	11.8	13.5	49.3	46.2	15.6	13.1	26.4	27.1	11.9	13.3
Tugela-DN	13.3	12.7	57.5	53.0	17.3	13.3	33.1	34.3	13.3	12.6
Avg KMC	12.3	13.0	52.2	49.2	16.1	13.1	27.8	30.1	12.3	12.9

KMC – Kernel moisture content (%) tmt – with treatment

hvst – with harvest

KMC at the various treatments was within acceptable range of 50 % at soft dough stage and 30 % at hard dough stage (Tottman, 1987) with exception of the higher harvest KMC of Tugela-DN at treatment 1 (soft dough stage) of the 2003/04 season. Final KMC with harvest for the soft dough treatments of 2003/04 season was higher compared to the control for all three cultivars evaluated due to too early harvesting and thrashing.

The strip split plot factorial analysis performed on the various parameters measured over the two seasons of evaluation (2003/04 and 2004/05) indicated that treatment-by-cultivar interaction was significant for TKM ($P \leq 0.01$), HLM ($P \leq 0.001$) and germination ($P \leq 0.05$) for the first season of evaluation (2003/04) only (Table 2).

Tables 3 and 4 give statistically significant values determined with the use of the LSD values for the individual cultivars at the various treatments over the two years of analysis (2003/04 and 2004/05).

Thousand kernel mass (TKM)

A significantly lower TKM was measured for all three cultivars after glyphosate application at the soft dough stage compared to the control for the 2003/04 season (Table 3). A similar effect was not observed with the 2004/05 season. Varying results were reported in literature with regard to the most sensitive stage of glyphosate application for wheat. Manthey *et al*. (2004) indicated that glyphosate application at soft dough stage resulted in a reduction in TKM. Yenish and Young (2000) and Darwent *et al.* (1994), however, indicated that kernel weight was only affected with glyphosate applications at milk stage, but differed in that Yenish and Young (2000) found a glyphosate rate effect whereas Darwent *et al.* (1994) reported none. The current study indicated that the mentioned effect was dependent on the season.

Hectolitre mass (HLM)

All the cultivars produced significantly poorer HLM at soft dough stage compared to the control during the 2003/04 season. This is concurrent with the findings of Manthey *et al.* (2004). In the second year of evaluation significantly higher HLM was measured for all three the cultivars when treated at soft dough stage. The reason for this phenomenon is not clear. Darwent *et al*. (1994) indicated that environmental conditions have an influence on the performance of the desiccant treatments, but indicated that the exact role was not clear.

Grain yield

Grain yield, with the exception of Tugela-DN (2003/04), was not affected by the various glyphosate applications, which corresponds with findings of O'Keefe and Makepeace (1985), Yenish and Young (2000) and Darwent *et al.* (1994). The latter indicated wheat yields were reduced only with glyphosate treatments at the milk stage. Yenish and Young, (2000), however, indicated that the percentage yield loss varied over season, variety as well as with glyphosate application. The significantly lower yield observed with Tugela-DN at soft dough stage, may be attributed to the higher than ideal KMCs at both treatment and harvest in the first year of evaluation.

Protein content

Darwent *et al*. (1994) indicated that protein was not affected by treatment at kernel moisture contents of below 41 %. Lower protein content was obtained with treatment at the soft dough stage (at approximately 50 % KMC) for all three cultivars compared to the control during the 2003/04 season. A similar effect was not observed for the 2004/05 season.

Hagberg Falling Number (HFN)

Tugela-DN treated at soft dough stage (2004/05) was the only cultivar that produced a significantly higher HFN compared to the control. This increase could possibly be explained by enhanced drying rates (Astbury and Kettlewell, 1992). Astbury and Kettlewell (1992), however, indicated that a poor relationship between drying rate and HFN in individual years exists. None of the other treatments and cultivars indicated a HFN response, which is concurrent with reports by O'Keefe and Makepeace, (1985).

Alpha-amylase activity (AA)

Elands had significantly higher AA than that of the control after treatment at the hard dough stage for both the 2003/04 and 2004/05 seasons (Table 4). Tugela-DN measured significantly higher AA at soft dough stage for the 2003/04 season. It is not clear why such high AA activity was measured within the first season of evaluation for Elands and Tugela-DN. The increased activity did not have a visible effect on the HFN.

Pre-harvest sprouting (PHS)

The application of glyphosate to wheat at various grain filling stages did not have any effect on the pre-harvest sprouting capability of the various cultivars. All three cultivars maintained their pre-assigned inherent pre-harvest sprouting resistance classes over the various treatments (Elands – high PHS resistance; Gariep – medium PHS resistance;

Tugela-DN – low PHS resistance). As with the case of HFN, the increased AA measured for Elands and Tugela did not have an effect on the PHS measured (table 4).

Days to harvest (DH)

DH was significantly affected by the various treatments in both seasons. The effect appeared to be more prominent in the 2003/04 season (Table 4), as the hard dough treatment of the 2004/05 season did not dry the wheat down to the same extent as was measured the previous season. The application of glyphosate at soft dough stage shortened the average growth period by approximately 10.1 days over the two years of evaluation. As previously mentioned, this may be slightly shorter (i.e. 7 to 8 days) as the harvest dates for e.g. Tugela-DN was not ideal for the 2003/04 season. Application at hard dough stage shortened the average growth period of the three cultivars evaluated by approximately 3.3 days.

Table 2. Summary of the mean square values obtained from the strip split plot analysis of variance for quality characteristics of three South African wheat cultivars treated with glyphosate at various stages of grain filling during a two-year evaluation.

	TKM (g)		HLM (kg/hl)		Yield (t/ha)		Protein (%)		HFN (s)	
	2003/04	**2004/05**	**2003/04**	**2004/05**	**2003/04**	**2004/05**	**2003/04**	**2004/05**	**2003/04**	**2004/05**
Treatment	207.569***	3.0147	92.3344***	8.1470**	0.54314	0.021115	4.3065***	0.0933	721.0	485.8
Cultivar	2.530	70.3297	85.2678***	4.5048*	0.05121	0.014559	4.0238**	0.9244	417.3	3568.4*
Treatment-by-cultivar	11.489**	0.0598	9.512***	0.2565	0.07643	0.008359	0.2692	0.1111	194.8	266.2

	AA (U/l)		PHS		DH		GDD		Germination (%)	
	2003/04	**2004/05**	**2003/04**	**2004/05**	**2003/04**	**2004/05**	**2003/04**	**2004/05**	**2003/04**	**2004/05**
Treatment	14258	1171.0	0.2327	0.03444	371.731***	137.815***	208704.9***	60857.0***	3640.15*	173.81*
Cultivar	25401*	4311.4*	11.4759***	41.4478***	4.1481*	0.0370	1781.1	17.44	99.15	114.37
Treatment-by-cultivar	3248	606.5	0.8369	0.02389	0.7037	0.0370	332.2	18.42	334.76*	22.81

TKM - Thousand kernel mass
HLM – Hectolitre mass
Prot - Protein
HFN – Hagberg Falling Number

AA - Alpha-amylase activity
PHS - Pre-harvest sprouting
DH - Days from anthesis to harvest
GDD - Growing degree days

*** - P≤0.001
** - P≤0.01
* - P≤0.05

Table 3. The effect of glyphosate treatment at soft and hard dough stages on the thousand kernel mass, hectolitre mass, yield, protein and Hagberg Falling Number of three South African cultivars.

	TKM (g)		HLM (kg/hl)		Yield (t/ha)		Protein (%)		HFN (s)	
	2003/04	2004/05	2003/04	2004/05	2003/04	2004/05	2003/04	2004/05	2003/04	2004/05
Elands										
Control	38.57[a]	28.77[a]	83.27[a]	75.97[a]	2.073[a]	1.210[a]	14.96[a]	13.600[a]	340.7[a]	345.0[a]
Soft dough	32.27[b]	27.83[a]	79.43[b]	77.33[b]	1.637[a]	1.133[a]	14.13[ab]	13.833[a]	329.3[a]	343.0[a]
Hard dough	37.97[a]	27.65[a]	83.83[a]	75.53[a]	1.900[a]	1.280[a]	15.31[a]	13.767[a]	341.0[a]	355.0[a]
Gariep										
Control	39.43[a]	27.94[a]	82.03[a]	75.40[a]	1.927[a]	1.227[a]	15.34[a]	13.800[a]	345.0[a]	335.0[a]
Soft dough	32.10[b]	27.06[a]	78.97[b]	77.13[b]	1.653[a]	1.190[a]	13.98[b]	13.367[a]	311.0[a]	351.7[a]
Hard dough	38.07[a]	27.17[a]	82.67[a]	74.73[a]	1.743[a]	1.270[a]	15.90[a]	13.500[a]	323.0[a]	345.7[a]
Tugela-DN										
Control	42.43[a]	33.29[a]	79.03[a]	74.53[a]	1.967[a]	1.237[a]	16.46[a]	14.400[a]	326.0[a]	368.3[a]
Soft dough	29.27[b]	32.32[a]	70.40[b]	75.67[b]	1.253[b]	1.097[a]	15.48[b]	14.000[a]	318.0[a]	397.0[b]
Hard dough	40.17[c]	32.00[a]	79.87[a]	74.43[a]	1.943[a]	1.120[a]	16.28[a]	14.133[a]	328.7[a]	375.7[ab]
CV (%)	3.3	3	0.7	0.8	9.5	7.2	3.9	3.3	8.2	3.1
LSD (0.05)	1.927	2.146	0.839	0.881	0.5199	0.2151	0.738	1.0148	57.02	27.4

TKM – Thousand kernel mass
HLM – Hectolitre mass
HFN – Hagberg Falling Number

Values followed by the same character ([a,b or c]) do not differ significantly

Table 4. The effect of glyphosate treatment at soft and hard dough stages on the *alpha*-amylase activity, pre-harvest sprouting, days to harvest, growing degree days and germination of three South African cultivars.

	AA (U/L)		PHS		DH		GDD		Germination (%)	
	2003/04	2004/05	2003/04	2004/05	2003/04	2004/05	2003/04	2004/05	2003/04	2004/05
Elands										
Control	182.4[a]	220.7[a]	2.23[a]	1.000[a]	58.00[a]	51.330[a]	1186.3[a]	1080.6[a]	79.0[a]	87.33[a]
Soft dough	252.4[ab]	233.4[a]	1.47[a]	1.033[a]	45.00[b]	44.4667[b]	878.9[b]	940.6[b]	60.0[a]	81.00[a]
Hard dough	307.7[b]	272.5[b]	1.83[a]	1.000[a]	51.67[c]	51.333[a]	1065.2[c]	1080.6[a]	62.7[a]	87.67[a]
Gariep										
Control	218.8[a]	279.2[a]	2.16[a]	2.700[a]	57.67[a]	51.333[a]	1180.9[a]	1080.6[a]	94.3[a]	84.67[a]
Soft dough	260.6[a]	268.4[a]	2.89[a]	2.567[a]	45.33[b]	44.667[b]	885.2[b]	940.6[b]	38.0[b]	74.33[b]
Hard dough	247.0[a]	281.6[a]	2.15[a]	2.700[a]	51.67[c]	51.333[a]	1031.4[c]	1080.6[a]	65.0[c]	87.67[a]
Tugela-DN										
Control	287.4[a]	279[a]	4.28[a]	5.200[a]	58.67[a]	51.333[a]	1164.8[a]	1080.6[a]	92.0[a]	92.67[a]
Soft dough	387.7[b]	283.1[a]	3.38[a]	5.167[a]	43.67[b]	44.330[b]	855.9[b]	933.2[b]	46.7[b]	86.33[a]
Hard dough	335.0[ab]	286.8[a]	4.39[a]	5.433[a]	51.67[c]	51.330[a]	1026.0[c]	1080.6[a]	77.7[a]	89.00[a]
CV (%)	16.4	6.2	22.7	10.2	1.6	0.9	1.5	0.7	13.3	4.7
LSD (0.05)	97.71	38.4	1.011	0.5259	1.178	0.8762	26.65	14.39	23.42	9.909

AA – *Alpha*-amylase activity
PHS – Pre-harvest sprouting
DH – Days to harvest
GDD – Growing degree days
Values followed by the same character ([a,b or c]) do not differ significantly

Growing degree days (GDD)

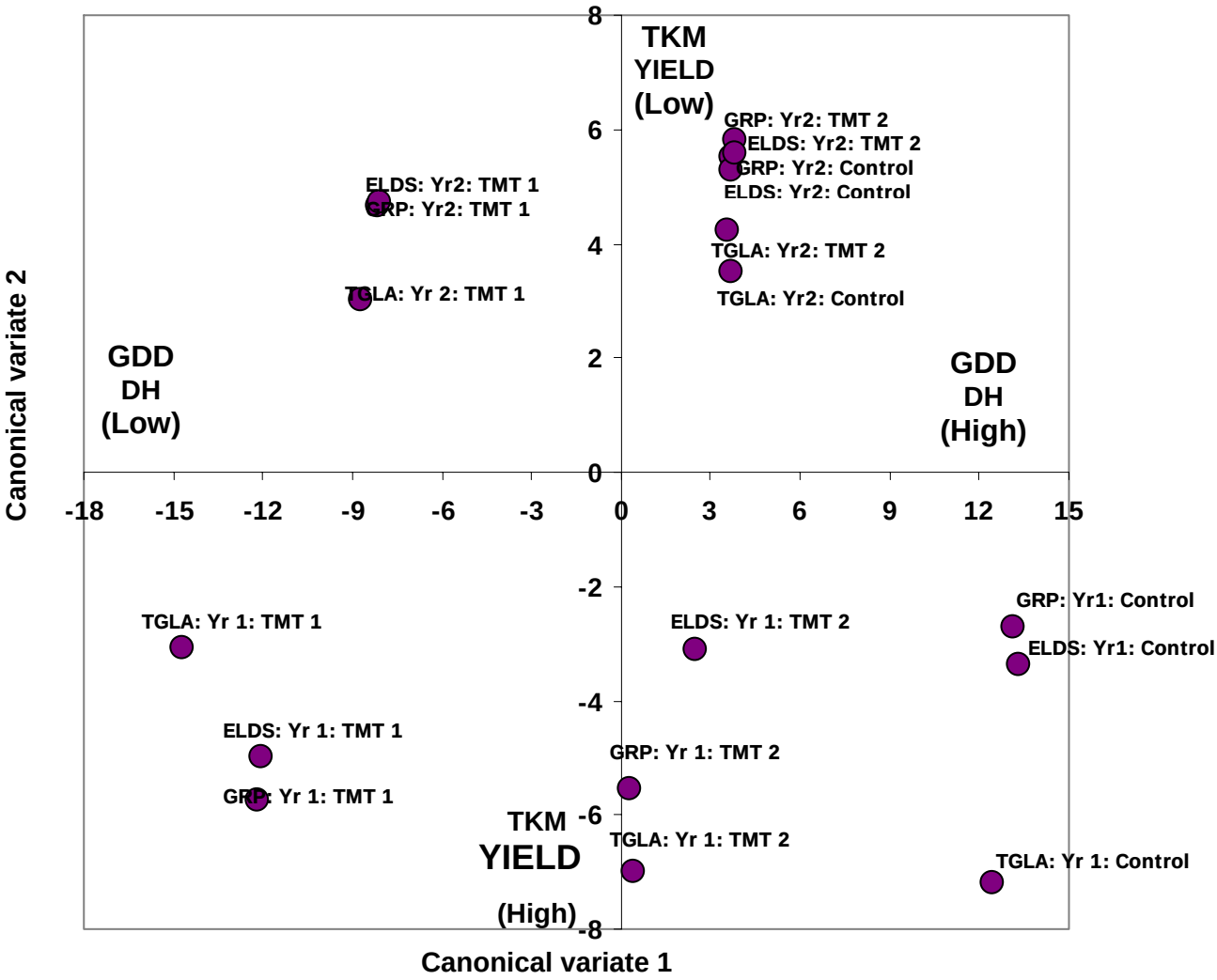
Similar to DH, GDD was significantly affected by the glyphosate application at soft dough stage in both seasons. Glyphosate application at the hard dough stage did not have the same effect during the two season of evaluation, as no reduction in GDD was measured during the 2004/05 season (Table 4). Clarke (1981) indicated that desiccants applied to grain at 43 % or lower kernel moisture content did not reduce grain moisture faster than the untreated control, which concurs with the data generated at the hard dough treatment in the second year of evaluation. Similarly McNeal *et al.* (1973) found that desiccation of wheat below 38 % grain moisture did not speed drying. Again the climatic effect appears to play an important role with the effectiveness of the glyphosate applications.

Germination capability

During the first year of evaluation, the soft dough treatment resulted in poor germination capability for all three cultivars (Elands – 54 %, Gariep – 26 % and Tugela-DN 34 %). Abnormalities recorded during the 2003/04 season included split coleoptiles, leaf and roots missing as well as general deformation, that may be attributed to residues of glyphosate and its major metabolite, aminomethylphosphonic acid (AMPA, Cessna *et al.,* 1994). The effect of glyphosate treatment on germination was not as extreme in the second year of evaluation (2003/04) as was the case with the first (2004/05; Table 4). Yenish and Young (2000) reported a similar phenomenon with regard to germination capability varying over seasons and application rate. Their research, however, indicated that glyphosate treatment later than milk stage did not have an effect on germination capability.

The CVA (Figure 2) performed on the parameters evaluated over a two year period indicated a clear environmental effect over the two years of analysis, as the 2003/04 data were grouped below the horizontal axis with the 2004/05 data above the horizontal axis. The drying rate of the wheat (days from anthesis to harvest as well as GDD) explained most of the variation that occurred on the horizontal axis. On the vertical axis, TKM and yield were responsible for most of the variation observed. The CVA therefore gives a visual confirmation that the 2003/04 season produced higher yields and TKM than the 2004/05 season. Secondly it confirms that treatment with glyphosate at soft dough stage shortens the drying rate of the wheat significantly, as all of the data for all three cultivars evaluated at treatment 1 (soft dough stage), fell to the left side of the vertical axis. Lastly, the control and treatment 2 (hard dough stage) exhibited similar performance in the

2004/05 season as these data points are grouped closely on the CVA in the upper right quadrant.



ELDS – Elands
GRP – Gariep
TGLA – Tugela-DN

Yr 1 - Year 1 (2003/04)
Yr 2 – Year 2 (2004/05)
TMT 1 – Treated at soft dough stage
TMT 2 – Treated at hard dough stage

TKM – Thousand kernel mass
T/Ha – Tons per hectare
GDD – Growing degree days
DH – Days to harvest

Figure 2. Canonical variate analysis on the influence of glyphosate application at soft and hard dough stage individually on various parameters of wheat.

Repeatability studies were conducted to determine whether the use of glyphosate at the specified stages would result in a more homogenic (stable) HFN. Twenty HFN measurements for each of the various replicates for all the treatments and controls were

generated for each of the two years. AMMIs were performed on the data generated for the 2003/04 and 2004/05 seasons (Figures 3 and 4) individually.

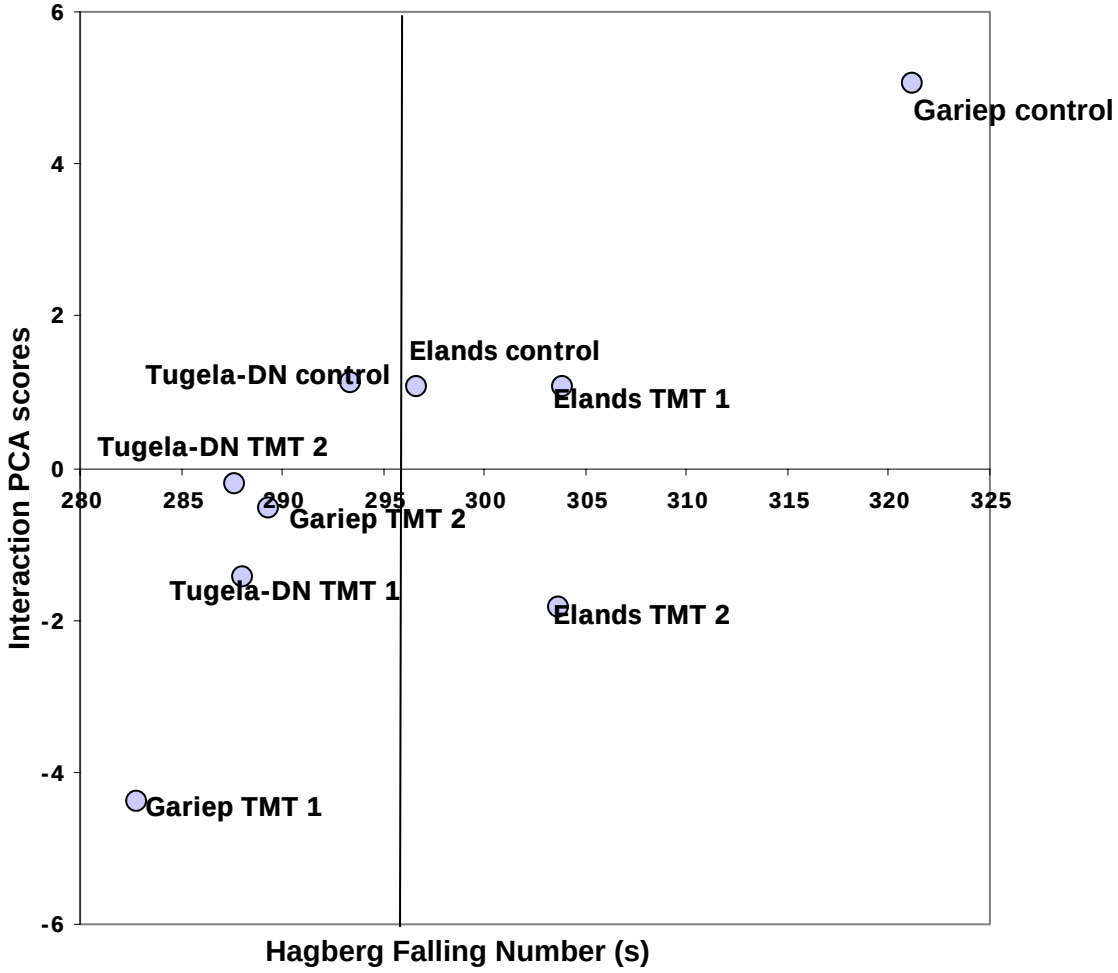


Figure 3. AMMI analysis indicating cultivar stability with glyphosate application at soft dough stage (TMT1) and hard dough stage (TMT2) during the 2003/04 season.

During the 2003/04 seasons, glyphosate application at hard dough stage resulted in a more stable HFN being measured for Tugela-DN and Gariep (Figure 3). Gariep TMT 1 (soft dough stage) and Gariep control produced the most unstable HFN. Similar results could not be obtained for the 2004/05 season as the glyphosate application at soft dough stage resulted in the most stable HFN measured for Tugela-DN and Gariep, followed by the Tugela-DN control and the Tugela-DN hard dough application (Figure 4). The Elands control and Elands soft dough application (TMT 1) produced the most unstable results

measured for the season. The reason for the observed instability observed by Gariep (control and TMT 1) during 2003/04 and the instability of Elands (control and TMT 1) during the following season is not quite clear. It is possible that cold weather that might have occurred during the growing season could have resulted in the production of LMA in the two cultivars. If this was, however, the case it is not clear as to why both cultivars did not show a similar response over both the seasons of evaluation as they both have similar LMA production capability.

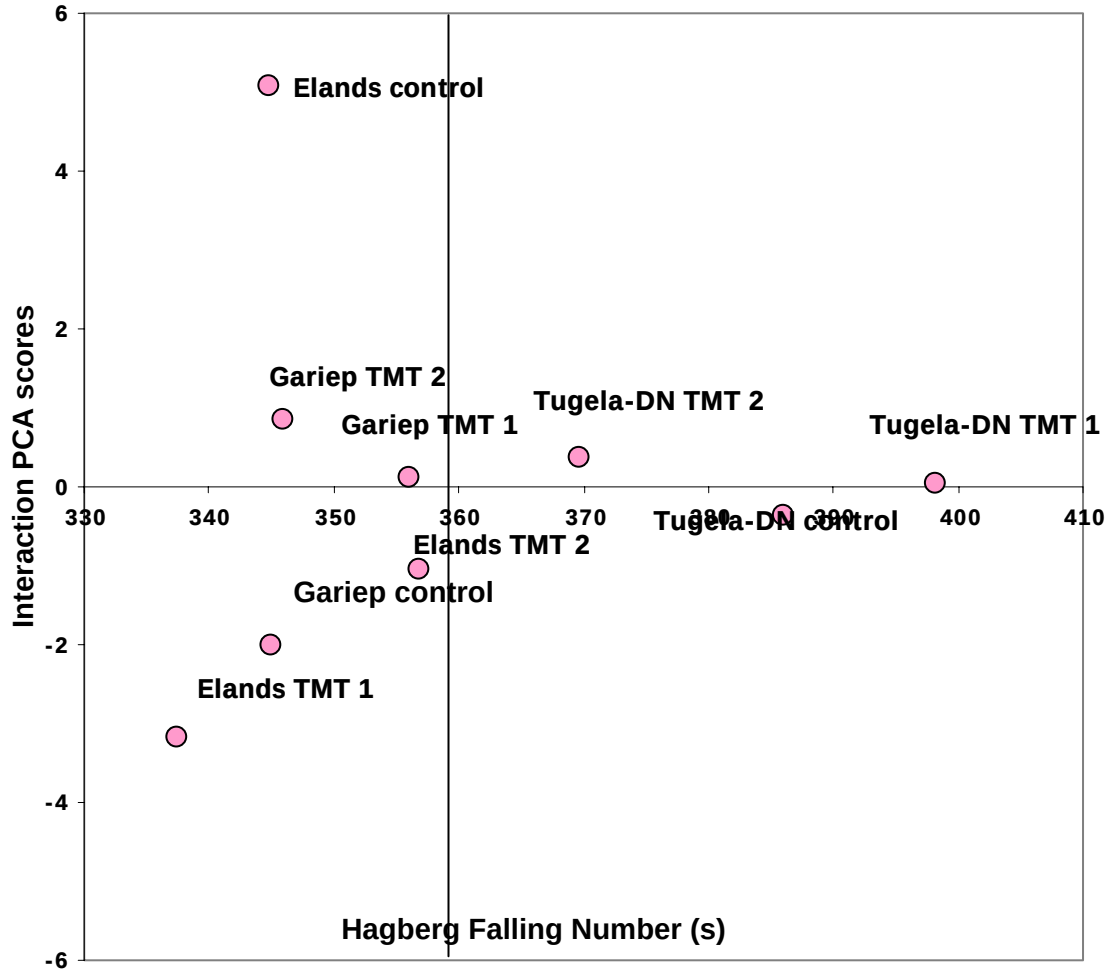


Figure 4. AMMI analysis indicating cultivar stability with glyphosate application at soft dough stage (TMT1) and hard dough stage (TMT2) during the 2004/05 season.

AMMI analysis over the two years indicated that the glyphosate applications at soft and hard dough stages produced varying successes with regard to stabilizing HFN, with the success rate varying over season as well as cultivar. Tugela-DN and Gariep showed the

largest reaction with regard to stability, with Elands showing minimal response in both years of evaluation. The response obtained varied over seasons with glyphosate application at the hard dough stage providing the optimal response for the 2003/04 season and application at soft dough stage the optimal response for the 2004/05 season. AA activity (Table 4) was determined on a single sample representative of the cultivar and treatment and not for each of the 20 HNF samples taken for each replication used in the analysis. It would therefore be wrong to assume that AA did not play a role in the results obtained with the repeatability study. Another possibility may lie in the GDD with the critical stage falling between 930 and 1030 GDD, as the HFN measured within the set GDD resulted in more stable HFN for Tugela-DN and Gariep.

Conclusions

The effect of the glyphosate treatments on wheat at various stages of grain filling varied over seasons. During the 2003/04 season TKM, HLM, Protein concentration, DH as well as GDD were significantly poorer with the soft dough treatment than that of the control for all three cultivars included in the study. The soft dough application reduced DH on average with 13.5 days (23.32 %) and with 6.6 days (11.4 %) at the hard dough stage. Yield, AA and PHS were not affected by any of the glyphosate applications during the 2003/04 evaluation. Tugela-DN was the only cultivar that managed a higher HFN during the 2004/05 season compared to the control. Germination was severely affected by the soft dough stage application.

DH and GDD were the only parameters investigated that were significantly affected by the soft dough application for all three the cultivars evaluated, but not to the same degree as with the 2004/05 season. DH was significantly reduced by 6.9 days (13.37 %), with the hard dough stage treatment showing no response. Similarly germination was also not affected to the same extent as with the previous season. An increase in the HLM was measured with the soft dough treatments that cannot be explained.

Studies on the repeatability of the wheat samples at the various treatments, indicated that glyphosate treatment managed to produce stable HFN in two of the three cultivars, but that the stage of application that resulted in the higher stability, varied over seasons. Both cultivar and environment therefore had an effect on the glyphosate treatments. Further studies to establish the correct stage of application are merited.

The current study therefore indicates that the practice of glyphosate application needs to be investigated extensively with regard to the effect of such applications on specific quality parameters (i.e. TKM, HLM and Protein concentration) as well as the correct physiological growth stage for application before it would hold any advantages within a wheat production system.

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Chapter 5
The classification of South African bread cultivars according to Hagberg Falling Number reaction to fertilizer application

Introduction

The Hagberg Falling Number (HFN) test was incorporated in the South African wheat grading regulations during June 1998. Before its incorporation, wheat was indirectly evaluated for low HFN through a visual screening test. The visual screening test required that a 25 g wheat sample should not contain more than 2 % sprouted wheat, as sprouted wheat has large amounts of *alpha*-amylase activity. Since the incorporation of the HFN test, it became obvious that various factors, other than sprouted wheat, had an influence on the HFN of wheat, as numerous reports of low HFN wheat without visual sprouting were received throughout the summer rainfall wheat producing areas of South Africa. With the release of the Falling Number Report (Anonymous, 2001), it was speculated that several cultivars performed poorly regarding HFN, due to insufficient nitrogen availability. Literature is, however, not clear as to the effect of fertilizer application on HFN. Tabl and Kiss (1983) as well as Oskarsen (1989) reported a negative association between N application and HFN, due to increased lodging and therefore elevated grain moisture content (Oskarsen, 1989). Hook *et al.* (1989) and Webb and Sylvester-Bradley (1995) reported no visible effect, with Gooding *et al.* (1986) and Kettlewell (1999) reporting a positive linear association between N application and HFN, due to delayed maturity, with such a delay aiding a high HFN. A decrease in *alpha*-amylase activity due to increased N is also speculated to be an explanation for high HFN (Pushman and Bingham, 1976). Various research studies have, however, indicated the presence of a cultivar effect linked to the HFN response to N application. Both Gooding *et al.* (1986) and Rule (1987) indicated that various N treatments increased the HFN of cv. Avalon but not cv. Mission. Biskupski (2000) also reported similar results. According to King (1989), N fertilizer will produce grain with higher protein and as a result, grain with an enhanced capacity to synthesize *alpha*-amylase.

The aim of this study was to investigate the effect of fertilizer application on the HFN of 15 South African bread wheats to establish the presence of a cultivar effect. This would result in the classification of all the current cultivated varieties into easily understood response groups.

Materials and methods

Field trials

The trials were planted over a two year period (in the month of July, 2001 and 2002) at the Small Grain Institute (Bethlehem, Eastern Free State) under rainfed conditions in a wheat-monoculture. The required soil analyses were performed beforehand to establish the soil statutes. Fifteen cultivars were evaluated at three different fertilizer treatments. The trial consisted of three replications for each treatment. The experiment was arranged as a split plot design in three randomized blocks with fertilizer treatment as the main plot factor and cultivar as the subplot factor. The three treatments included 0 N, P, K kg/ha (sub-optimal), 20 N, 5 P, 5 K kg/ha (standard) and 60 N, 15 P and 15 K kg/ha (optimal). The fertilizers were applied as LAN, superphosphate and potassiumchloride. The specific treatment levels were chosen to simulate fertilizer applications by producers in the dryland summer rainfall wheat producing areas. The plot size in all years was 2.4 m x 5 m with a 40 cm interrow-spacing. Fertilizer treatments were band placed at planting. Weather data (rainfall, maximum and minimum temperatures) was recorded within 0.4 km of the trial.

Sampling and laboratory analysis

All cultivars were harvested at approximately 12 % kernel moisture content. Cleaned wheat samples were milled and the protein content determined with Near Infrared Reflectance (NIR, AACC39-10) and HFN (ICC no 107/1, 1995) determined within two weeks after harvest. Yield was determined as ton/ha.

Statistical analysis

A split plot factorial analysis was used to establish a cultivar effect for yield, protein content and HFN for each of the two years of evaluation. Once the environmental effect was determined, data was pooled into a two year analysis to determine cultivar response over seasons that would allow cultivars to be grouped according to their HFN response to fertilizer treatments. The main grouping of cultivars was done according to their HFN performance as established by this analysis. In addition to the split plot analysis for the combined seasons, a Canonical Variate Analysis (CVA) was used to determine which of the three characteristics (HFN, yield and protein content) discriminated most between the cultivar-by-treatment combinations. The CVA, also better known as linear discriminant analysis, is used when it is of more interest to show differences between groups (such as cultivar-by treatment combinations) than between individuals (Digby and Kempton 1987).

The variability in a large number of variables is firstly reduced to a smaller set of variables called canonical variates, that are linear combinations of the original measurements, and are thus given as vectors of loadings for the original measurements. With this approach a set of directions is obtained in such a way that the ratio of between group variability to within group variability in each direction is maximised. In this study the variates were the three characteristics that were measured (HFN, protein and yield) for each cultivar and treatment combination (45 cultivar-by-treatment combinations). The scores calculated for each of the canonical variates were then correlated with the original variates to find those that were the most important in discriminating between the groups. Plots of the canonical variate means for each group indicated the group positions relative to one-another. In such a plot, points closer together are similar and points further apart are dissimilar with respect to the variates that discriminate between them. The criteria used for describing the optimum classification was the minimum within-group sum of squares method. Data analysis was performed with the statistical program GenStat (GenStat Committee, 2000).

Results and discussion

Climatic conditions

The average monthly minimum and maximum temperatures experienced during 2001 and 2002 are indicated in Figure 1. The 2002/03 season had lower minimum temperatures in October, November and December, but higher maximum temperatures than in 2001/02. The 2001/02 season had higher rainfall (Figure 2), in comparison to the relatively drier 2002/03 season. Increased rainfall during December (2002), however, increased the risk of preharvest sprouting. The 2001/02 season was therefore a cooler moist season, with the 2002 season (for the larger period) being warm and dry.

The individual performance of the cultivars with regard to yield, protein content and HFN over the two years is indicated in Table 1 (2001/02) and Table 2 (2002/03).

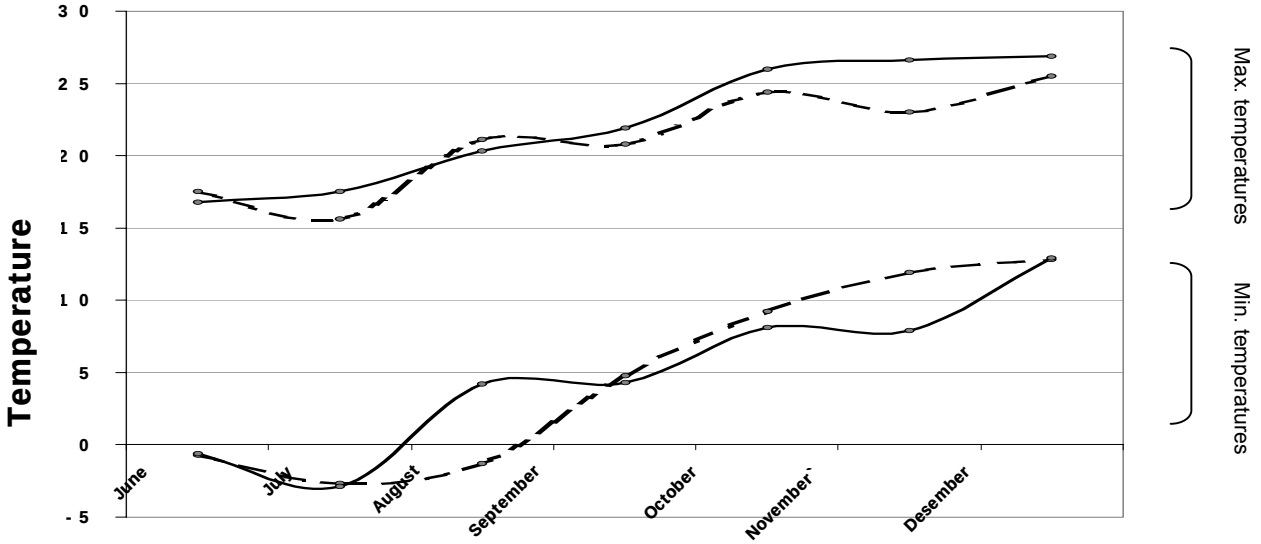


Figure 1. Monthly averaged minimum and maximum temperatures for the 2001/02 (dashed line) and 2002/03 (solid line) seasons (Bethlehem, Eastern Free State).

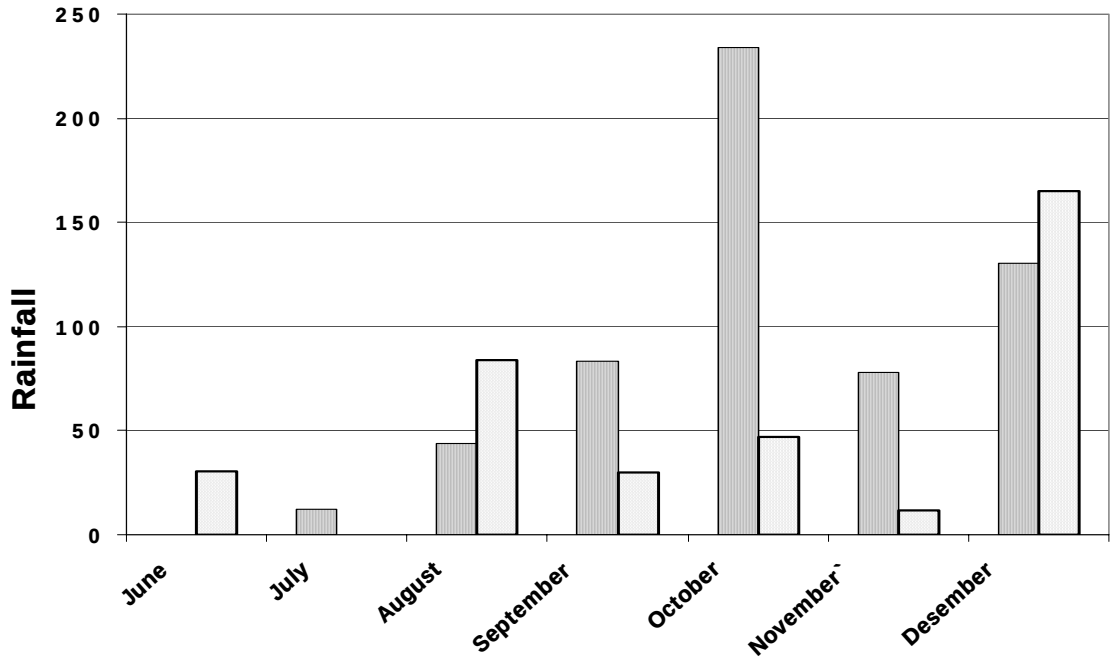


Figure 2. Total rainfall (mm) measured for the 2001/02 (lined bars) and 2002/03 (dotted bars) growing season.

Yield

Most of the cultivars produced significantly better yield with the optimal fertilizer application during the 2001/02 season (Table 1). The standard application also resulted in increased yield with most of the cultivars evaluated, but the increases were not significantly higher than that of the control. Limpopo, PAN 3377, SST 124, SST 363 and SST 399 did not show any significant increase in yield, as the fertilizer application increased (Table 2). During the following season, nine of the 15 cultivars evaluated did not show any response to the fertilizer applications. This observation may be attributed to the climate conditions that were warmer and drier than the previous season, resulting in stressed growing conditions. This is indicated by lower average yield being measured over the 15 cultivars evaluated for the 2002/03 season, compared to that of the 2001/02 season (Figure 3).

Protein content

The average protein production for the 15 cultivars evaluated was higher for the 2002/03 season, compared to the 2001/02 season (Figure 3). This is consistent with the higher maximum temperatures experienced during the 2002/03 season. This overall higher protein content measured for the 2002/03 season resulted in little differences being observed between the various fertilizer treatments (Table 2). Reaction to the various treatments for the 2001/02 season was, however, obtained and was most prominent in the optimum application (Table 1).

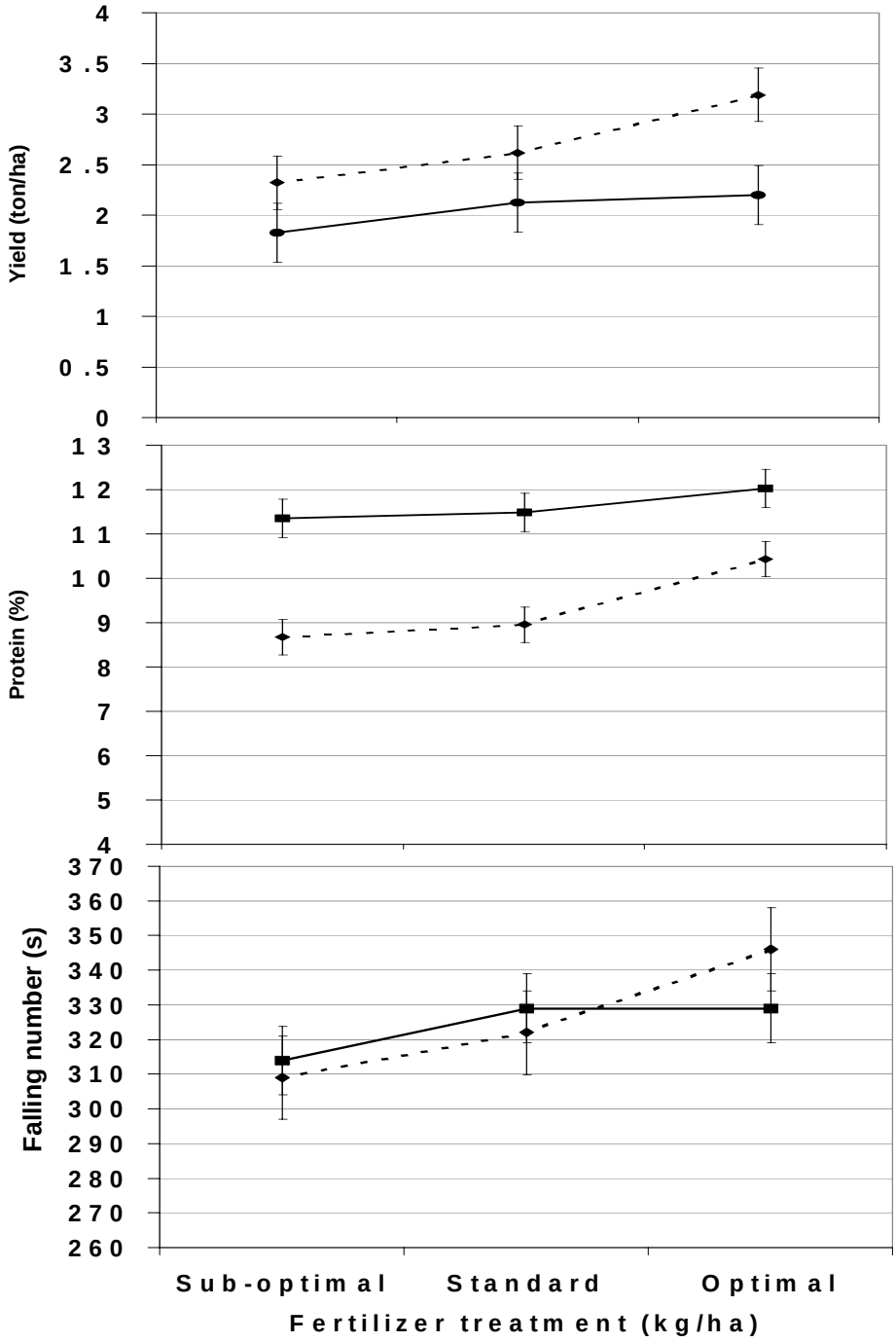
Hagberg Falling Number (HFN)

The HFNs for all the cultivars at the various treatments were above the 220 s cut-of mark for grade for both seasons. The cooler moist conditions during the 2001/02 season resulted in an average increase in HFN for the 15 cultivars evaluated with an increase in fertilizer level (Figure 3). A similar trend could not be obtained during the warmer 2002/03 season.

During the 2001/02 season, Gariep and PAN 3377 produced significantly better HFN with the optimum fertilizer application, with the standard application not differing significantly from the control or optimum application (Table 1). Limpopo gave significantly better HFN for both the standard and optimum applications compared to the control. PAN 3235, SST 399 and SST 966 all gave significantly higher HNF with the optimum fertilizer application. The eight remaining cultivars did not show any response to the fertilizer application during

the 2001/02 season. During the 2002/03 season the HFN response was limited to only two cultivars, Caledon and PAN 3191 (Table 2).

To investigate the probability of a cultivar effect, the two year data was combined and statistical analysis performed on the two year average of the various cultivars. With a comparison of interaction means for each of the cultivars individually with the use of least significant differences (LSDs), significant cultivar differences were visible, which allowed for the classification of the 15 cultivars into four main groups regarding their sensitivity to fertilizer treatment to their individual HFN performance. The four groups identified were high, medium, low to medium and low response (Table 3).



Sub-optimal = 0 N,P,K (kg/ha) Standard = 20 N, 5 P, 5 K (kg/ha) Optimal = 60 N, 15 P, 15 K (kg/ha)

Figure 3. Average yield, protein and Hagberg Falling Number response of 15 dryland cultivars to fertilizer level during 2001/02 (dashed line) and 2002/03 (solid line). Standard error of means is indicated.

Table 1. Average yield, protein content and Hagberg Falling Number for 15 dryland cultivars evaluated at three fertilizer levels for the 2001/02 season.

Cultivar	Yield (t/ha)			Protein content (%)			Falling Number (s)		
	Sub-optimal	Standard	Optimal	Sub-optimal	Standard	Optimal	Sub-optimal	Standard	Optimal
Betta-DN	2.059[a]	2.527[a]	3.133[b]	8.330[a]	8.200[a]	10.567[b]	282.0[a]	314.3[a]	311.7[a]
Caledon	2.203[a]	2.420[a]	3.597[b]	8.800[a]	9.100[a]	11.100[b]	312.7[a]	342.3[a]	343.0[a]
Elands	2.253[a]	2.813[a]	3.382[b]	7.967[a]	9.267[b]	10.000[b]	365.3[a]	378.0[a]	335.3[a]
Gariep	2.337[a]	2.300[a]	3.206[b]	8.000[a]	8.700[a]	10.933[b]	302.7[a]	332.7[ab]	355.0[b]
Limpopo	2.303[a]	2.609[a]	2.867[a]	8.467[a]	8.600[a]	10.067[b]	300.3[a]	364.7[b]	336.7[b]
PAN 3191	2.520[a]	2.850[a]	3.696[b]	8.433[a]	8.567[a]	10.733[b]	326.0[a]	323[a]	355.0[a]
PAN 3235	2.400[a]	2.465[a]	3.505[b]	8.500[a]	10.400[b]	10.367[b]	289.7[a]	296.7[a]	343.7[b]
PAN 3349	1.789[a]	2.698[b]	3.855[c]	9.067[a]	8.133[a]	9.267[a]	327.0[a]	338.3[a]	367.3[a]
PAN 3364	2.586[a]	2.918[a]	3.140[b]	8.500[a]	8.733[a]	9.933[b]	279.7[a]	282.3[a]	311.0[a]
PAN 3377	2.847[a]	3.170[a]	2.979[a]	9.033[ab]	8.567[b]	9.967[a]	310.0[a]	325.7[ab]	352.3[b]
SST 124	2.013[a]	2.203[a]	1.874[a]	9.467[a]	9.733[a]	11.500[b]	351.0[a]	344.0[a]	342.0[a]
SST 333	1.985[a]	2.054[a]	2.811[b]	10.500[a]	10.667[a]	12.000[b]	346.3[ab]	340.0[a]	375.0[b]
SST 363	1.902[a]	1.995[a]	2.056[a]	8.867[a]	9.267[a]	10.667[b]	333.0[a]	328.0[a]	358.3[a]
SST 399	2.619[a]	2.743[a]	3.089[a]	8.033[a]	8.300[a]	9.833[b]	307.7[a]	314.3[a]	350.3[b]
SST 966	3.001[a]	3.487[a]	4.648[b]	8.100[a]	8.367[a]	9.600[b]	299.0[a]	299.3[a]	357.7[b]
CV (%)		15.4			7.5			6.2	
LSD (0.05)		0.6054			1.1242			33.07	

Sub-optimal = 0 N,P,K (kg/ha) Standard = 20 N, 5 P, 5 K (kg/ha) Optimal = 60 N, 15 P, 15 K (kg/ha)

Individual cultivar values for yield, protein and Hagberg Falling Number, indicated with the same letters ([a,b, or c]) do not differ significantly from each other

Table 2. Average yield, protein content and Hagberg Falling Number for 15 dryland cultivars evaluated at three fertilizer levels for the 2002/03 season.

Cultivar	Yield (t/ha)			Protein content (%)			Falling Number (s)		
	Sub-optimal	Standard	Optimal	Sub-optimal	Standard	Optimal	Sub-optimal	Standard	Optimal
Betta-DN	1.432[a]	2.461[b]	2.115[ab]	11.867[a]	11.833[a]	12.167[a]	335.7[a]	344.7[a]	353.7[a]
Caledon	2.216[a]	2.143[a]	2.269[a]	10.833[a]	11.333[a]	11.733[a]	330.0[a]	342.7[ab]	370.3[b]
Elands	1.977[a]	2.315[a]	2.234[a]	10.667[a]	10.600[a]	11.533[a]	325.7[a]	331.0[a]	337.7[a]
Gariep	1.930[a]	2.283[a]	2.392[a]	10.667[a]	10.467[a]	11.100[a]	328.3[a]	346.0[a]	319.0[a]
Limpopo	2.360[a]	1.805[a]	2.240[a]	10.867[a]	11.333[a]	11.533[a]	320.3[a]	316.0[a]	339.0[a]
PAN 3191	1.495[a]	2.263[b]	2.068[ab]	11.833[a]	12.067[a]	12.833[a]	264.0[a]	337.0[b]	291.7[a]
PAN 3235	1.853[a]	1.861[a]	1.861[a]	10.500[a]	12.000[b]	11.700[ab]	321.7[a]	349.0[a]	331.7[a]
PAN 3349	2.062[a]	2.500[a]	2.836[ab]	10.733[a]	10.667[a]	11.200[a]	312.3[a]	329.0[a]	326.3[a]
PAN 3364	1.638[a]	2.562[b]	2.233[ab]	12.467[a]	12.033[a]	12.600[a]	296.0[a]	325.3[a]	328.7[a]
PAN 3377	2.291[a]	2.676[ab]	3.187[b]	10.567[a]	11.500[ab]	11.967[b]	291.3[a]	296.0[a]	326.3[a]
SST 124	1.409[a]	1.797[a]	1.893[a]	12.467[a]	12.467[a]	12.667[a]	327.3[a]	346.0[a]	346.3[a]
SST 333	2.401[a]	2.075[a]	2.087[a]	12.600[a]	12.133[a]	13.100[a]	330.3[a]	337.3[a]	336.3[a]
SST 363	1.034[a]	1.485[a]	1.978[a]	11.330[a]	10.933[a]	11.433[a]	302.3[a]	326.3[a]	331.3[a]
SST 399	0.526[a]	1.190[b]	0.884[ab]	12.000[a]	12.300[a]	13.467[b]	318.7[a]	305.0[a]	286.3[a]
SST 966	2.820[a]	2.474[a]	2.716[a]	10.867[a]	10.733[a]	11.367[a]	298.3[a]	302.7[a]	322.0[a]
CV (%)		20.0			6.2			6.6	
LSD (0.05)		0.7123			1.3139			36.93	

Sub-optimal = 0 N,P,K (kg/ha) Standard = 20 N, 5 P, 5 K (kg/ha) Optimal = 60 N, 15 P, 15 K (kg/ha)

Individual cultivar values for yield, protein and Hagberg Falling Number, indicated with the same letters ([a,b, or c]) do not differ significantly from each other

Table 3. The classification of 15 cultivars into low, low-medium, medium and high response groups according to Hagberg Falling Number reaction to fertilizer treatment over two seasons of evaluation. Similar classifications for protein content and yield are indicated within the set response groups for HFN.

Main HFN response groups	Cultivar	Protein content (%)	Yield (t/ha)
High	SST 966	High	High
	PAN 3377	High	Low-Medium
	Elands	Low-Medium	Low-Medium
Medium	PAN 3191	High	Medium
	Limpopo	Low-Medium	Low
Low to medium	Caledon	High	High
	Betta-DN	High	Medium
	SST 363	High	Low-Medium
	PAN 3235	Medium	High
	PAN 3349	Low	High
	PAN 3364	Low	Medium
Low	Gariep	High	High
	SST 124	High	Low
	SST 333	High	Low
	SST 399	High	Low

As the main objective of this study was to investigate HFN, attention was given to the grouping of cultivars according to their performance regarding HFN at various fertilizer treatments. The definitions of the four groups are as follows (see Figure 4 for visual criteria).

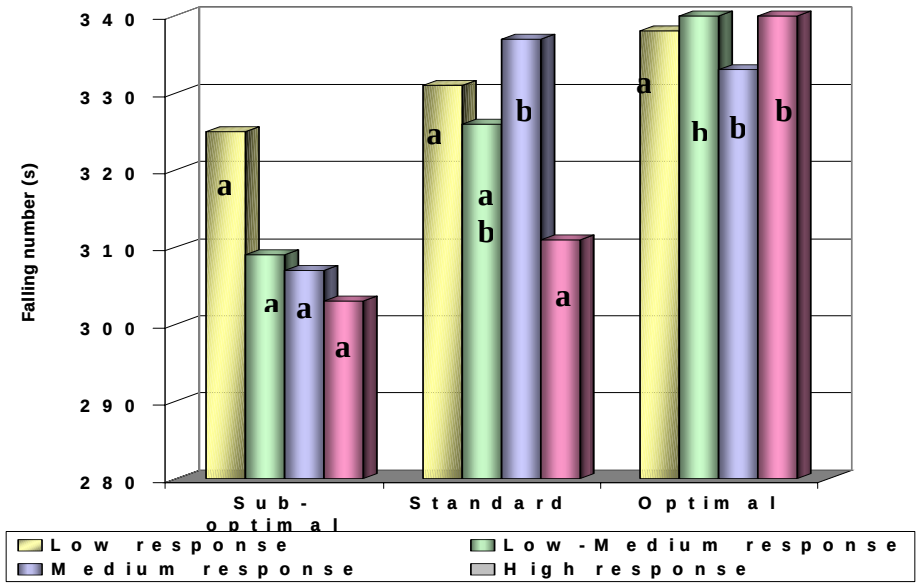


Figure 4. Cultivar grouping into low, low to medium, medium and high response groups at the different fertilizer treatments according to Hagberg Falling Number (s) performance (LSD=23.7).

- The low response cultivars did not show any significant differences over all the treatment levels. It therefore appears that fertilizer application does not have any effect on the HFN. Cultivars included in this group were Gariep, SST 124, SST 333 and SST 399.

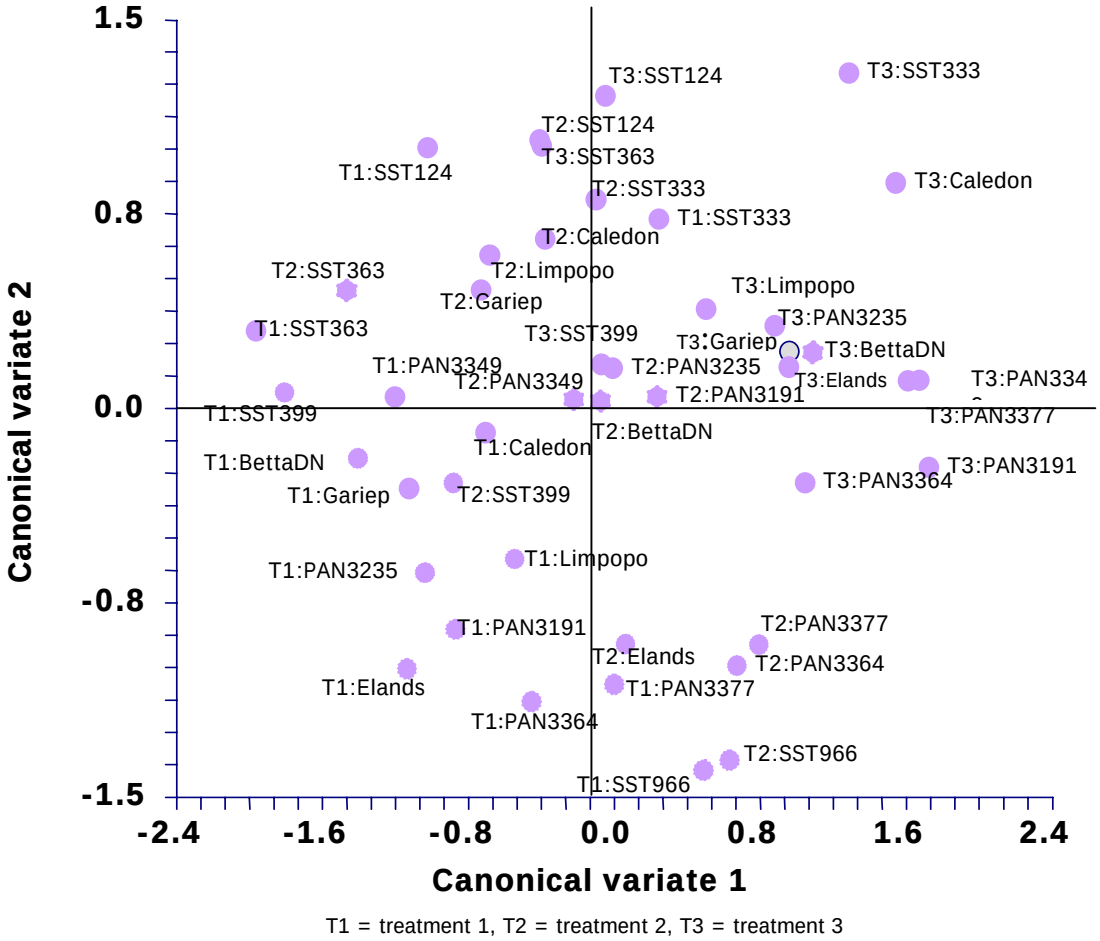
- In the second group (low to medium response) the sub-optimum and the standard treatments did not differ significantly, with the standard and optimum treatments also not differing significantly. Only the sub-optimum and optimum treatments therefore differed significantly. These cultivars were grouped into the low to medium response class, as there appears to be a very small but gradual improvement of HFN with increased N application. Cultivars included in this group were Caledon, Betta-DN, SST 363, PAN 3235, PAN 3349 and PAN 3364.

- The medium response cultivars performed significantly better with the standard and optimum treatment than with the sub-optimum treatment. These cultivars were grouped together as they could generally perform equally well at the standard and

optimum fertilizer applications regarding their HFN, but may experience problems (a lower than expected HFN) should the N (fertilizer) availability be limited. Cultivars included under this response group were PAN 3191 and Limpopo.

- The last group (high response) was classed due to its tendency to perform effectively at optimum fertilizer treatment. This group should be seen as a high response group as their optimum performance regarding their HFN is linked to only optimum levels of fertilizers. Inaccessibility or insufficient N may result in a reduced HFN, lower than the potential for HFN for these cultivars. Cultivars included in this group were SST 966, PAN 3377 and Elands.

This study also indicated that yield as well as protein content is related to fertilizer applications. The grouping of sensitive cultivars should therefore not only allow for HFN, but also should allow the incorporation of yield and protein responses. For this purpose, a Canonical Variate Analysis (CVA) was performed. The CVA (Figure 5) indicated that the horizontal axis (CVA 1) explained 62.42 % of the measured variation. Correlation between the scores (CVA 1 + CVA 2) and the parameters of investigation (yield, protein content and HFN) indicated that yield had the highest correlation (0.778). This indicates that yield was responsible for the variation between the different points on the horizontal level. On a vertical level (CVA 2), due to the highest correlation (0.863), HFN was responsible for most of the variation observed on the vertical axis and explained 28.98 % of the total variation measured. In conclusion, yield and HFN were the main factors distinguishing between the 45 treatment-by-cultivar combinations on the graph (Figure 5) and explained 91.4 % of the total variation.



T1 = treatment 1, T2 = treatment 2, T3 = treatment 3

Figure 5. Canonical variate analysis (CVA) of the Hagberg Falling Number response of 15 South African wheat cultivars to various fertilizer applications over two seasons of evaluation.

With the use of the CVA, the initial classification according to HFN via split plot ANOVA, could be extended to yield as well as protein response. Table 4 represents the final classification of the cultivars. As the data obtained from the CVA gave a combined reaction of yield, protein as well as HFN to fertilizer treatment, the initial grouping was altered. As this specific study was, however, centred on the HFN response of various cultivars to fertilizer treatment, the four main response groups (high, medium, medium-low, low response) were maintained. The combined performance of each cultivar is indicated with the use of the canonical variate means, used to plot the different cultivars on the CVA graph (Fig. 7). By adding the X and Y-axis coordinates for each cultivar, a potential response value is obtained (Table 4).

The more negative the value, the poorer the performance of the cultivar for the combined factors. The closer the value is to zero, the more average the response that was obtained. The more positive the value the higher the performance of the cultivar for all three factors. A good example is 'T1: Elands' in the bottom left quadrant and 'T3: SST 333' in the top right quadrant of Figure 5. 'T1: Elands' represents the performance of Elands at the sub-optimum level (treatment 1), and indicates that the cultivar is situated further from the 0:0 point of all the cultivars in the lower than average yield and HFN quadrant. 'T3: SST 333' represents SST 333 at the optimum fertilizer treatment (treatment 3). This cultivar is also situated further from the 0:0 point, but in the higher than average yield and HFN quadrant. According to Table 4, Elands (sub-optimum fertilizer treatment) indicated a performance potential of -2.1425, the lowest potential of all the cultivars, with SST 333 (optimum) the highest potential (2.581). According to Figure 5, these two cultivars represent the lowest and highest potential cultivars, which is reflected in the potential value assigned to each in Table 4.

Table 4. Classification of 15 dryland cultivars for their HFN, yield and protein response to fertilizer treatments calculated from the CVA.

		Sub-optimal		Standard		Optimal	
†HFN classification*	Cultivar	Potential	Rank	Potential	Rank	Potential	Rank
High response	Elands	-2.1425	15	-0.8481	13	1.1144	11
	PAN 3377	-1.0675	5	-0.1193	8	1.7151	5
	SST 966	-0.9095	4	-0.7277	12	2.1294	3
Medium response	PAN 3191	-1.7236	13	0.2799	3	1.4960	6
	Limpopo	-1.1289	6	-0.0900	7	0.8858	12
Medium to low response	PAN 3235	-1.6723	12	0.1467	5	1.1982	8
	SST 363	-1.6652	11	-1.0110	14	0.6160	14
	Betta-DN	-1.5970	10	-0.0465	6	1.1803	9
	PAN 3364	-1.5848	9	-0.3221	11	0.7582	13
	PAN 3349	-1.1581	7	-0.1867	9	1.7770	4
	Caledon	-0.7993	3	0.2760	4	2.4132	2
Low response	SST 399	-1.7459	14	-1.1684	15	0.0998	15
	Gariep	-1.4338	8	-0.2732	10	1.3051	7
	SST 124	-0.0175	2	0.6269	2	1.1578	10
	SST 333	0.9755	1	0.7065	1	2.5810	1

Classification according to HFN response to fertilizer treatment – spit plot analysis

Sub-optimal = 0 N, P,K (kg/ha) Standard = 20 N, 5 P, 5 K (kg/ha) Optimal = 60 N, 15 P, 15 K (kg/ha)

† HFN is the main factor, with the combined HFN, yield and protein performance as sub-factors. The rank indicated represents the position of the cultivars with regard to all three factors.

These results should be seen as a guideline to the cultivation of these cultivars. It is general practice to fertilize in order to obtain high yields with good protein content. Should fertilizer application, however, be insufficient, the high response cultivars (i.e. Elands, PAN 3377 and SST 966) will not be able to obtain their optimum HFN for the season. This data is also important should leaching of nitrogen due to heavy rain be a problem in certain areas.

The reason for the increase in HFN with optimum fertilizer treatment, as observed in the response group, is unknown. Both Jönsson (1966) and Kettlewell (1999) reported that increased N application resulted in increased HFNs due to decreased *alpha*-amylase activity in glasshouse as well as field trials. Kettlewell (1999) further speculated that pre-maturity *alpha*-amylase (PMAA) is responsible for the increase in HFN by N in the absence

of sprouting. PMAA can arise in certain cultivars (Flintham and Gale, 1988) and is speculated to be simulated when grains dry slowly during ripening (Gale *et al.,* 1983). Pushman and Bingham (1976) found that N application resulted in decreased *alpha*-amylase in some of the cultivars evaluated, with no effect in the others, which could explain the cultivar effect indicated in the current study. Retained pericarp *alpha*-amylase activity (RPAA) refers to the type of enzymes located in the pericarp of immature grains and which activity declines greatly by harvest (Olered and Jönsson, 1970). RPAA is able to reduce the HFN of cultivars that contain unripe grains at harvest (Lunn *et al.,* 1997). According to Kettlewell (1999), crops that tend to suffer from such RPAA, for example where a second, later, population of tillers develop, the effect of N on the HFN may be of commercial significance as the effect may result in the HFN being lowered to unacceptable levels.

Clare *et al*. (1990) concluded that as the response to HFN to N was similar to the response of yield, crops would unlikely suffer reduced HFNs, due to the fact that crops are generally fertilized for yield. This is confirmed by the current study. What makes the classification of cultivars into response groups necessary in the South African wheat industry, is the varying climatic conditions experienced over the whole of the wheat producing area. Cultivars that remain constant with regard to their HFN irrespective of their fertilizer application may be ideal for areas with limited N availability.

Conclusions

An investigation into the effect of fertilizer application indicated that fertilizer had, in general, no statistically significant effect on the HFN of wheat. Cultivar differences did, however, occur that allowed for the individual effect of fertilizer on the HFN of these cultivars to be identified. This allowed for the grouping of cultivars into four response groups namely low, low to medium, medium and high response cultivars. Classification was refined with the use of CVA (HFN, yield and protein). HFNs measured were, however, never below the 220 s cut-off mark for grade. The research conducted indicated that crops are unlikely to suffer reduced HFNs (i.e. 220 s), due to insufficient plant nutrition. In addition the risk of reduced HFNs is minimized by the fact that the current level of fertilization followed by most commercial farmers is aimed at yield and protein content.

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Chapter 6
A study into the effect of moderately high temperature exposure during grain filling on the Hagberg Falling Number of wheat.

Introduction

During the second and fourth week of October 2002, the south western Free State suffered four to six consecutive days of temperatures ranging between 32°C and 34°C. Depending on the planting date, wheat planted in that specific region was at various stages of early grain filling. Shortly after harvest, complaints were received from the area regarding low Hagberg Falling Number (HFN) without visual sprouting. As extreme cold conditions (-4°C night temperature) also occurred during the same period, it was not certain whether the reduced HFN measured could be attributed to the high or low temperature occurrence. The weather data, however, provided the necessary proof, that the climatic conditions in certain wheat producing areas, though not frequently, are capable of causing high temperatures that could result in heat stress early enough during the season to coincided with the early grain filling stages of wheat and therefore might be responsible for the poor wheat quality measured in certain wheat producing areas.

Genotype, environmental factors, and the interaction between genotype and environment influence wheat quality (Busch *et al.,* 1969; McGuire and McNeal, 1974). Grain yield, protein content, dough strength, and the size and number of starch granules are some of the factors affected by temperature (Sofield *et al.,* 1977; Bhullar and Jenner, 1985; Randall and Moss, 1990; Tester *et al*., 1995). Little or no data, however, exists on the effect of high temperature exposure on the Hagberg Falling Number (HFN) of wheat. Data on the possible effect of other quality parameters, affected by high temperature exposure, on the HFN of wheat is also limited. Matsuki *et al.* (2003) reported that by increasing the maturation temperature from 15ºC to 30ºC, kernel weight and starch content is significantly reduced but also indicated that cultivars differed in their reaction or sensitivity to increased temperatures during grain filling. In addition Shi *et al.* (1994) concluded that increased temperature during grain filling results in increased starch gelatinisation temperatures. It is therefore clear that starch, either in its content or its properties, is influenced by temperature, therefore the possibility is created that unexplained Hagberg Falling Numbers (HFN) that occur in the absence of pre-harvest sprouting (PHS) might be attributed to changes within the starch composition of the wheat samples as a result of high

temperature exposure, especially since Olered and Jonsson (1970) indicated that different starch properties can cause considerable variation within HFN.

A slight positive correlation between increased temperatures during early stages of grain filling and protein content was found by Johnson *et al.* (1972), with higher average temperatures late in grain filling showing no effect on protein content. Various authors also reported on the effect of very high temperatures during grain filling on protein composition (Blumenthal *et al.,* 1991; Graybosch *et al.,* 1995; Stone and Nicolas *et al.,* 1995). According to Blumenthal *et al.* (1990) heat-inducible accumulation of gliadins and heat shock proteins (HSPs) in developing grain lead to deterioration of flour and subsequently bread making quality. HSPs are generally known to function as chaperones, playing an important role in the folding and assembly of protein (Boston *et al.,* 1996; Waters *et al.,* 1996). Earlier research has shown that the gluten components of flour have little effect on the HFN of wheat (Perten, 1964), but the effect of the so-called stress induced proteins on the HFN of wheat are to a large extent still unknown.

The effects of heat stress on wheat yield and quality are speculated to be the result of lengthy periods of above optimal temperatures i.e. chronic heat with daily maxima of 20°C to 32°C. The effect could also be attributed to short periods of heat shock i.e. a few days with maximum temperatures of over 32°C (Skylas *et al.,* 2002). According to Stone and Nicolas (1996) two distinct temperature ranges are distinguished, the moderately high (25-32°C) and the very high temperatures (33-40°C), with responses to moderately high temperatures being the result from changes in rate and duration of existing processes. At very high temperatures, new physiological processes may be initiated. The aim of the current study was to determine whether a short period of moderately high temperature exposure (32°C) at either late milk stage or soft dough stage would result in a reduced HFN and whether such a response could be linked to cultivar sensitivity. In addition, other quality parameters would be investigated that could possibly be correlated to HFN.

Material and methods

Temperature model

Temperature data recorded in the Bloemfontein area during October of 1995 to 2002 were analysed to create a temperature protocol that would simulate representative high

temperature scenarios as they occur in the field. Once a temperature model was created and the required temperature settings for the frost chambers decided on, the X21 Datalogger (Campbell Scientific, Inc.) was used to determine the accuracy of the growth chamber. Temperature sensors were placed at ear level and temperatures logged at five-minute intervals over the duration of the treatment.

Wheat trial

Three wheat cultivars (Tugela-DN, Elands and Gariep) were selected to form part of the study performed at the University of the Free State. The trial consisted of two treatments (in addition to the control) replicated three times. Each replication consisted of 20 pots. Seedlings were vernalised for a period of six weeks before being planted out 2 l pots (two plants per pot). Pots were placed in a completely randomised block design. Greenhouse temperatures were set at 15°C/22°C. Main tillers were labelled for all the treatments. Fertilizer was administered every second week (3:2:1: N:P:K, 10 g/l and 3g/l Kan: 50 ml per pot). Fungicides, pesticide as well as micronutrients were administered as required.

The main tiller of each plant was individually studied with the initiation of the treatments, to ensure that it was at the correct stage of development required for the specified treatment. From the temperature analysis, a protocol was decided on that entails a 32ºC day temperature (8 h period) and a 15ºC night time period (16 h period) for a period of 96 hours (4 days). Wheat was treated at two different grain filling stages individually. The first treatment was administered when the main tillers were at late milk stage, the second treatment when the main tillers were at soft dough stage. After treatment, the plants were allowed to mature naturally in the greenhouse. With harvest, the main and side tillers were pooled and sent for quality analysis.

Quality analysis

Quality analysis tests were performed by the Quality Laboratory at the Small Grain Institute (Bethlehem). Quality parameters investigated included Hagberg Falling Number (HFN: ICC no 107/1, 1995), SDS sedimentation volume (SDS: AACC 56-60), flour protein content (FPC: Determined with NIR – Near Infrared Reflectance: AACC 39-10), thousand kernel mass (TKM), kernel diameter (KD) and kernel hardness (KH). TKM, KD and KH were determined with SKCS (Single Kernel Characterization System: AACC 55-31). Starch damage (SD) was determined with the use of the Starch Damage kit (Megazyme).

Statistical analysis

Data analysis was performed with the statistical program GenStat (GenStat, 2003). Data was analysed as a completely randomised block design.

In addition, a Canonical Variate Analysis (CVA) was used to determine which of the quality parameters investigated discriminated most between the cultivar-by-treatment combinations. The CVA, also better known as linear discriminant analysis, is used when it is of more interest to show differences between groups (such as cultivar-by treatment combinations) than between individuals (Digby and Kempton, 1987).

Results and discussion

Temperature analysis

Temperature analysis indicated 14 days during the seven years investigated that reached 32°C and more during the day during the month of October. Only three of these days (during 2002) reached 34°C. This indicated that such extreme temperatures rarely occur. A screening temperature protocol of 32°/15°C was decided on.

Quality analysis

The ANOVA indicated that the three cultivars as well as the various treatments differed significantly ($P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$) from each other for the majority of the parameters evaluated (Table 1). The cultivar-by-treatment interactions did not differ significantly at any of the quality parameters investigated.

Hagberg Falling Number (HFN)

The biggest response with regard to the various treatments was obtained by Elands, as both the heat stress treatments at late milk (TMT 1) and soft dough stages (TMT 2) resulted in a significantly poorer HFN compared to that of the control (Table 2). A very low LSD value (26.63 s) was, however, used in the determination of significant differences that would probably not be considered as significant in the general agricultural practise. TMT 2 resulted in a significantly poorer HFN in Tugela-DN, with Gariep indicating no response. Gariep in general obtained poorer HFN in both the control and the treatments when compared to that of the other two cultivars included in the study. Although significant differences were obtained in two of the three cultivars included in the study, none of the treatments resulted in a HFN below 220 s. Auld and Paulsen (2003) indicated that neither

drought nor high temperature stress affected *alpha*-amylase enzyme activity, which is of great importance as high *alpha*-amylase is mostly responsible for low HFN (Chamberlain *et al.,* 1981) and is commonly observed in pre-harvest sprouted wheat kernels or with normal germination (Sargeant, 1980). *Alpha*-amylase activity within the various cultivars and treatments could not be confirmed in the current study, as insufficient amounts of material were available for *alpha*-amylase determination. Various literature, however, report on shifts within gliadin:glutenin ratios after a period of heat shock. Blumenthal *et al*. (1990) reported an increase in gliadin content in grain that had been heat-shocked at >35°C for 1h or longer. Their results therefore supported the hypothesis that a heat-shock during ripening may produce grain with weaker gluten. It is possible that such changes within protein composition might be responsible for reduced HFN measured after heat shock. SE-HPLC analysis on protein composition should be considered in follow-up studies. Alternatively the effect of starch properties on HFN in the current should be more closely investigated, as various research reports on a possible association (Olered and Jonsson, 1970; Matsuki *et al.,* 2003). HFN was significantly correlated (Table 3) with FPC (P≤0.001), TKM (P≤0.01), DM (P≤0.01), KH (P≤0.001) and SD (P≤0.001).

SDS sedimentation volume (SDS)

The SDS sedimentation test is normally used to estimate flour strength (Preston *et al.,* 1991) and protein quality (Graybosch *et al*., 1995). According to Graybosch *et al*. (1995) protein quality as measured by the SDS sedimentation test, is more susceptible to environmental modification than the flour protein content or the level of the different protein fractions as measured by SE-HPLC. Randall and Moss (1990) demonstrated that a daily average temperature up to 30°C increased dough strength, while temperatures above this threshold value, even applied for periods of only a few days, tended to decrease dough strength. Gariep was the only cultivar evaluated in the current study that showed any response to the various treatments (Table 2) as it managed to produce significantly lower SDS at both TMTs 1 and 2 compared to the control. This is contradictory to reports of Bencze *et al.* (2004) that reported an increase in SDS sedimentation volume (35°C for 15 days during grain filling). Comparatives studie are, however, difficult as the treatments differed from the current study. SDS was significantly correlated to DM (P≤0.05), KH (P≤0.05) and SD (P≤0.05).

Flour protein content (FPC)

TMT 1 resulted in a significant poorer FPC in Tugela-DN (Table 2). Significantly better FPC was measured for both the treatments compared to that of the control for Gariep at both treatments, with no effect measured for Elands. This data therefore partly contradicts Johnson *et al*. (1972) which reported that increased temperatures during the early stages of grain filling was positively correlated with protein content, but that higher temperatures during later stages of grain filling had no effect on protein content. According to Graybosch *et al.* (1993), an increase in gliadin content with increased flour protein content is a commonly observed effect. FPC could only be significantly correlated with HFN ($P \leq 0.001$; Table 3).

Thousand kernel mass (TKM)

Gariep was the only cultivar that produced a significantly poorer TKM with TMT 1 (Table 2). Elands obtained a similar response, although not significant. Wardlaw *et al.* (2002) reported a reduced kernel weight in cultivars exposed to short periods of heat shock, which was most evident at day/night temperatures above 30/25°C. Matsuki *et al.* (2003) reported similar results. They demonstrated that by increasing the maturation temperature to 30°C, kernel weight is reduced. A cultivar effect was also reported that is verified with the current study. According to Bhullar and Jenner (1985) the reduction in grain weight observed at high temperature is the result of the effect of high temperatures on starch deposition, as the conversion of sucrose to starch is impaired at high temperature and therefore limits starch synthesis TKM was significantly correlated with HFN ($P \leq 0.001$), DM ($P \leq 0.001$), KH ($P \leq 0.01$) and SD ($P \leq 0.01$; Table 3).

Kernel diameter (KD)

A significantly poorer KD was measured for Gariep at TMT 1 (Table 2). As with TKM, Elands also indicated a similar response although not significant. Both Batey *et al.* (1990) and Blumenthal *et al*. (1994) concluded that the reduced grain size for heat-stressed wheat is a result of a reduction in the proportion of the smaller starch granules. Jenner (1994), however, speculated that a lack of activity for the soluble starch synthase enzyme as a result of heat-stress during grain filling results in reduced grain size. KD was significantly correlated with all quality parameters evaluated with the exception of FPC (Table 3).

Kernel harness (KH)

Elands was the only cultivar evaluated that showed any response to the treatments regarding its KH. TMT 2 (at soft dough stage) produced a significant lower KH to that of the control. As was the case with KD, KH was significantly correlated with all quality parameters evaluated with the exception of FPC (Table 3).

Starch damage (SD)

No response to the various treatments was obtained by any of the cultivars evaluated (Table 2). This can be expected as SD is affected by KH (Beecher *et al.,* 2002) as harder kernels are more difficult to crush, resulting in higher levels of SD (Jolly *et al.,* 1993). SD was significantly correlated to all quality parameters evaluated with the exception of FPC (Table 3).

Canonical variate analysis (CVA)

The results of the CVA performed on the data generated are indicated in Figure 1. CVA mean scores were used to plot the nine points seen in Figure 1. These points are each a compilation of all the quality parameters investigated in the current study (i.e. HFN, SDS, FPC, TKM, KD, KH and SD). The CVA is a very useful tool within data analysis as it not only gives an indication of groupings that occur within data generated, but also provides information regarding which factor or quality investigated was most responsible for the grouping obtained.

The horizontal and vertical axes of the CVA were individually responsible for 83.13 % and 9.24 % of the variation measured. To determine which of the quality parameters investigated were the most responsible for the variation measured, the correlation between scores and the quality parameters are taken into consideration. For the current study, all the correlations were very high as well as negative for al seven quality parameters investigated [i.e. HFN (r = -0.945), KH (r = -0.771), FPC (r = -0.713), SD (r = -0.706), KD (r = -0.622), TKM (r = -0.558) and SDS (r = -0.520)] for the horizontal axis. All the parameters therefore contributed to the way the data was grouped on the horizontal axis. Of the seven parameters, HFN contributed the most as it had the highest correlation. Only HFN will therefore be indicated in Figure 1, but it should be kept in mind that the other parameters played an almost equally important role. Regarding the vertical axis, SDS was the only parameter evaluated that contributed significantly to the variation (or grouping of data) observed (r = -0.585).

According to Figure 1 a clear cultivar effect can be observed, as the various cultivars were grouped together. The general poorer performance of Gariep compared to Tugela-DN and Elands can clearly be seen, as both its control and treatments are grouped in the low HFN and low SDS quadrant of the graph.

Table 1. Mean square values for quality parameters analysed as a completely randomised block design.

Source of variation	Mean squire values						
	HFN	**SDS**	**FPC**	**TKM**	**KD**	**HD**	**SD**
Rep.Cult.TMT stratum							
Cultivar	35431.3***	80.037***	2.9544***	35.374**	0.13370**	239.32***	0.83903***
Treatment	1251.8*	21.148	0.0433	31.323**	0.13481**	45.13*	0.18840
Cultivar.Treatment	638.1	2.815	0.4628	6.381	0.02315	15.29	0.07545
CV (%)	5.1	3.0	2.9	8.5	6.5	5.5	9.4

Cult – Cultivar
TMT – Treatment
HFN – Hagberg Falling Number
SDS – Sodium Dodecyl Sulphate sedimentation volume
FPC – Flour Protein Content
TKM – Thousand Kernel Mass
KD – Kernel Diameter
KH – Kernel Hardness
SD - Starch Damage
* - P≤0.05
** - P≤0.01
*** - P≤0.001

Table 2. Average values obtained for selected quality parameters investigated.

	HFN (s)	SDS (ml)	FPC (%)	TKM (g)	KD (mm)	KH (%)	SD (%)
Tugela-DN							
Control	388.0[a]	85.33[a]	16.367[a]	28.40[a]	2.067[a]	68.27[a]	3.430[a]
Treatment 1	381.7[ab]	84.33[a]	15.700[b]	27.33[a]	1.967[a]	69.40[a]	3.387[a]
Treatment 2	357.0[b]	83.67[a]	15.900[ab]	28.20[a]	2.000[a]	65.33[a]	2.993[a]
Gariep							
Control	257.7[a]	83.00[a]	14.333[a]	28.20[a]	2.000[a]	59.97[a]	2.913[a]
Treatment 1	252.3[a]	78.67[b]	15.100[b]	21.37[b]	1.567[b]	56.27[a]	2.520[a]
Treatment 2	260.0[a]	78.33[b]	15.100[b]	25.23[a]	1.833[a]	57.80[a]	2.723[a]
Elands							
Control	308.3[a]	87.00[a]	15.267[a]	25.70[a]	1.900[a]	63.93[a]	2.860[a]
Treatment 1	264.3[b]	85.33[a]	15.467[a]	22.50[a]	1.700[a]	59.57[ab]	2.820[a]
Treatment 2	271.3[b]	84.67[a]	15.367[a]	24.57[a]	1.800[a]	55.60[b]	2.620[a]
LSD	26.62	4.253	0.7622	3.769	0.2088	5.836	0.4711
CV (%)	5.1	3	2.9	8.5	6.5	5.5	9.4

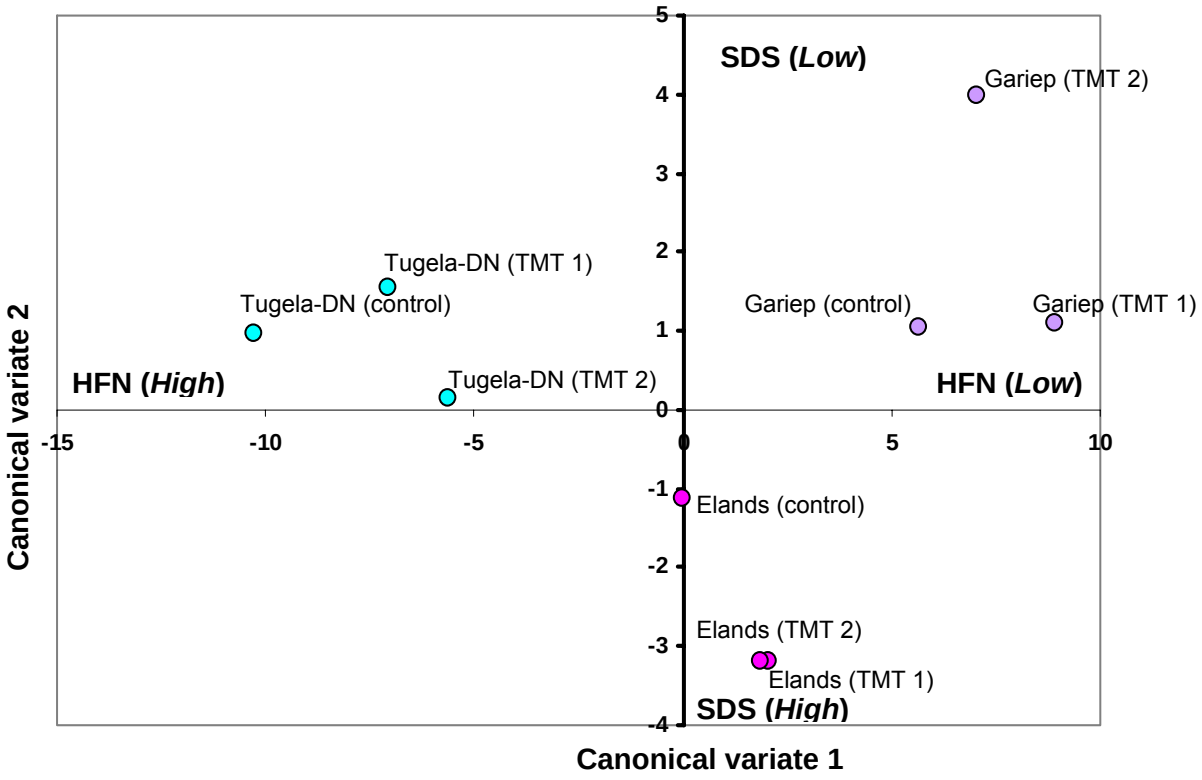
HFN – Hagberg Falling Number
SDS - Sodium Dodecyl Sulphate sedimentation volume
FPC – Flour Protein Content
TKM – Thousand Kernel Mass
KD – Kernel Diameter
KH – Kernel Hardness
SD – Starch damage
Treatment 1 - 32°C at late milk stage
Treatment 2 - 32°C at soft dough stage

Values followed by the same letter ([a. b or c]) did not differ significantly

Table 3. Correlation matrix obtained for quality parameters evaluated from wheat main tillers subjected to 32°C at individually the late milk and soft dough stage.

HFN						
SDS	0.364					
FPC	0.648***	0.079				
TKM	0.524**	0.364	0.099			
KD	0.567**	0.450*	0.148	0.968***		
KH	0.806***	0.484*	0.306	0.510**	0.604**	
SD	0.698***	0.442*	0.169	0.533**	0.529**	0.762***
	HFN	**SDS**	**FPC**	**TKM**	**KD**	**KH**

HFN – Hagberg Falling Number
SDS - Sodium Dodecyl Sulphate sedimentation volume
FPC – Flour Protein Content
TKM – Thousand Kernel Mass
KD – Kernel Diameter
KH – Kernel Hardness
SD – Starch damage
* - P≤0.05
** - P≤0.01
*** - P≤0.001



TMT 1 - Treatment 1 (32°C at late milk stage) TMT 2 - Treatment 2 (32°C at soft dough stage)

Figure 1. Canonical variate analysis indicating the impact of moderately high temperatures (32°C) at late milk and soft dough stage individually on quality parameters evaluated for Tugela-DN, Elands and Gariep.

Conclusions

Data generated mostly contradicted available literature on the effect of high temperatures during grain filling on selected quality parameters. This might partly be attributed to the treatments administered in the current study not being as extreme as that of international literature. An investigation into the effect of moderately high temperatures (32°C for four days) on the HFN of wheat indicated that such rather short and less extreme treatments did have an effect on HFN. In addition, cultivars varied in their sensitivity with regard to HFN reaction to such conditions, as Elands appeared to be sensitive at both late milk and soft dough stage. Tugela-DN showed sensitivity at only the soft dough stage with Gariep showing no response. It should, however, be noted that a rather low LSD value was used in the determination of significant differences (LSD = 26.62 s). This is a rather important observation, as HFN is known to lack stability and normally have deviations of between 30 and 40s within the same sample. Even though Gariep was not affected by the heat treatments with regard to HFN, it appeared to be the most sensitive of the three cultivars evaluated with regard to other quality parameters investigated (SDS sedimentation volume, FPC, TKM and KD). The effect is most prominent in the 32°C treatment at late milk stage. This might be an indication of a general lower tolerance to heat stress than Tugela-DH and Elands. The fact that its HFN was not affected might be attributed to an unexplained general lower HFN for the control, TMT 1 and TMT 2 being measured compared to the other cultivars included in the study. Further studies, that include SE-HPLC are, however, a necessity before accurate conclusions can be made as to the effect of heat stress on the HFN of selected cultivars.

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Chapter 7
Assessment of the effect of freezing stress on Hagberg Falling Number and selected quality parameters of wheat

Introduction

During 2002, 5.34 % of all cultivars evaluated within the cultivar evaluation programme (Small Grain Institute, Bethlehem) produced Hagberg Falling Numbers (HFNs) below the 220 s cut-off mark for grade under the current South African wheat grading regulations. Of these cultivars, 31 % contained more than 2 % sprouted grain, 38 % contained less than 2 % sprouted grain and 7 % produced low HFNs due to green kernels. In nearly a quarter of the cultivars that produced low HFNs the occurrence of the low HFNS could not be attributed to sprouting. This becomes more concerning as the 38 % of the samples that indicated less than 2 % sprouting, did not justify the low HFNs obtained (wheat with less than 2 % sprouting generally results in acceptable HFNs). An unknown factor was therefore responsible for approximately 62 % of the low HFN measured during the 2002/03 season.

No known research has investigated the effect of sudden cold spells during grain filling on the HFN of South African wheat. During the first week of October 2002, minimum temperatures of –1.8°C to as low as –4°C were measured in the Bloemfontein (Central Free State) area. Similar temperatures were measured for the Bethlehem and Frankfort (Eastern Free State) areas. These unexpected low temperatures all occurred approximately 5 to 10 days after anthesis in most of the trials (depending on the various planting dates). No visual frost damage could be observed in any of the samples.

Various researchers have reported on the effects of frost damage on wheat quality (Dexter *et al.,* 1985; Single, 1985; Preston *et al.*, 1991). These quality defects are dependant on the temperature of the frost, severity, duration and the growth stage of the plant, all of which will influence the amount of damage to the seeds in the emerged ear (Single, 1985). As the visual degree of frost damage increases, ash and colour increases, while loaf volume decreases and crumb and crust characteristics become progressively poorer. In addition, physical dough properties weaken, flour starch damage increases (therefore a decrease in HFN) and farinograph absorption increases (Dexter *et al.,* 1985).

The effect of frost on quality test results is more evident during the early maturity stages. Very high starch damage was reported above 37 % moisture, but little change in starch below 37 % (Preston *et al.,* 1991*)*. According to Tottman (1987) the soft dough stage contains approximately 50 % moisture and the hard dough stage approximately 30 % moisture. The critical stages will therefore be during the late milk, early dough, and soft dough stages, with the early hard dough stages also being subject to frost damage. A temperature below approximately –3°C is required to bring out this response and maximum response is attained over a narrow temperature range (Preston *et al.,* 1991).

The objectives of this study were 1) to create accurate climatic models for the administration of cold/frost stress conditions, 2) to investigate the effect of various cold/frost treatments during late milk and soft dough grain filling stages on selected quality parameters and 3) to establish a protocol for future screening purposes to identify cultivars prone to poor quality as a result of cold/frost stress at a specified critical stage.

Material and Methods

Temperature model

The focus of this part of the study was simulation of cold stress conditions that occur randomly at critical stages during grain filling of wheat and might, as a result, have an influence on the quality of the wheat. Such conditions do not occur annually and can be described as 'freak cold spells'. Such temperatures occurred during October 2002 in the Bloemfontein area.

Five-minute interval data (obtained from the South African weather service) of temperatures experienced in the Bloemfontein area during the months July and October of 1995 to 2002 were analysed. As limited data of the required temperature drops was available for the month of October, July was included. Nights that experienced temperatures down to –1°C and –4°C individually were used to create average temperature curves. During July (1995-2002), nine nights (data sets) experienced temperatures at or below –4°C compared to the one night logged over the same period for the month of October. (03/10/2002). The 10 data sets were combined and an average temperature curve created that indicated an average temperature at five-minute intervals over a period of 24 hours. Similar analysis was performed for nights that reached –1°C

(lowest temperature) during July and October (1995-2002). Seven data sets were identified for July and one for the month of October (02/10/2002). The eight data sets obtained were used to create a combined curve for the general temperature drop experienced when temperatures reached –1°C. Figure 1 gives the average temperature curves created for –4°C and –1°C respectively. The curves were used to create temperature models that would mimic the time period spent at the critical minimum temperatures as well as the temperature drop over a specified time period to reach the required minimum temperature. Once a temperature model was created and the required temperature settings for the frost chambers decided on, the X21 Datalogger (Campbell Sientific, Inc.) was used to determine the accuracy of the frost chambers. Temperature sensors were placed at ear level and temperatures logged at five-minute intervals over the duration of the treatment.

Wheat trial

Three wheat cultivars (Tugela-DN, Elands and Gariep) were selected to form part of the study performed at the University of the Free State. The selected cultivars also varied with regard to their capability to produce Late maturity *alpha*-amylase (LMA), with Tugela-DN having the lowest capability (12.5 %) and Gariep the highest (100 %). Elands demonstrated an 87.5 % capability to produce LMA (Anonymous, 2004). The trial consisted of four treatments (in addition to the control) each containing 20 pots per replication for each of the three cultivars. Seedlings were vernalised for a period of six weeks before being planted out in 2 l pots (two plants per pot). Pots were placed in a pseudo split plot design. Greenhouse temperatures were set at 15°C/22°C (night and day). Night temperatures, however, dropped to between 10°C and 15°C depending on the temperatures experienced outside the greenhouse, during the time period (May, June and July) the trial was conducted. Main tillers were labelled for all the treatments. Fertilizer was administered every second week (3:2:1: N:P:K, 10 g/l and 3g/l Kan: 50 ml per pot). Fungicides, pesticide as well as micronutrients were administered as required.

The main tiller of each plant was individually studied with the initiation of the treatments, to ensure that it was at the correct stage of development required for the specified treatment. The first treatment (-4°C) was administered when the main tillers were at soft dough stage, the second treatment (-4°C) when the main tillers were at late milk stage. The third treatment (-1°C) was administered when main tillers were at soft dough stage and the fourth treatment (-1°C) when main tillers were at the late milk stage. The various

treatments were administered over a nine hour period (the equivalent of one night), as the analysis of temperature data indicated that such rare cold spells seldom occur for two consecutive nights. After treatment, the plants were allowed to mature naturally in the greenhouse.

At harvest, the main tillers were harvested and threshed separately from the side tillers. The main tillers and side tillers for the various cultivars (at various treatments) were pooled (main tiller together and side tillers together) and sent for quality analysis.

Quality analysis

Quality analysis tests were performed by the quality laboratory at the Small Grain Institute (Bethlehem). Quality parameters investigated included Hagberg Falling Number (HFN: ICC no 107/1, 1995), SDS sedimentation volume (SDS: AACC 56-60), flour protein content (FPC: Determined with NIR – Near Infrared Reflectance: AACC 39-10), thousands kernel mass (TKM), kernel diameter (KD) and kernel hardness (KH). TKM, KD and KH were determined with SKCS (Sinlge Kernel Characterization System: AACC 55-31). Starch damage (SD) was determined with the use of the Starch Damage kit (Megazyme).

SE-HPLC analysis

Chromatography (SE-HPLC) was performed at the University of the Free State, to establish whether a shift in protein composition had occurred with any of the treatments. Proteins were extracted from wheat kernels with a two-step extraction procedure developed by Gupta *et al.* (1993). The following fractions were obtained with the calculation of the areas of the different peaks: (a) SDS-soluble (b) SDS-insoluble, where A = large polymeric proteins (LPP), B = smaller polymeric proteins (SPP), C = larger monomeric proteins (LMP) mainly gliadins, D = smaller monomeric proteins (SMP) mainly albumins and globulins.

The percentage of total unextractable polymeric protein (%TUPP) in the total polymeric protein was calculated. %TUPP = [(SDS-insoluble large and smaller protein polymers)/(SDS-soluble and insoluble large and smaller proteins polymers)]*100 (Gupta *et al.,* 1993).

The percentage of large unextractable polymeric protein (%LUPP) in the total large polymeric protein was calculated. Percentage LUPP = [(SDS-insoluble large protein polymer)/SDS-soluble and insoluble large protein polymers)]*100 (Gupta *et al.,* 1993).

Gliadin/glutenin ratios were determined for SDS-soluble and SDS-insoluble fractions as well as for the SDS-insoluble fractions alone:
Gliadin/glutenin ratio 1 = (LMP SDS-soluble + LMP SDS-insoluble)/(LPP SDS-soluble + LPP SDS-insoluble + SPP-soluble + SPP-insoluble)
Gliadin/glutenin ratio 2 = (LMP SDS-insoluble)/(LPP SDS- insoluble + SPP SDS-insoluble)

Statistical analysis
Data analysis was performed with the statistical program GenStat (GenStat, 2003). Data was analysed as a completely randomised pseudo split plot design, with tillers as sub-plots.

In addition, a Canonical Variate Analysis (CVA) was used to determine which of the quality parameters investigated discriminated most between the cultivar-by-treatment combinations. CVA was performed separately for main and side tillers. The CVA, also better known as linear discriminant analysis, is used when it is of more interest to show differences between groups (such as cultivar-by treatment combinations) than between individuals (Digby and Kempton, 1987).

Results and discussion

Temperature model
The frost simulation administered in frost chambers is indicated in Figure 2, as logged by the X21 Datalogger (Campbell Scientific, Inc.). The models are based on the average curves indicated in Figure 1. Five-minute interval data of the frost chambers (Figure 2) indicated that the temperature obtained within the frost chambers, was not a constant temperature, but rather fluctuated around the set temperature.

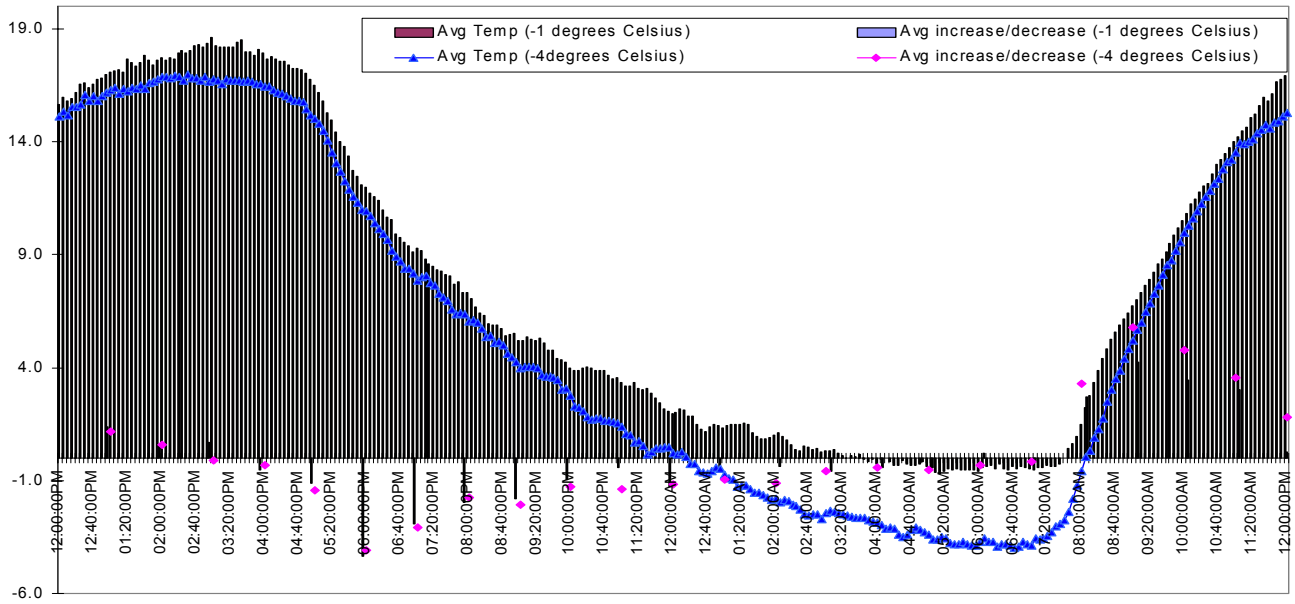

Figure 1. Average temperature curves for –1°C and –4°C frost nights experienced during the months of July and October (1995-2002) in the Bloemfontein area. Average temperature increase/decrease for each curve is indicated.

The cold stress treatments were administered over a nine hour period, that accommodated a realistic drop and rise in temperatures (2-3°C/h) as well as a 3 hour period at which temperatures stabilised in the vicinity of the minimum temperature (either –1°C or –4°C). These temperature specifications were selected based on the analysis of the two standard temperature curves.

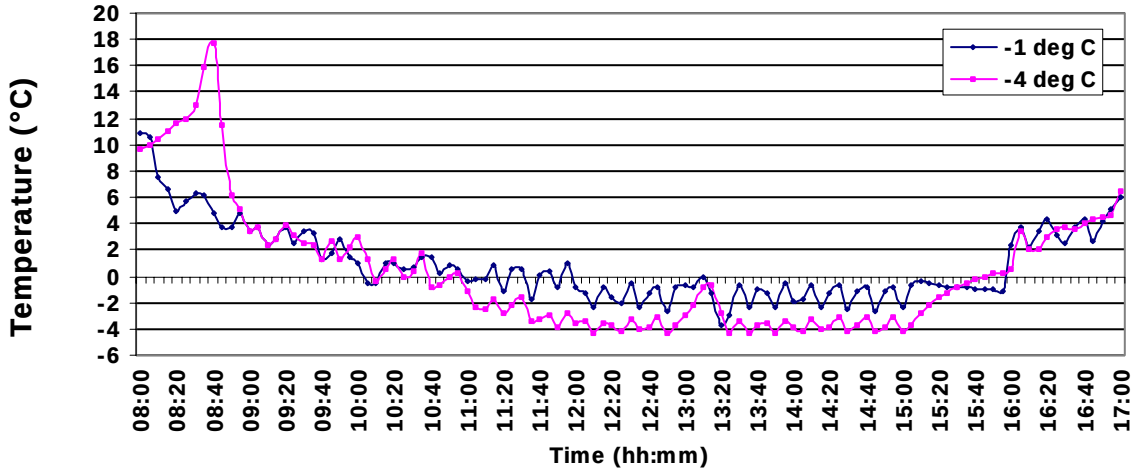

Figure 2. Temperature models used to administer -1°C and -4°C to wheat ears at selected grain filling stages.

Wheat trial

Results obtained by the ANOVA performed on quality parameters investigated are indicated in Table 1. Significant differences obtained within the various parameters evaluated are indicated in Table 2. Main and side tillers were analysed independently of each other within the various treatments (i.e. pooled main tillers for each cultivar were evaluated over the various treatments and side tillers were investigated over the various treatments).

The pseudo split plot analysis of variance indicated that the three cultivars as well as the various treatments differed significantly ($P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$) from each other for the majority of parameters evaluated (Table 1). The cultivar-by-treatment interaction was significant for starch damage (SD; $P \leq 0.01$) together with both the gliadin:glutenin ratios ($P \leq 0.01$ and $P \leq 0.05$), indicating that these quality parameters were the most affected within the various cultivars when they were subjected to the cold stress treatments. The analysis further indicates that, with the exception of kernel hardness, percentage LUPP and percentage TUPP, main and side tillers differed significantly for all the parameters investigated. The cultivar-by-treatment-by-tiller interaction was only significant for the two gliadin:glutenin ratios ($P \leq 0.001$).

Table 1. Mean square values for quality parameters analysed as a pseudo split plot design with tillers as sub-plots.

Source of variation	Mean square values										
	HFN	**SDS**	**FPC**	**TKM**	**KD**	**KH**	**SD**	**%LUPP**	**%TUPP**	**GGsi**	**GGs**
Rep.Cult.TMT stratum											
Cultivar	32687***	155.51**	3.1323*	338.967***	0.95278***	580.69***	3.8251***	84.94**	3.49	0.0735863***	0.0369522***
Treatment	15617**	338.29***	4.6084***	63.742*	0.19933*	96.11***	4.3829***	113.31**	44.60*	0.0027625	0.0045098
Cultivar.Treatment	2759	43.79	0.3822	29.54	0.07458	16.92	0.4634**	15.01	10.06	0.0060359**	0.0052866*
CV (%)	12.3	3.8	3.7	9.1	7.3	4.5	4.8	4.9	5.4	5.0	7.7
Rep.Cult.TMT.Splot stratum											
Tillers	16349**	384.40***	21.7071**	517.92***	0.961***	33.70	2.2095***	8.53	22.80	0.0491296***	0.0150479***
Cultivar.Tillers	12600**	28.93	2.0541**	232.533***	0.79633***	13.8	2.2204***	102.03*	57.36*	0.0046834**	0.0013659
Treatment. Tillers	17701***	79.62***	1.7604**	120.413***	0.26544***	49.06*	1.7405***	79.87*	35.96	0.0126043***	0.0137914***
Cultivar.TMT.Tillers	1830	23.99	0.4295	8.712	0.02286	6.23	0.2055	22.22	30.23	0.0056544***	0.0052495***
CV (%)	11.8	4.2	3.5	7.6	5.5	7.2	6.6	8.7	7.8	5.1	5.5

Cult – Cultivar
TMT – Treatment
HFN – Hagberg Falling Number
SDS – Sodium Dodecyl Sulphate sedimentation volume
FPC – Flour Protein Content
TKM – Thousand Kernel Mass
KD – Kernel Diameter
KH – Kernel Hardness
SD - Starch Damage
%LUPP – percentage large unextractable polymeric proteins
%TUPP – percentage total unextractable polymeric proteins
GGsi – sonicated and not sonicated fractions
GGs – sonicated fractions only
* - P≤0.05
** - P≤0.01
*** - P≤0.001

Table 2. Average values obtained for selected quality parameters investigated. Main and side tillers were analysed independently of each other within the various treatments.

	HFN (s)		SDS (ml)		FPC (%)		TKM (g)		KD (mm)		KH (%)		SD (%)	
	MT	ST	MT	ST	MT	ST	MT	ST	MT	ST	MT	ST	MT	ST
Tugela-DN														
Control	387.0	355.7	88.00	84.67	16.433	16.633	33.97	37.60	2.233	2.467	59.07	55.92	4.200	5.333
Treatment 1	404.0	319.7	80.67*	83.33	16.367	14.667*	26.57*	40.27	1.900*	2.600	63.63	62.50*	6.033*	5.667
Treatment 2	230.0*	364.7	78.67*	85.33	17.633*	14.667*	26.10*	39.70	1.867*	2.600	62.27	61.47*	6.067*	6.233*
Treatment 3	410.0	370.3	94.67*	86.67	16.533	14.733*	29.80	46.47*	2.100	2.900*	57.07	63.27*	5.433*	7.000*
Treatment 4	371.7	370.3	90.67	83.33	17.367	15.833	32.23	37.77	2.200	2.500	61.73	60.17	5.200*	6.900*
Gariep														
Control	332.0	267.7	82.00	72.00	16.600	15.933	27.73	20.70	1.933	1.533	50.80	45.97	3.967	4.767
Treatment 1	359.7	277.7	76.67	80.67*	15.100*	14.500*	26.33	34.40*	1.933	2.300*	54.37	56.30*	5.800*	5.400*
Treatment 2	269.0	256.0	84.00	78.67*	16.567	14.900	27.07	35.67*	1.933	2.267*	49.17	54.23*	5.400*	5.300
Treatment 3	366.3	212.7	91.33*	82.00*	15.067*	14.833	26.07	33.87*	1.900	2.233*	53.10	58.73*	5.133*	5.700*
Treatment 4	352.0	293.7	84.67	79.33*	16.233	15.167	28.80	32.53*	2.000	2.167*	48.43	53.73*	4.733*	5.600*
Elands														
Control	347.7	317.0	88.00	78.00	16.333	16.433	34.53	28.23	2.300	1.933	53.67	51.03	4.667	4.467
Treatment 1	311.3	237.7*	77.33*	72.00	15.767	15.100*	35.37	35.50*	2.333	2.267*	57.3	56.97	6.233*	5.267*
Treatment 2	195.3*	306.0	82.67	82.67	16.133	15.133*	31.37	35.97*	2.200	2.333*	54.53	55.40	5.867*	5.567*
Treatment 3	355.7	342.3	94.67*	90.00*	16.133	15.600	37.63	38.70*	2.467	2.433*	52.57	57.30*	4.933	5.500*
Treatment 4	315.7	311.7	91.33	84.67*	17.000	16.400	38.40	36.57*	2.533	2.400*	48.53	51.60	4.9000	4.567
LSD (0.05)	78.14		6.513		1.1538		5.738		0.3026		6.148		0.5947	
CV (%)	11.8		4.2		3.5		7.6		5.5		7.2		6.6	

HFN – Hagberg Falling Number
SDS - Sodium Dodecyl Sulphate sedimentation volume
FPC – Flour Protein Content
TKM – Thousand Kernel Mass
KD – Kernel Diameter
KH – Kernel Hardness
SD – Starch Damage
MT – Main tillers
ST – Side tillers
Treatment 1 - -4°C at soft dough stage
Treatment 2 - -4°C at late milk stage
Treatment 3 - -1°C at soft dough stage
Treatment 4 - -1°C at late milk stage

* - Differed significantly from control (P≤0.05)

Table 3. Average values for the four SE-HPLC parameters investigated. Main and side tillers were analysed independently of each other within the various treatments.

	%LUPP		%TUPP		GGsi		GGs	
	MT	ST	MT	ST	MT	ST	MT	ST
Tugela-DN								
Control	45.97	50.28	47.20	50.03	0.4680	0.5220	0.4387	0.4240
Treatment 1	58.04*	48.83	54.31*	45.58	0.5560*	0.5043	0.4367	0.4160
Treatment 2	50.36	40.99*	47.80	38.68*	0.6323*	0.5627	0.4980*	0.4657
Treatment 3	53.19*	50.12	48.59	44.99	0.4903	0.5520	0.4147	0.4513
Treatment 4	50.32	50.55	47.57	47.19	0.4687	0.5727	0.3670*	0.4750
Gariep								
Control	48.23	52.72	49.62	46.50	0.5057	0.6736	0.4717	0.5201
Treatment 1	55.63*	54.92	49.74	48.21	0.5763*	0.5500*	0.4070*	0.4313*
Treatment 2	49.18	50.08	46.51	46.44	0.5427	0.5190*	0.4637	0.3717*
Treatment 3	51.67	56.93	46.75	48.12	0.5377	0.5427*	0.4377	0.3997*
Treatment 4	52.61	49.96	47.15	45.71	0.4677	0.6027*	0.3333*	0.4960
Elands								
Control	41.71	54.84	43.17	53.29	0.4003	0.4639	0.3460	0.3857
Treatment 1	49.84*	49.51	47.69	49.83	0.4110	0.4945	0.3280	0.4205
Treatment 2	43.97	45.14*	46.13	45.10*	0.4597*	0.4923	0.4233*	0.3750
Treatment 3	50.10*	48.79	49.49*	43.67*	0.3854	0.5143	0.2884	0.3863
Treatment 4	49.48*	55.86	48.55	51.81	0.4620*	0.4980	0.3883	0.4120
LSD (0.05)	6.5		6.022		0.05221		0.05920	
CV (%)	8.7		7.8		5.1		5.5	

MT – Main tillersST – Side tillers
%LUPP – percentage large unextractable polymeric proteins
%TUPP – percentage total unextractable polymeric proteins
GGsi – sonicated and not sonicated fractions
GGs – sonicated fractions only
Treatment 1 - -4°C at soft dough stage
Treatment 2 - -4°C at late milk stage
Treatment 3 - -1°C at soft dough stage
Treatment 4 - -1°C at late milk stage

* - Differed significantly from control (P≤0.05)

Table 4. Average values of SDS soluble and insoluble proteins measured for three red wheat cultivars subjected to -1°C and -4°C at soft and hard dough stages individually.

	SDS soluble Proteins (%)								SDS insoluble proteins (%)							
	LPP		SPP		LMP		SMP		LPP		SPP		LMP		SMP	
	MT	ST	MT	ST	MT	ST	MT	ST	MT	ST	MT	ST	MT	ST	MT	ST
Tugela-DN																
Control	15.00	11.98	32.6	32.54	23.5	27.59	3.434	4.179	12.74	12.01	29.81	32.27	18.67	18.74	4.83	4.02
Treatment 1	10.12*	14.48*	28.8	42.56*	27.12	32.99*	4.708	4.575	14.01	13.97	32.26	34.43	20.20	20.18	3.89	4.57
Treatment 2	11.94*	14.22	34.91	41.02*	35.55*	34.49*	5.537*	4.736	12.06	9.92	30.56	25.12*	21.18	16.21	3.77	4.05
Treatment 3	11.24*	13.63	32.65	40.87*	24.71	34.58*	3.278	4.814	12.85	13.76	28.85	31.05	17.19	20.25	3.9	4.41
Treatment 4	14.26	10.56	39.30*	33.93	30.05*	29.44	4.106	4.039	14.33	10.68	33.95	28.79	17.69	18.75	4.12	4.64
Gariep																
Control	12.51	10.38	33.13	41.31	24.57	41.71	3.06	4.6	11.61	11.67	33.34	33.22	21.14	23.24	5.36	4.4
Treatment 1	10.22	11.21	38.22	44.49	36.17*	36.81*	4.66*	4.34	12.77	13.59	34.71	38.52	19.26	22.55	4.62	4.71
Treatment 2	12.35	11.56	40.62*	44.59	32.37*	36.21*	3.66	4.29	11.76	11.72	33.89	37.23	21.11	18.29*	5.44	4.34
Treatment 3	12.22	11.11	45.17*	44.35	36.00*	37.40	4.17	4.26	13.02	14.75*	37.93	36.92	22.46	20.66	6.28	5.28
Treatment 4	13.29	10.67	44.62*	43.80	34.10*	37.76	4.65*	4.43	14.99*	10.74	36.71	35.40	17.14	22.71	2.88*	6.14*
Elands																
Control	17.51	11.04	42.26	35.49	26.53	25.84	3.96	3.62	12.47	12.59	33.27	35.58	15.62	18.57	4.26	4.05
Treatment 1	13.06*	11.04	37.9	31.9	26.22	24.97	3.95	3.6	13.77	10.73	35.45	32.65	16.05	18.23	4.1	4.81
Treatment 2	14.35*	13.93	37.37	42.5*	25.19	33.33*	3.48	4.81	11.04	11.48	32.82	35.02	18.56	17.26	5.38	4.2
Treatment 3	15.10	12.83	46.27	43.15*	31.40*	34.35*	3.92	4.55	15.69*	12.23	45.24*	31.16	17.62	16.77	6.64*	4.6
Treatment 4	12.47*	9.13	37.55	34.21	26.46	25.57	3.4	2.81	12.05	11.58	34.64	35.17	18.14	19.33	5.12	5.37
LSD (0.05)	2.45		6.33		4.62		1.31		2.45		6.33		4.62		1.31	
CV (%)	11.5		10.2		11.6		19.4		11.5		10.2		11.6		19.4	

LPP – Large polymetric proteins
SPP – Smaller polymetric proteins
LMP – Larger monomeric proteins
SMP – Smaller monomeric proteins
MT – Heat tillers
ST – Side tillers
Treatment 1 - -4°C at soft dough stage
Treatment 2 - -4°C at late milk stage
Treatment 3 - -1°C at soft dough stage
Treatment 4 - -1°C at late milk stage
* - Differed significantly from control (P≤0.05)

Table 5. Correlation matrix obtained for quality parameters evaluated from wheat main tillers subjected to -1°C and -4°C cold stress conditions at individually the late milk and soft dough stage.

	HFN	SDS	FPC	TKM	Diameter	Hardness	Starch	% LUPP	% TUPP	GGsi
SDS	0.280									
FPC	-0.162	0.065								
TKM	0.071	0.384*	0.070							
KD	0.057	0.407**	0.048	0.984***						
KH	0.029	-0.133	0.138	-0.120	-0.103					
SD	-0.310*	-0.393**	-0.103	-0.176	-0.115	0.447**				
%LUPP	0.282	-0.062	-0.212	-0.254	-0.240	0.136	0.319*			
%TUPP	0.121	-0.139	-0.049	-0.148	-0.146	0.268	0.190	0.789***		
GGsi	-0.099	-0.405**	0.019	-0.709***	-0.683	0.263	0.358*	0.364*	0.217	
GGi	-0.258	-0.273	0.054	-0.529***	-0.518	0.264	0.138	0.024	0.080	0.763***

HFN – Hagberg Falling Number
SDS - Sodium Dodecyl Sulphate sedimentation volume
FPC – Flour Protein Content
TKM – Thousand Kernel Mass
KD – Kernel diameter
KH – Kernel hardness
SD – Starch damage
%LUPP – percentage large unextractable polymeric proteins
%TUPP – percentage total unextractable polymeric
GGsi – soluble and insoluble fractions
GGi – insoluble fractions
* - P≤0.05
** - P≤0.01
*** - P≤0.001

Table 6. Correlation matrix obtained for quality parameters evaluated from side tillers obtained from wheat cultivars subjected to -1°C and -4°C cold stress conditions when main tillers were at individually late milk and soft dough stage.

SDS	0.322*									
FPC	0.121	0.004								
TKM	0.477**	0.477**	-0.265							
KD	0.538***	0.481**	-0.281	0.982***						
KH	0.437**	0.353*	-0.412**	0.534***	0.583***					
SD	0.320*	0.354*	-0.528***	0.515***	0.550***	0.561***				
%LUPP	-0.532***	-0.169	0.233	-0.301	-0.323*	-0.309*	-0.346*			
%TUPP	-0.328*	-0.319*	0.377*	-0.285	-0.306*	-0.276	-0.447**	0.835***		
GGsi	-0.185	-0.188	-0.163	-0.316*	-0.271	-0.127	0.321*	-0.024	-0.232	
GGi	0.062	-0.099	-0.084	-0.112	-0.058	0.059	0.330*	-0.105	-0.207	0.824***
	HFN	SDS	FPC	TKM	Diameter	Hardness	Starch	% LUPP	% TUPP	GGsi

HFN – Hagberg Falling Number
SDS - Sodium Dodecyl Sulphate sedimentation volume
FPC – Flour Protein Content
TKM – Thousand Kernel Mass
KD – Kernel diameter
KH – Kernel hardness
SD – Starch damage
%LUPP – percentage large unextractable polymeric proteins
%TUPP – percentage total unextractable polymeric
GGsi – soluble and insoluble fractions
GGi – insoluble fractions
* - P≤0.05
** - P≤0.01
*** - P≤0.001

Hagberg Falling Number (HFN)

Main tillers of both Tugela-DN and Elands produced significantly lower HFNs, compared to that of the controls, when subjected to –4°C for 3 hours at late milk stage (Table 2). A slightly reduced effect (not significant) was observed in the main tillers of Gariep with no effect being measured in the side tillers. Elands, however, managed significantly poorer HFN than the control in the side tillers at –4°C at soft dough stage. A similar, but not significant effect, was observed for the Tugela-DN side tillers. The decline in HFN observed within the side tillers for Elands and Tugela-DN support the observation of the effect of –4°C at late milk stage of wheat, as the side tillers were most likely close to or at late milk stage with TMT 1 (-4°C at soft dough stage of main tillers). Tugela-DN and Elands appeared to be more sensitive to sudden cold spells, with regard to HFN, than Gariep. It should, however, be stressed that the effect was observed in the main tillers only and that the side tillers were most affected at the –4°C treatment when the main tillers were at soft dough stage.

Preston *et al*. (1991) reported no visible effect of frost and maturity stage on HFN. Dexter *et al.* (1994) however indicated a continuous decline ($P \leq 0.05$) in HFN as the CWAD (Canadian Western Amber Durum) grade wheat, assigned on basis of frost damage and immaturity against primary standard samples, declined. Dexter *et al*. (1985) observed similar trends for hard red winter wheat, which they attributed to the relatively high level of *alpha*-amylase activity present in immature wheat kernels. This observation could not be confirmed with the current study, as insufficient amounts of flour were available for *alpha*-amylase determination. The current study indicates that cultivar response differed with regard to HFN performance when subjected to cold stress, but also that the effect was mainly limited to main tillers. Not enough seed was available to determine the effect of the main and side tillers combined and it can only be speculated whether the side tillers with normal HFNs would be able to increase the HFN of the plant as a whole by limiting the effect of the main tillers. Past research has indicated that a very small amount of low HFN wheat (6 %) will be able to reduce the HFN of a sound wheat sample to below acceptable levels. This, however, applied to sprouted wheat samples (>2 % sprouted kernels). In addition it will be difficult to link LMA to the observations made in the current study as Tugela-DN has a general low LMA producing capability (12.5 %), but indicated the similar strong HFN reaction to that of Elands with high LMA producing capability (87.5 %). As it is the temperature range of 17°C day and 13 °C night that appears to induce LMA production

(Mrva and Mares 2001), the controls, as well as the -1°C treatments of the current study should also have produced low HFNs.

SDS sedimentation volume

The general response obtained by the main tillers was similar for all three cultivars over the various treatments, with the degree of response varying. Side tiller response varied over treatments and cultivars. Tugela-DN main tillers produced significantly poorer SDS sedimentation volumes with TMTs 1 and 2 (-4°C at soft dough and late milk stage) compared to the control, with the side tillers not affected to the same extent (Table 2). Gariep indicated a more pronounced effect in the side tillers, as all the treatments gave significantly higher SDS sedimentation volumes than the control. Significantly lower SDS volumes were obtained by the main tillers of Elands with TMT 1 (-4°C at soft dough stage) compared to that of the control. Both the main and side tillers gave significantly higher SDS volumes at TMT 3 (-1°C at soft dough stage).

The SDS sedimentation test is normally used to estimate flour strength (Preston *et al.,* 1991). No correlation between SDS-sedimentation and degree of frost damage could be found by Dexter *et al.* (1994). Preston *et al.* (1991), however, reported that SDS sedimentation values (at –5°C treatments) were much lower than that of the controls during the earliest stages of grain filling (1-8, 9-12 and 13-16 days after anthesis, approximately >40% kernel moisture content), but also that significantly lower SDS sedimentation values were produced for 17-24 days after anthesis, similar to what has been found with the current study with the two cultivars, Tugela-DN and Elands at TMT 1 and 2 (-4°C at soft dough and late milk stage).

Flour protein content (FPC)

The response varied between the three cultivars evaluated. Main tillers of Tugela-DN gave significantly higher FPC at TMT 2 (-4°C late milk stage) compared to the control, with the side tillers of TMTs 1, 2 and 3 giving significantly poorer FPC. Gariep main tillers gave significantly lower FPC with TMT 1 and 3 (-4°C and –1°C at soft dough stage respectively). Side tillers were negatively affected at TMT 1. Elands had no visible effect in the main tillers but its side tillers produced reduced FPC with TMTs 1 and 2 (-4°C at soft dough and late milk stages).

FPC response to frost conditions appears to be more pronounced in the main tillers of Gariep at soft dough stage (-1°C and –4°C). With both Tugela-DN and Elands the effect was, however, more pronounced in the side tillers, of especially the –4°C treatments. Tipples (1980) showed that wheat protein content is relatively stable once the wheat kernel desiccates to 50 % moisture, explaining the effect observed in the side tillers of Tugela-DN and Elands in the current study. Similarly, Preston *et al.* (1991) reported significantly lower flour protein content, as well as other protein related parameters that included wheat protein and wet gluten content at the early stages of kernel maturity (up to 16 days maturity i.e. approximately at higher than 37 % kernel moisture content). The reason for the poorer FPC observed with Gariep at –4°C and –1°C at the soft dough stage is, however, not clear. Cultivar sensitivity at the mentioned treatments might be an explanation for the observation.

Thousand kernel mass (TKM)

Again the response measured varied between the cultivars and treatments. Cold stress treatment of –4°C at both late milk and soft dough stage had a negative effect on the TKM of Tugela-DN main tillers. According to Marcellos and Single (1984) and Single (1985), ice nucleation that occurs below –4°C causes disruption of immature seed cell membranes and the tracheary element of the rachis and rachilla, where translocation of nutrients from vegetative tissue to the growing seed would occur, resulting in reduced kernel mass. Both Gariep and Elands had no significant effect on the main tillers, with the side tillers of all four treatments giving significantly higher TKM. Dexter *et al.* (1985) reported a decrease in kernel weight as the degree of frost damage increased. Preston *et al*. (1991) reported a larger effect as kernel weight was strongly affected by frost at the most immature stage of wheat development. Allen *et al.* (2001) reported similar results. The increase in TKM observed in the side tillers of Gariep and Elands at all the treatments was unexpected and the reason for the response not clear.

Kernel diameter (KD)

A similar response was obtained for diameter as measured for TKM for all three cultivars, indicating that TKM and KD are highly correlated (Table 5 and 6).

Kernel hardness (KH)

The effect of the treatments on KH was most prominent in the side tillers of Tugela-DN and Gariep. The side tillers of Tugela-DN gave significantly higher KH with TMTs 1, 2 and 3,

with Gariep also experiencing higher levels of KH at all four treatments. Elands did not manage the same result, as only the side tillers of TMT 3 showed increased KH. Both Dexter *et al.* (1985) and Preston *et al.* (1991) demonstrated that KH of wheat increased with an increase in the degree of frost damage that resulted in decreased first break release and increased energy requirements.

KH refers to whether the endosperm is physically hard or soft (Giroux and Morris, 1998) and therefore gives an indication of the kernel texture. Harder kernels are difficult to crush, resulting in higher levels of SD (Jolly *et al*., 1993), and explains the significant correlation observed between KH and SD in both the main and side tillers ($P \leq 0.01$ and $P \leq 0.001$ individually). International reports on correlation studies between KD and KH show varying results (Chung, 1972; Fowler and De la Roche, 1975; Williams *et al.*, 1987; Pomeranz *et al.,* 1988). With the current study, no correlation was seen between KH and KD within the main tillers evaluated. A positive correlation was, however, present between the two parameters with the side tillers.

KH together with kernel weight is known for their sensitivity to maturity and frost (Tipples, 1980; Dexter *et al*., 1985). As the effect of the various treatments on both KH and kernel weight was most prominent in the side tillers, it can be assumed that the phenomenon of increased weight in the side tillers (TKM) can be attributed to the effect of cold stress.

Starch damage (SD)

Both Tugela-DN and Gariep were significantly affected by all four of the cold stress treatments, in especially the main tillers, with Elands' main tillers only being affected by TMT 1 and 2. Tugela-DN appears to be the most sensitive of the three cultivars (Table 2). TMTs 1 and 2 gave significantly higher SD in the main tillers than that of both the control and TMTs 3 and 4, but TMT 3 and 4 also measured significantly higher SD than that of the control, but to a lesser extent than that of TMTs 1 and 2. Gariep's main tillers were also negatively affected by all four the treatments. Elands' main tillers were only significantly affected by TMT 1 and 2 (i.e. at –4°C at late milk and soft dough stage). The percentage SD was higher in the side tillers in most of the treatments of all three cultivars compared to the control.

Preston *et al.* (1991) reported that flour SD was much higher ($P \leq 0.05$) at –5°C than for the corresponding controls during early maturity (>37 % kernel moisture content) stages. The

current study partly confirms the findings of Preston *et al.* (1991), but also indicates that cultivar response differs. This study also indicates that SD after milling might be altered by temperatures as low as –1°C, depending on cultivar sensitivity to cold stress. Dexter *et al.* (1985) indicated that increased KH led to dramatically increased flour SD. This is reflected in the current study with the significant correlation observed (P≤0.01) between KH and SD (Table 5) even though the current study focused on material that did not have the same extreme levels of frost damage reported on by Dexter *et al*. (1985). In addition to KH, SD was also significantly negatively correlated to HFN (P≤0.05; Table 5).

Percentage Large Unextractable Polymeric Proteins (% LUPP)

The percentage LUPP observed for the main tillers was significantly higher that the control at TMT 1 and 3 for Tugela-DN, TMT 1 for Gariep and TMT 1, 2 and 3 for Elands (Table 3). The increases observed can mostly be attributed to a reduction in LPP (soluble) fractions (Table 4). A reduction in percentage LUPP was observed within the side tillers of Tugela-DN and Elands at TMT 2, that might be attributed to a reduction in LPP (insoluble) fractions within Tugela-DN, with an increase in LPP (soluble) fractions in the case of Elands. Cold stress treatments (-1°C and –4°C) at soft dough stage therefore resulted in an increase in percentage LUPP in two of the three cultivars evaluated (Tugela-DN and Elands), with Gariep only being positively affected at –4°C soft dough stage (main tillers).

Percentage LUPP determined within the main tillers was positively correlated to percentage SD (P≤0.05), percentage TUPP (P≤0.001) and GGsi ratio (P≤0.05; Table 5). Within the side tillers (Table 6) correlations were observed, that were not present within the main tillers, indicating the effect of physiological growth stage of the kernel at the various treatments on the factors investigated. The percentage LUPP was significantly negatively correlated to HFN (P≤0.001), diameter (P≤0.05) and SD (P≤0.05), and positively correlated to percentage TUPP (P≤0.001).

Percentage Total Unextractable Polymeric Proteins (%TUPP)

Cultivar response varied. Tugela-DN and Elands gave significantly higher percentage TUPP at different treatments (TMT 1 and TMT 3 individually – Table 3). In each of the two cases, a decrease in LPP (soluble) fractions might be responsible for the increase in percentage TUPP observed. The reduction in percentage TUPP observed in the side tillers of Tugela-DN and Elands is attributed to the increase in SPP (soluble) fractions.

Gliadin:glutenin ratio - soluble and insoluble fractions (GGsi ratio)

A significantly higher gliadin:glutenin ratio than that of the control was obtained with the main tillers of Tugela-DN at TMTs 1 and 2 (-4°C at soft dough and late milk stage) with Gariep producing a higher ratio at TMT 1. In both cases general higher LMP soluble fractions (gliadins) together with reduced LPP soluble fractions (low polymeric glutenins) might be responsible for the ratio shift. Elands indicated a shift in the GGsi ratios for TMT 2 and 4 (-4°C and –1°C late milk stage) as a result of reduced LPP soluble fractions (Table 4). These findings are contradictory to the findings by Dexter *et al.* (1994) who reported significantly lower proportions of gliadins in wheat with extreme degrees of frost damage. The material used in the current study, however, do not have the extreme levels of frost damaged as those reported on by Dexter *et al*. (1994). Dexter and Dronzek (1975), however, indicated that gliadins synthesis is very rapid up to about a week before physiological maturity and also that gliadins and glutenins are synthesised at different rates (Huebner *et al.,* 1990). Severe damage as a result of severe or killing frost before the final stages of development will result in significant reduction or more probably termination of gliadins and glutenin synthesis. The ratio of gliadins to glutenins can therefore be significantly influenced, depending on the developmental stage at which the frost damage occurs (Dexter *et al.,* 1994). The answer to the increase in gliadins may partly lie in research performed by Stone and Nicolase (1996), which indicated that gliadin is less temperature sensitive than most of the other remaining protein fractions (especially SDS-insoluble polymer and albumin/globulin). Only Gariep side tillers indicated a significant reduction in the ratio as a result to a reduction in the LPM soluble fractions together with an increase in the SPP soluble fractions (Table 3).

The GGsi ratio was negatively correlated to SDS ($P \leq 0.05$) and TKM ($P \leq 0.001$) of the main tillers (Table 5).

Gliadin:glutenin ratio (insoluble fractions – GGi ratio)

The general trend observed differed slightly from the GGsi ratio. An increase in the ratio was observed within the main tillers of Tugela-DN for TMT 2 with a reduction observed in TMT 4. A reduction in the ratio was observed for both TMTs 1 and 4 of Gariep main tillers, with an increase observed in TMT 2 for Elands. Compared to the SDS soluble proteins, limited statistical significant differences were obtained from the SDS insoluble proteins. The reported shifts in the GGi ratios are therefore attributed to subtle changes in all four protein fractions.

As can be expected, the GGi ratio within the main tillers was highly correlated (P≤0.001) with the GGsi ratio. The ratio for insoluble fractions was, however, also correlated to TKM (P≤0.001) but not to SD, indicating that the correlation observed between the GGsi ratio and SD is based on the shifts, or the lack of it, within the soluble fractions of proteins (Table 5).

Canonical variate analysis (CVA)

The results of the CVA performed on the main tillers of the various cultivars at the various treatments are indicated in Figure 2. Fifteen points were plotted according to the CVA mean scores of which each is a compilation of all the quality parameters investigated in the current study (i.e. HFN, SDS, FPC, TKM, KD, KH, SD, percentage LUPP, percentage TUPP, GGis ratio and GGi ratio). The CVA gives an indication of groupings within data generated as well as which factor or quality investigated was responsible for the grouping obtained.

The horizontal and vertical axes of the CVA were individually responsible for 73.07 % and 18.86 % of the variation measured. SD was responsible for most of the variation measured within the main tillers on the horizontal axis, with the GGi ratio responsible for most of the variation on the vertical axis. Together these two parameters explained 91.93 % of the variation measured.

Figure 2 indicates the grouping of the various treatments from right to left on the horizontal axis as control, TMT 4, TMT 3, TMT 2 and TMT 1, indicating that the SD increased from the control to TMT 1 (-4°C in the soft dough stage). Vertically, the cultivars were grouped according to their GGi ratio, indicating that Elands generally had a lower GGi ratio than the trial average for all the treatments, as all the allocated points are above the zero line. Most of the points for Gariep and Tugela-DN falls below the vertical zero line indicating a higher than average GGi ratio for all the treatments (with the exception of TMT 4).

From CVA analysis it is possible to determine which of the treatments differed the most from the control for each of the cultivars individually. By adding the means of each of the horizontal and vertical axis for the individual point on the CVA, a numerical value is allocated to each point which will be referred to as the plot value (PV). The more negative the PV, the poorer the general performance of the cultivar at the specific treatment. For Tugela-DN, TMT 2 (-4°C at late milk stage) produced the lowest value (-8.071) compared

to the control (4.401). For Gariep, TMT 1 (-4°C at soft dough stage) resulted the lowest PV (poorest performance -5.493) compared to the control (2.605). TMT 2 (-4°C at late milk stage) produced the lowest value (-0.102) compared to the control (5.585) for Elands.

The CVA for the side tillers is indicated by Figure 3. The horizontal and vertical axes of the CVA were individually responsible for 45.99 % and 28.69 % of the variation measured. Together these two parameters explained 74.68 % of the variation measured. The lower percentage obtained by the side tillers (compared to the main tillers), is an indication that the quality parameters investigated each contributed to a larger extent to variation observed than was the case with the main tillers where most of the variation observed were as a result of SD and the GGi ratio. According to the analysis KD was responsible for most of the variation measured within the side tillers on the horizontal axis, with the GGsi ratio responsible for most of the variation on the vertical axis.

From Figure 3 the observation is made that the cultivars appeared to be grouped together, opposed to being grouped according to treatments as was observed with the CVA of the main tillers. Similar to the main tiller CVA, the values of the vertical and horizontal axis values were added to determine which of the treatments had the most negative impact on the wheat quality. TMT 1 (-4°C at soft dough stage) resulted in the poorest performance within Tugela-DN as it had the most negative value, with treatment 3 (-1°C at soft dough stage) producing the poorest performance within Gariep. As for Elands, none of the treatments performed poorer that the control as all the values were very similar to that of the control, indicating that the side tillers generally performed similar or slightly better than that of the control at the various cold stress treatments.

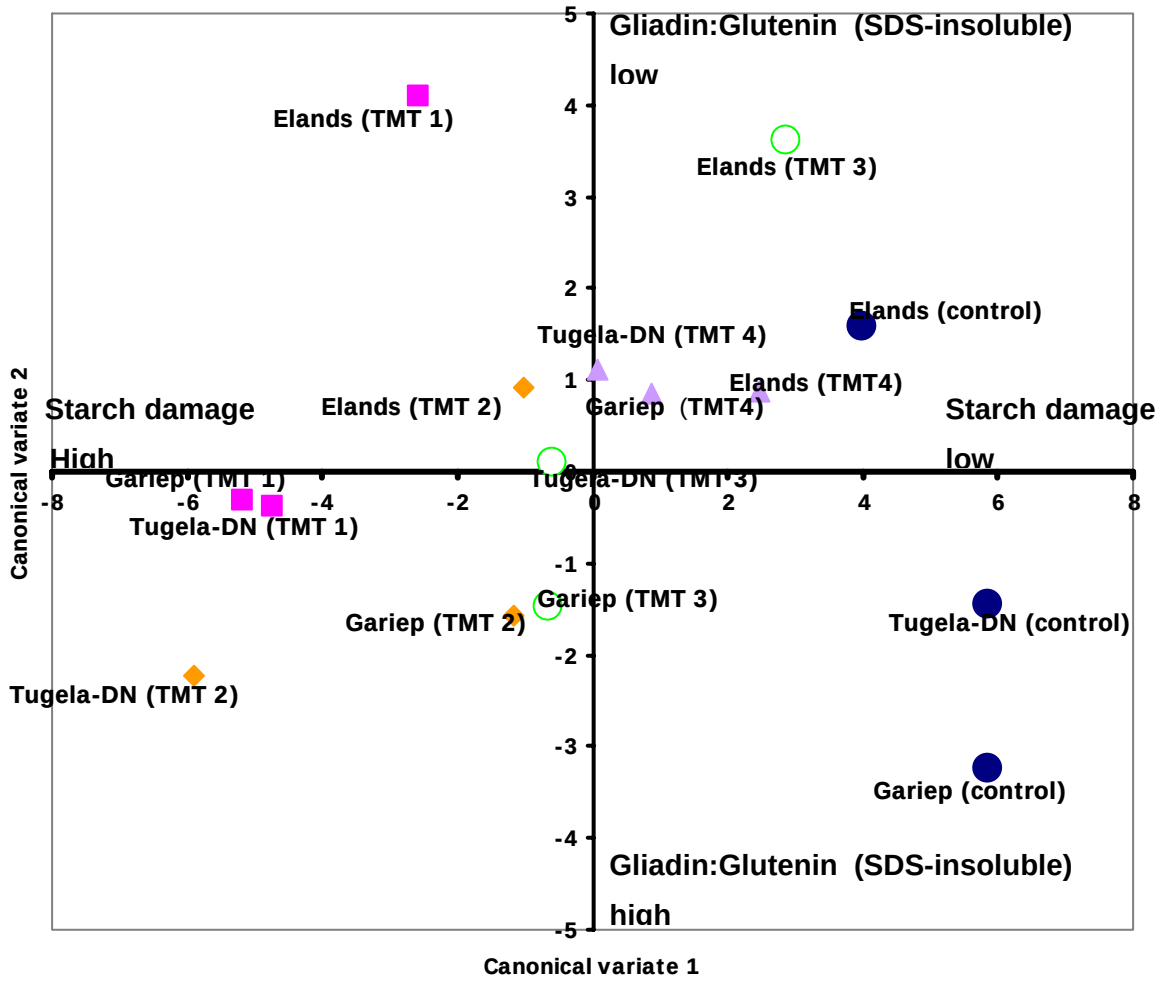

Figure 3. Canonical variate analysis indicating the impact of cold stress temperatures (-1°C and -4°C) at late milk stage and soft dough stage individually on the main tillers of Tugela-DN, Gariep and Elands (TMT 1 = -4°C at soft dough stage; TMT 2 = -4°C at late milk stage; TMT 3 = -1°C at soft dough stage; TMT 4 = -1°C at late milk stage).

Plot value = X-axis value + Y-axis value

Tugela-DN control = 4.401	Gariep control = 2.605	Elands control = 5.585
Tugela-DN TMT 1 = -5.123	Gariep TMT 1 = -5.493	Elands TMT 1 = 1.515
Tugela-DN TMT 2 = -8.071	Gariep TMT 2 = -2.745	Elands TMT 2 = -0.102
Tugela-DN TMT 3 = -0.497	Gariep TMT 3 = -2.13	Elands TMT 3 = 6.488
Tugela-DN TMT 4 = 1.177	Gariep TMT 4 = 1.713	Elands TMT 4 = 3.346

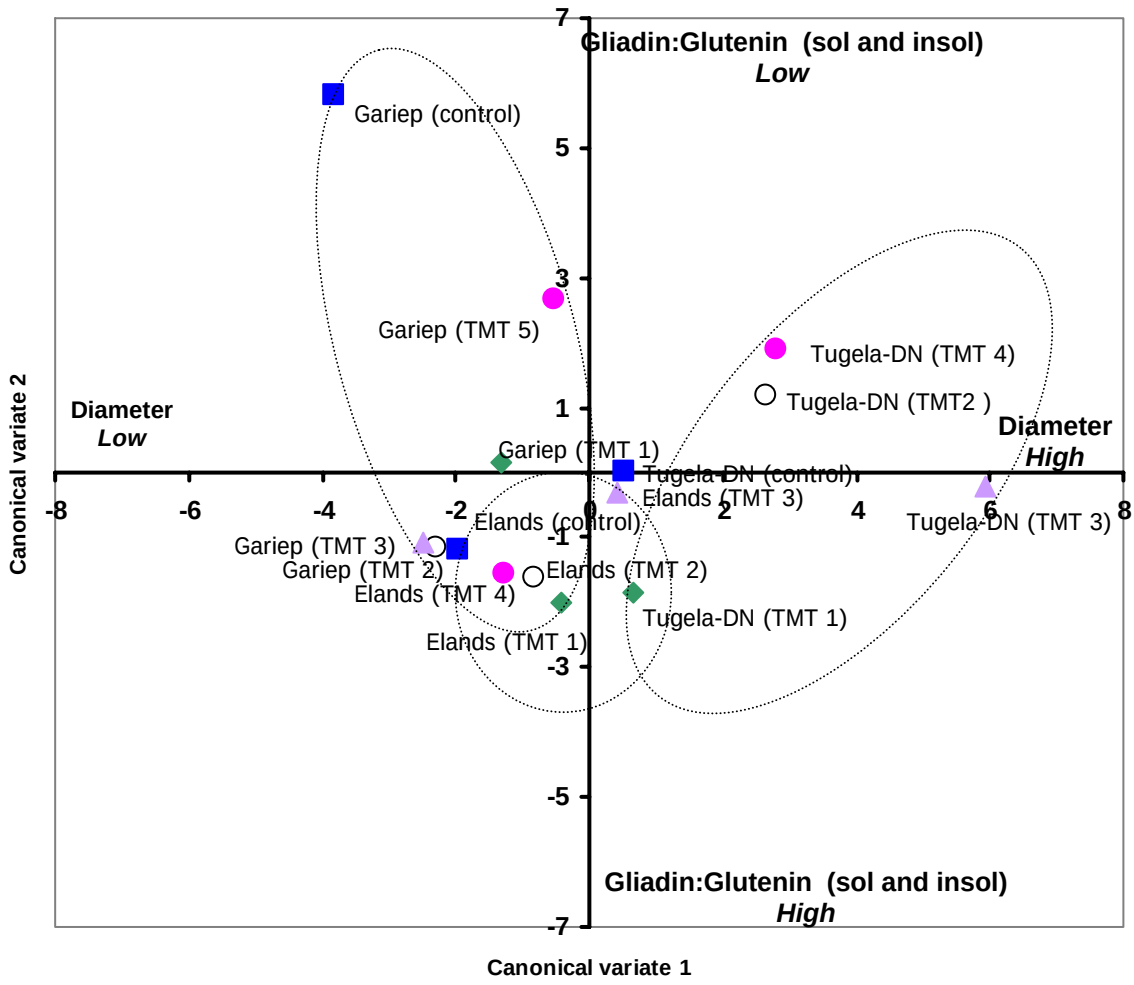

Figure 4. Canonical variate analysis indicating the impact of cold stress temperatures (-1°C and -4°C) at late milk stage and soft dough stage individually on the side tillers of Tugela-DN, Gariep and Elands (TMT 1 = -4°C at soft dough stage; TMT 2 = -4°C at late milk stage; TMT 3 = -1°C at soft dough stage; TMT 4 = -1°C at late milk stage).

Plot value = X-axis value + Y-axis value

Tugela-DN control = 0.528	**Gariep control = 2.009**	**Elands control = -3.144**
Tugela-DN TMT 1 = -1.173	**Gariep TMT 1 = -1.156**	**Elands TMT 1 = -2.407**
Tugela-DN TMT 2 = 3.826	**Gariep TMT 2 = -3.467**	**Elands TMT 2 = -2.437**
Tugela-DN TMT 3 = 5.704	**Gariep TMT 3 = -3.587**	**Elands TMT 3 = 0.116**
Tugela-DN TMT 4 = 4.716	**Gariep TMT 4 = 2.126**	**Elands TMT 4 = 2.834**

Conclusions

The primary aim of the current study was to determine the influence of sudden cold spells during grain filling of wheat on the quality traits of three South African hard red wheat cultivars. Secondly it was attempted to establish a screening protocol for such conditions that could be used to enhance current wheat breeding programmes.

Treatments were administered over a nine hour period, with temperatures remaining at the set minimum temperature for a three hour period as to simulate one night of frost. This was done in accordance to an average temperature curve created out of selected nights as they occurred over a seven year period where temperatures reached –1°C and –4°C respectively. This, however, created difficulty in comparing the data generated to that of international literature as results obtained had to be compared to research with wheat being treated over a 28 day period (Preston *et al.*, 1991). Nonetheless, statistical significant effects were observed that in most cases concurred with international studies of severe frost damaged material.

Of the three cultivars included in the study, quality traits of Tugela-DN were the most affected. Pseudo split plot analysis of variance indicated that with Tugela-DN, HFN, SDS sedimentation volume, TKM, KD as well as SD were negatively affected by the –4 °C at late milk stage treatment. This effect was, however, in most cases limited to the main tillers. HFN (main tillers), FPC (side tillers) and starch damage (main and side tillers) was negatively affected by the –4°C treatments at late milk stage in Elands. SD (in both main and side tillers) and FPC (main and side tillers) were negatively affected with Gariep, mostly at the –4°C and –1°C soft dough stage treatment. The cultivars therefore differed in their response to the various treatments but also with regards to quality traits most affected.

According to the CVA performed on the main tillers of the three selected cultivars, SD and the GGi ratio were the two quality traits most affected by the various cold stress treatments. With the creation of plot values with the use of the CVA axis values, it was concluded that a -4°C treatment at late milk stage resulted in the poorest quality performance in both Tugela-DN and Elands. In the case of Gariep, a –4°C treatment at soft dough stage would result in poor quality being obtained.

A separate CVA conducted on side tillers, however, indicated that DM and GGsi ratio were the quality factors most affected by the treatments. The results obtained differed completely for that of the main tillers, which can be expected, as the side tillers would be at a younger physiological stage that the main tillers. It is, however, not certain why a general increase in the KD of the kernels was measured compared to the control. Plot values indicated that a –4°C treatment at soft dough stage had the most significant effect on the quality of Tugela-DN kernels. A –1°C treatment at soft dough stage had the most significant effect on the side tillers of Gariep, with the side tillers of Elands not showing any difference to the control.

Under South African climatic conditions, frost conditions that occur during grain filling stages of wheat, are very rare. When they do occur, ideal conditions for frost are limited to one night only. This study confirmed that such conditions, although limited in duration, did have an effect on the quality of three South African wheat cultivars. As can be expected, main and side tillers differed in their response to the various treatments, as they were at different stages of development at the time of treatment. For protocol purposes, starch damage and GGs ratios might be considered as screening parameters. HFN should also be included as it is not only a fast screening method but it was also the only quality trait evaluated that produced values that would have an influence on the grading of the wheat samples. It also confirmed that the low HFNs measured during the 2002 season in the Bloemfontein area might have been the result of sudden cold stress that occurred during early grain filling stages. A minimum temperature of –4°C at late milk stage should be used as protocol for screening of cultivars that might be prone to frost/cold stress conditions as this treatment produced significant differences in quality traits evaluated in two of the three cultivars included in the study.

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Chapter 8
General discussion

It has previously been determined that the majority of the commercially available South African wheat cultivars, with the exception of SST 363, SST 124 and Tugela-DN, are highly prone to Late maturity *alpha*-amylase (LMA) production. Although Tugela-DN is no longer commercially available, it has, however, been widely incorporated into South African wheat breeding programmes. It would therefore be interesting to determine the LMA status within the South African gene pool. It, however, remains a concern that a large amount of South African cultivars are prone to LMA production and the resultant problems associated with reduced Hagberg Falling Numbers (HFNs). It can therefore not be denied that the most probable cause of unexplained HFN can be attributed to LMA. International literature has, however, reported that HFN is affected by various factors other than cool weather, which induces LMA production. The question therefore remains whether HFN can be successfully managed within the boundaries set by the LMA status of South African material?

The aim of the current study was to investigate various agricultural practices by producers that might explain the reduced HFNs that are obtained in the absence of pre-harvest sprouting, and accordingly to determine whether HFN could be managed through specific management practices. Data generated in the current study, however, suggested that cultivar response couldn't be ignored within such agricultural practices.

Agricultural practises

The first logical approach to this study was to investigate the effect of early kernel termination (i.e. early harvest) on the final HFN obtained. In fear of late rain that might hamper the harvesting process, producers harvest their wheat at high kernel moisture content (KMC). As 'green *alpha*-amylase' could still be present in the kernels at high kernel moisture content, reduced HFNs in the absence of sprouted wheat might be obtained. Data generated with three cultivars (Elands, Gariep and Tugela-DN) over a four year period indicated that the effect of KMC with harvest on the HFN of wheat was, to a large extent, dependant on the season. The observation was, however, made that although not statistically significant, most of the cultivars produced higher HFNs at the lower KMC regimes. The grouping of data into two KMC regimes therefore probably created a masking effect that resulted in no subsequently statistical significance being

indicated. During the 2003/04 season, evaluation of SST 966 produced a HFN below the 220s cut-off mark for grade when evaluated at 15-15.9 % KMC, confirming the danger associated with harvesting at high KMC. An additional observation was made with regard to the existence of 'high' and 'low' HFN seasons. Clear differences in the HFN performance of the various cultivars could be seen over the various seasons. The HFNs measured for the individual cultivars during the 2004/05 season were higher than those of the 2003/04 season. This could explain why farmers can harvest their material early at high KMC, season after season without any HFN problems, as those seasons are most probably 'high' HFN seasons. A reduction in HFN due to high KMC would therefore not be noticed. With a low HFN season, HFNs are already rather low to begin with. Should a farmer therefore harvest early, the reduction effect brought about by the early harvest practise would be noticed, and suddenly a low HFN is obtained without any visual sprouting. The effect of *alpha*-amylase activity with reference to both *alpha*-AMY 1 (and more specific LMA) and *alpha*-AMY-2 at the KMC investigated in the study could unfortunately not be successfully linked to reduced HFN.

Second to unexplained low HFN, the lack of repeatability of the HFN test is most complained about by wheat producers. Most researchers agree that this phenomenon is mostly attributed to the presence of *alpha*-amylase activity in green kernels. Should a way therefore be found to dry the whole wheat plant with its side tillers simultaneously to a set moisture content, *alpha*-amylase activity that might be present in late tillers could be reduced. A study was accordingly conducted to investigate the possibility of managing HFN by creating more homogenous wheat through glyphosate applications, without compromising wheat quality. The effect of the glyphosate treatments on wheat at various stages of grain filling (soft and hard dough) was accordingly investigated. The effect of the treatments varied with regard to the various seasons. Thousand kernel mass (TKM), hectolitre mass (HLM) and protein content were some of the quality parameters that were negatively affected by the treatments at especially the soft dough stage. As can be expected days to harvest (DH) as well as growing degree days (GDD) were significantly reduced with the soft dough treatment compared to the control for all three cultivars included in both seasons of evaluation. Yield, *alpha*-amylase activity (AA) and pre-harvest sprouting (PHS) were not affected. HFN measured for Tugela-DN treated at soft dough stage produced a significantly higher HFN compared to the control (2004/05). According to literature this might be attributed to the enhanced drying rate. None of the other cultivars at the various treatments indicated any HFN response to the glyphosate application. Germination was severely affected by the soft dough stage glyphosate

application in the first year of evaluation. Studies into the effect of the various treatments on the repeatability of the HFN test, indicated that glyphosate treatment managed to produce a certain amount of stability for two of the three cultivars, but the treatment that resulted in the stability varied between seasons. The glyphosate treatment at hard dough stage resulted in a more stable HFN during the first season for Gariep and Tugela-DN. In the second season of evaluation, glyphosate application at soft dough stage resulted in a more homogeneous wheat sample for Gariep and Tugela-DN. Both cultivar and environment therefore had an effect on the glyphosate treatments. The current study therefore indicated that the practice of glyphosate application would hold few advantages within a wheat production system as the optimum application stage for a more stable HFN varied between seasons. In addition is the success of the applications linked to cultivar choice, as Elands remained unresponsive to the treatments regarding its HFN stability, compared to Tugela-DN and Gariep that showed a positive reaction.

Various researchers speculated that several cultivars performed poorly regarding HFN, due to insufficient nitrogen availability. To test this hypothesis, 15 South African wheat cultivars were subjected to evaluation for their HFN response to various degrees of fertilizer application. No statistical significant effect on the HFN of wheat in general could be seen. Cultivar differences did, however, occur that allowed for the individual effect of fertilizer on the HFN of these cultivars to be identified. This allowed for the grouping of cultivars into four response groups namely low, low to medium, medium and high response cultivars. Classification was refined with the use of a CVA (Canonical Variate Analysis) that included the HFN, yield and protein response to fertilizer application. HFNs measured were, however, never below the 220s cut-off mark for grade. The research therefore indicated that crops are unlikely to suffer reduced HFNs (i.e. 220 s), due to insufficient plant nutrition. In addition, the risk of reduced HFNs is minimized by the fact that the current level of fertilization followed by most commercial farmers is aimed at yield and protein content. The grouping of the cultivars should, however, be used as guidelines for areas that are prone to leaching. Of importance here is the possibility of a high HFN season (as already discussed). The complex interactions within the environment determines what type of HFN will be experienced. If a lack of sufficient nitrogen due to leaching is brought into the equation, a cultivar that falls into the high response group (i.e. shows a high HFN response to fertilizer application), might produce an even lower HFN than the low response group cultivars under the combined effect of insufficient nitrogen and a general low HFN season .

The effect of moderately high temperatures on wheat quality was investigated. Again Elands, Gariep and Tugela-DN were exposed to four days of 15/32°C at both late milk stage and soft dough stage. Results obtained indicated that such rather short and less extreme treatments did have an effect on HFN. In addition cultivars varied in their sensitivity with regard to HFN reaction to such conditions, as Elands appeared to be sensitive at both late milk and soft dough stage. Tugela-DN showed sensitivity at only the soft dough stage with Gariep showing no response. It should, however, be noted that a rather low LSD value was used in the determination of significant differences (LSD = 26.62 s), compared to the LSD value used for the frost treatments of 78.14 s. This is a rather important observation, as HFN is known to lack stability and normally have deviations of between 20 and 30s within the same sample.

Under South African climatic conditions, frost that occurs during grain filling stages of wheat, is very rare. When it does occur, ideal conditions for frost are limited to one night only. A study into the influence of such sudden cold spells during grain filling of wheat on the quality traits of three South African hard red wheat cultivars (Gariep, Elands and Tugela-DN) was initiated. Four treatments were investigated (-1°C at soft dough stage, -1°C at late milk stage, -4°C at soft dough stage and -4°C at late milk stage) and were administered for one night only. From the results obtained, the conclusion was made that, although limited in duration, such frost conditions did have an effect on the quality of South African wheat cultivars. Although various quality parameters were investigated during the study, the general discussion will be limited to HFN responses obtained. It was especially the -4°C at late milk stage that resulted in significantly reduced HFN being measured for both Elands and Tugela-DN. A similar effect was observed for Gariep, but was not statistically significant. It is possible that LMA might have played a role in the results obtained, but not probable, as Tugela-DN indicated a similar response to that of Elands, this when it is known that Tugela-DN is technically a low LMA producing cultivar.

Although the response was limited to the main tillers only, the conclusion was made that it is quite probable that the reduced HFN would be visible in the pooled sample of head and side tillers, as research has shown that only a small amount of low HFN wheat is required to reduce the HFN of a high HFN sample to below acceptable levels. As all three cultivars showed a rather prominent reaction to the -4°C late milk stage treatment, it would be interesting to see the reaction of other South African cultivated varieties to such treatments. During the course of the study a screening protocol was suggested that would

allow classification of cultivars for frost tolerance. Until such a time as cultivars of which the HFN is not subject to frost conditions can be identified, producers in areas that are known for their late frost or cold spells should avoid planting too early, as a single night of low temperatures (–4°C) at late milk stage is able to reduce HFN to below acceptable levels.

Cultivar response

Throughout all the trials performed, it was attempted to include three basic cultivars – Tugela-DN, Gariep and Elands. These cultivars were chosen specifically for the various degrees of pre-harvest sprouting resistance they possess (Tugela-DN – poor resistance, Gariep – good resistance and Elands – excellent resistance) and LMA producing capability. This was done due to the close relationship that exists between pre-harvest sprouting as well as LMA and HFN. Accordingly, a clear indication of the three cultivar's qualities and HFN response to various agricultural practises could be obtained.

Elands proved to be unaffected by the early harvest practise over a four year analysis. The question arose whether this might be linked to its PHS resistance. The cultivar, however, appeared to be heavily affected by frost conditions (-4°C) during late milk stage, as well as with moderately high temperatures (32°C for four days) at both the late milk and soft dough stages. The use of glyphosate treatments to create a more stable HFN did not have merit with this cultivar, as it showed no response to the various treatments. With the fertilizer trial, the cultivar was grouped into the high response group, indicating that should the fertilizer level fall below that of the standard practise due to leaching for example, it might result in a reduced HFN.

Gariep appeared to be moderately sensitive to early harvest as it was affected by the practise in one of four seasons of evaluation. With regard to frost conditions (-4°C, late milk stage), the cultivar can be classed as moderately to highly sensitive, as its HFN was reduced by 63 s compared to the control (although this was not statistically significant). The use of glyphosate treatments to create a more stable HFN might have merit with this cultivar, as it responded to the various treatments. The correct stage of application should, however, be established first. According to the fertilizer trial, Gariep falls in the low response group, indicating that it might be a good choice for areas prone to leaching.

Tugela-DN was, for two out of four seasons, negatively affected by the early harvest practice and could therefore be classed as a moderately to highly sensitive cultivar. Temperature stress at both low (-4°C at late milk) and high (32°C at soft dough stage) resulted in a reduced HFN, with the low temperature effect being more severe. The cultivar was, similar to Gariep, responsive to glyphosate application. Unfortunately the fertilizer response group op the cultivar is not known.

Conclusions

All of the agricultural practices investigated indicated some form of HFN reaction, mostly due to cultivar reaction. No clear link to LMA could be made in any of the cases of reduced HFN observed. This is mostly based on the fact that Tugela-DN (low LMA cultivar) was the cultivar most affected by the various treatments investigated.

The effect of frost conditions (-4°C) during late milk stage, produced the most significant effect on the HFN of the wheat cultivars evaluated, and further studies are certainly merited. As for the creation of an integrated management practice, the following can be said:

Sufficient data have been generated to recommend that allowing wheat to dry naturally on the field would probably be the first step in a HFN management system. To limit the threat of pre-harvest sprouting due to late rain, pre-harvest sprouting tolerant cultivars should be considered. Further studies into cost effective practices that would result in a more stable HFN should be initiated. Although fertilizer application might not be the most probable cause for unexplained HFN, cultivars appear to react differently regarding their HFN reaction to fertilizer application. Producers in areas prone to leaching should therefore consider cultivars that fall into the low and low to medium response groups created in Chapter 5. Additional studies into more localities would be merited. Although significantly lower HFN were obtained at 32°C treatments during grain filling, further studies are suggested before any recommendation in this regard could be made. In addition, producers in areas that are known for their late frost or cold spells should avoid planting too early, as a single night of low temperatures (–4°C) at late milk stage can reduce HFN. The screening protocol created in Chapter 6 should be utilized to screen all cultivated varieties for such reactions, so that recommendations could be made as to which cultivars would be the most tolerant.